RPOS REGULON MODULATION BY ENVIRONMENTAL SELECTION

RPOS REGULON MODULATION BY ENVIRONMENTAL SELECTION

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ABSTRACT

Regulatory interactions evolve to incorporate new genomic material and contribute to bacterial diversity. These regulatory interactions are flexible and likely provide bacteria with a means of rapid environmental adaptation. In this thesis, the RpoS regulon is used as a model system to investigate the hypothesis that regulon composition and expression are modified according to environmental pressures. Several novel findings are presented, namely the distribution of RpoS homologs in bacteria, the flexibility of the RpoS regulon, and the effect of diverse environmental pressures on RpoS regulon expression. Based on phylogenetic and reciprocal best hits analyses, RpoS was determined to be conserved in γ-, β-, and δ-proteobacteria, likely because it confers a selective advantage in many bacterial niches. Regulon composition, however, was highly flexible. Even between species of the same class, *Escherichia coli* and *Pseudomonas aeruginosa*, only 12 of 50 orthologs were regulated in common by RpoS. RpoS regulon flexibility may thus be the result of adaptation to different bacterial habitats. Indeed, mutations in *rpoS* and differential regulon expression could be identified among environmental *E. coli* isolates collected from diverse sources. Among environmental *E. coli* isolates, RpoS mutant frequency was found to be 0.3%, and activity of KatE, a prototypical RpoS regulon member, was undetectable in some isolates despite the presence of functional RpoS. Modulated RpoS regulon expression among environmental *E. coli* isolates is consistent with environment as a key factor shaping regulatory interactions. Regulon flexibility was similarly apparent in oxidative stress regulons, OxyR and SoxRS, of *E. coli*. SoxRS regulon function is weakly conserved, possibly due to low selective pressure for a superoxide stress response regulon in some bacterial species. Environment, therefore, is a crucial element that defines the dynamics of regulatory networks.

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PREFACE

This thesis is structured in sandwich format and includes three published articles. Chapter 1 (Introduction) provides the necessary background and context for the published articles (Chapters 2-4). Chapter 2, published in Journal of Molecular Evolution in 2010, elucidates the evolution of RpoS and the flexibility of the RpoS regulon between species. Chapter 3, published in Applied and Environmental Microbiology in 2011, demonstrates environmental selection of RpoS mutants and regulon modulation in environmental *E. coli* isolates. Chapter 4, published in Archives of Biochemistry and Biophysics in 2012, reviews the major oxidative stress regulons of *E. coli*, OxyR and SoxRS, and their functional conservation in bacteria. Lastly, Chapter 5 (Discussion) discusses how the previous chapters constitute one body of work and support regulon flexibility as a consequence of environmental pressures.

S. M. Chiang is the sole contributor of all published articles included in this thesis. Dr. Herb E. Schellhorn supervised and provided direction for Chapters 2-4. Dr. Tao Dong helped in the initial screening for RpoS mutants among environmental *E. coli* isolates in Chapter 3. Dr. Thomas A. Edge provided environmental *E. coli* isolates and source details in Chapter 3. The published articles are modified according to thesis format requirements and are printed in this thesis under license.

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LIST OF ABBREVIATIONS AND SYMBOLS

σ	sigma factor
ATP	adenosine 5’-triphosphate
BLAST	Basic Local Alignment Search Tool
BLASTp	BLAST for protein-protein alignment
bp	base pair
cAMP-CRP	3', 5'-cyclic AMP-cAMP response protein
CFU	colony forming unit
DNase	deoxyribonuclease
ECOR	*Escherichia coli* reference collection
E-value	Expect value
FAD	flavin adenine dinucleotide
HGT	horizontal gene transfer
HPI	hydroperoxidase I
HPII	hydroperoxidase II
INH	isoniazid
LB	Luria-Bertani medium
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
mRNA	messenger RNA
NADPH	nicotine adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NJ	Neighbor-joining
OD_{600}	optical density at 600nm
ORF	open reading frame
PAGE	polyacrylamide electrophoresis
PCR	polymerase chain reaction
ppGpp	guanosine 3’, 5’-bispyrophosphate
PVDF	polyvinylidene difluoride
RNAP	RNA polymerase
ROS	reactive oxygen species
rpm	revolutions per minute
rRNA	ribosomal RNA
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SEM	standard errors of the means
SOD	superoxide dismutase
SPANC	self preservation and nutritional competence
sRNA	small non-coding RNA
Suc^{++}	strains with enhanced growth on succinate minimal media
TCA	tricarboxylic acid
UP	upstream promoter element
UV	ultraviolet

CHAPTER 1:

Introduction

1.1 Bacterial evolution

The high genetic diversity of bacteria is increasingly apparent as more prokaryotic genome sequences become available. This diversity is attributable to three primary processes in bacterial evolution: (i) gene duplication, (ii) gene decay, and (iii) horizontal gene transfer (HGT) (Snel *et al.*, 2002; Zhi *et al.*, 2012). A comparative study using 21 archaeal and bacterial genomes of diverse genera determined that 5-33% of genomic coding sequence is a result of lineage-specific expansion, such as through gene duplication (Jordan *et al.*, 2001). Duplications occur at a small scale, in particular of single genes, and are most often of genes important for transcription, metabolism, and defense (Gevers *et al.*, 2004). The second process, gene decay or loss due to mutation, is perhaps the primary factor shaping bacterial genetic diversity, since a new gene sequence is typically deleted unless it confers a selective advantage (Mira *et al.*, 2001). In *Escherichia coli*, approximately 950-1,500 genes are lost from the common ancestor of proteobacteria (Snel *et al.*, 2002). As well, genome size, which remains small across prokaryotes despite acquisition of new genes by duplication and HGT (Mira *et al.*, 2001), is maintained by a balance of gene deletion with gene gain (Kunin & Ouzounis, 2003). The third mechanism, horizontal gene transfer, results in rapid acquisition of novel functions by the incorporation of foreign DNA (Lawrence, 1997). Indeed, the transfer of genetic material can confer an immediate selective advantage to bacteria, such as the transfer of antibiotic resistance genes (Ochman *et al.*, 2000), while gene duplication (Teichmann & Babu, 2004) and gene decay (Mira *et al.*, 2001) are traditionally considered as means for long-term evolutionary divergence. Recently however, horizontal transfer was shown to have a more substantial role in evolution because transferred genes persist longer than gene duplications (Treangen & Rocha, 2011). Since the divergence of *E. coli* from *Salmonella*, 755 out of *E. coli*'s 4,288 open reading frames (ORFs) were transferred through at least 234 individual horizontal transfer events (Lawrence & Ochman, 1998). As genetic changes accrue through duplication, gene decay, and HGT, adaptation of regulatory networks must also accommodate for novel functions.

1.1.1 Regulon development

New genomic material must be incorporated into regulatory networks for expression, and even slight changes to existing networks can dramatically impact cell phenotype. Therefore, the development of gene regulation is a necessary component for bacterial evolution. Duplication and horizontal gene transfer are two elements that shape the development of regulatory networks. Considering gene duplications, as determined by conserved protein domain structure, and known regulatory interactions, approximately one-half of regulatory interactions in *E. coli* and yeast resulted from the duplication of either a transcription factor or target gene(s) (gene(s) regulated by the transcription factor), followed by regulatory diversification, and approximately one-third resulted from duplication with inherited regulatory interaction (Teichmann & Babu, 2004). Secondly, while global regulators are predominantly gained by vertical inheritance, many target genes are acquired through horizontal transfer (Price *et al.*, 2008). Transcription factors located beside target genes, which may be functionally coupled and provide a selective advantage to the cell, are also frequently gained through horizontal transfer (Price *et al.*, 2008). The transfer of regulators is likely facilitated by their incorporation into an existing metabolic pathway of the recipient organism (Maslov *et al.*, 2009). Regulatory divergence by duplication and transfer helps to shape the organization of transcriptional networks.

Transcriptional networks of bacteria are relatively flexible and provide the cell with the ability to quickly adapt to the environment (Lozada-Chavez *et al.*, 2006). Indeed, only 30% of transcription factors and target genes from *E. coli* are conserved in proteobacteria (Lozada-Chavez *et al.*, 2006). A comparison of 176 genomes, used as a representative set of all sequenced prokaryotic genomes, indicates that transcription factors are less conserved, and therefore evolve more rapidly, than target genes (Madan *et al.*, 2006). While transcription factors and target genes often evolve independently, species with similar lifestyles possess a higher number of homologous regulatory interactions compared to species with dissimilar lifestyles (Madan *et al.*, 2006). One means for expression of a newly acquired gene is by its incorporation into extant regulons, in which a group of genes are co-ordinately regulated by the same factor. Regulons are characteristically composed of variable genes and core genes, the latter of which are conserved in the regulon across several species. Regulons with a defined core gene set include the PhoP regulon that functions in Mg^{2+} homeostasis and has two conserved operons, *phoPQ* and lipoprotein-encoding *slyB*, in both *Salmonella* and *Yersinia* (Perez *et al.*, 2009) and the LexA regulon that expresses DNA repair genes and has five conserved operons among alpha- and gammaproteobacteria (Erill *et al.*, 2007).

Therefore despite the predominance of independent evolution, it is probable that regulation of the core gene set evolves, in part, by the co-evolution of the transcription factor and target gene(s). Sigma factors, which are subunits of the RNA polymerase that recognize promoter sequences, may also control expression of core gene sets.

As an aside, the evolution of gene regulation is difficult to determine with currently available *in silico* methods. Orthologous transcription factors and target genes across more than one bacterial species are occasionally used as an indicator of regulatory conservation (Madan *et al*., 2006; Mironov *et al*., 1999). However, because transcription factors rapidly diverge in function, transcription factor orthology alone does not indicate regulatory conservation (Baumbach, 2010; Price *et al*., 2007). As well, the presence of binding motifs and promoter recognition sites have been used to elucidate regulatory interactions (Monsieurs *et al.*, 2005). While a useful method in predicting direct protein-DNA interaction, a binding motif is often difficult to identify due to the low number of known recognition sites, and in cases where a binding motif is identifiable, these motifs are poorly conserved (Mironov *et al.*, 1999) and provide little information on the magnitude of regulation. Therefore, the evolutionary development of gene regulation is difficult to fully elucidate by sequence comparison due to the rapid functional divergence of transcription factors and motif dissimilarity.

1.1.2 Sigma factor conservation

Bacterial transcription consists of the recognition and transcription of genes by RNA polymerase (RNAP). The core enzyme of RNAP transcribes non-specifically, but associated sigma factors guide RNAP to cognate genes by specific interaction with gene promoters and accessory transcription factors (Ishihama, 2000). Among 291 bacterial genomes that were used as a non-redundant, representative set of all sequenced bacterial genomes, sigma factors were found to be 10× less abundant than other transcription factors, suggesting possible constrained evolution of sigma factors (Perez-Rueda *et al*., 2009). The necessity of sigma factors and the limited means by which sigma factors affect transcription, i.e., by interaction only with core RNAP and promoter recognition sites (not with multiple proteins or DNA sequences), has likely limited the evolutionary divergence of sigma factors and cognate genes (Perez-Rueda *et al*., 2009). There are two families of sigma factors in bacteria, sigma 70 and sigma 54, which are evolutionarily distinct (Merrick, 1993; Wosten, 1998). Sigma factors from both families are broadly found in bacteria, but the most prevalent of these families is the sigma 70 family (Wosten, 1998). Genes encoding sigma 70 factors have high sequence similarity, in particular in regions that encode sigma 70 regions that bind core RNAP or promoter DNA (Lonetto *et al*., 1992). In phylogenetic analyses, sigma 70 factors with equivalent functions, such as housekeeping functions or heat shock response, further cluster together (Lonetto *et al*., 1992; Wosten, 1998), suggesting predominantly vertical inheritance of functionally related sigma factors. Both sigma factor families are found in the commonly used model organism, *E. coli*.

1.2 *E. coli* sigma factors

In *E. coli*, there are seven sigma factors that guide core RNAP to promoter recognition sites and, consequently, control gene expression. These factors regulate the transcription of genes in growth or in response to environmental cues, such as nitrogen metabolism or hyperosmolarity (Bianchi & Baneyx, 1999; Merrick, 1993).

1.2.1 *E. coli* sigma 70 factors

Six of the seven sigma factors present in *E. coli* are from the sigma 70 family. These factors are further subdivided into four groups based on sequence and function (Gruber & Gross, 2003; Lonetto *et al*., 1992). Group 1 sigma factors are primary sigma factors and are highly conserved across bacterial species (Lonetto *et al*., 1992). Belonging to group 1 sigma factors, the primary sigma factor of *E. coli* is RpoD (σ^{70}), which directs the transcription of ~1,000 housekeeping genes (Ishihama, 2000). RpoD recognizes the –10 region "TATAAT" and the –35 region "TTGACA" of dependent promoters and has preference for a 17 ± 1 bp spacer length between these two regions (Harley & Reynolds, 1987; Typas & Hengge, 2006). As the primary sigma factor, RpoD is the most abundant sigma factor in *E. coli* and has the highest affinity for core RNAP (Jishage *et al*., 1996; Maeda *et al*., 2000).

An alternative sigma factor, RpoS (σ^{38}), belongs to group 2 of sigma 70 factors. RpoS is primarily active during the transition to stationary phase and in response to stress, such as oxidative stress (Schellhorn, 1995) and acidic pH (Mukhopadhyay & Schellhorn, 1994; Small *et al*., 1994). The RpoS regulon consists of at least 200 positively regulated genes and an almost equal number that are negatively regulated (Patten *et al*., 2004). Characteristic of group 2 sigma factors, RpoS sequence is highly conserved with RpoD, in particular a 65 amino acid region with 85% similarity (Mulvey & Loewen, 1989), but is dispensable for growth (Lonetto *et al*., 1992).

Despite sequence similarity, RpoS and RpoD regulate predominantly distinct regulons, largely due to differences in promoter recognition sequences (Gaal *et al*., 2001; Typas *et al*., 2007b). For example, a cytosine preceding the –10 region at position –13 is conserved in many RpoS-dependent promoters and increases the specificity of RpoS recognition. For *csiD* and *aidB* promoters, both of which are highly characterized RpoS-dependent genes, transcriptional induction by RpoS is strongest in the presence of C-13 and impaired when C-13 is mutated (Becker & Hengge-Aronis, 2001; Lacour *et al*., 2002). A guanine at the same –13 position, conserved in many RpoD-dependent promoters, has no effect on RpoD specificity (Becker & Hengge-Aronis, 2001). RpoS is also more tolerant of deviations from the promoter consensus than RpoD by recognizing spacer lengths between 15 and 19 bp (Typas & Hengge, 2006). Other factors that favour RpoS promoter recognition over RpoD is the presence of the distal portion of the upstream promoter elements (UP) as opposed to the full UP (Germer *et al*., 2001; Typas & Hengge, 2005) and of promoters with intrinsic curvature (Espinosa-Urgel & Tormo, 1993).

RpoH (σ^{32}) and FliA (σ^{28}) of *E. coli* are categorized as group 3 sigma 70 factors (Gruber & Gross, 2003). Group 3 sigma factors generally respond to a specific cellular stimulus (Lonetto *et al*., 1992). RpoH, for instance, is induced during heat shock (Grossman *et al*., 1984). After five minutes of RpoH expression from an inducible promoter, approximately 130 genes increase in expression, including heat shock proteins that aid in protein repair (Zhao *et al*., 2005). The RpoH-dependent chaperone protein, DnaK, functions in negative feedback regulation of RpoH activity, since *rpoH* and RpoH-dependent transcripts are more abundant in a *dnaK* mutant compared to a wild type control (Zhao *et al.*, 2005). Secondly, FliA controls the last developmental stage of flagellar biosynthesis (Helmann, 1991). Flagellar biosynthesis occurs through a three-step cascade of gene expression, termed early, middle and late phases. The early phase genes, *fhlDC*, are transcriptional activators for the genes in middle phase, which, in turn, assemble the hook-basal body of the flagellum. FliA, and its anti-sigma factor FlgM, regulate late phase genes and, consequently, the final stage of flagella formation (Chilcott & Hughes, 2000). FlgM both inhibits FliA and protects FliA from degradation until after the hook-basal body of the flagellum is formed (Barembruch & Hengge, 2007). Once formed, FlgM is secreted from the cell, which induces FliA regulon expression (Karlinsey *et al.*, 2000) and simultaneously allows for FliA degradation, thereby providing transient expression of late phase genes (Barembruch & Hengge, 2007).

Group 4 sigma factors constitute the largest percentage of known sigma factors and are typically induced in response to cues at the cell surface (Gruber & Gross, 2003). In *E. coli*, there are two group 4 sigma factors: RpoE (σ^{24}) and FecI (σ^{fecI}). RpoE regulates over 40 genes in response to extracytoplasmic stress (Dartigalongue *et al.*, 2001). The RpoE regulon, along with the RpoH regulon, repairs protein misfolding (Mecsas *et al.*, 1993). Hence, RpoE activates genes in response to heat-shock and hyperosmotic shock when protein damage or rapid protein synthesis occurs (Bianchi & Baneyx, 1999). FecI, in the presence of ferric citrate, controls genes that respond to iron deprivation (Braun *et al.*, 2003). FecA, an outer membrane receptor protein, detects the presence of ferric citrate and relays a signal to FecR, a transmembrane protein. FecR, in turn, activates FecI to transcribe the iron transport genes, *fecABCDE*, and import iron (Enz *et al.*, 2000). When iron is in excess, expression of *fecIR* and the neighbouring *fecABCDE* operon are repressed by Fur (Angerer & Braun, 1998).

1.2.2 *E. coli* sigma 54 factor

RpoN (σ^{54}) is structurally distinct from the sigma 70 factors (Lonetto *et al.*, 1992). RpoN, which is widely distributed in α- and γ-proteobacteria (Morett & Segovia, 1993), has several functions, including response to nitrogen limitation (Hirschman *et al.*, 1985) and heat shock (Kuczynska-Wisnik *et al.*, 2001), as well as flagellar biosynthesis (Zhao *et al.*, 2010). Next to RpoD, RpoN has the highest affinity for core RNAP (Maeda *et al.*, 2000), but unlike the other sigma factors, it requires an enhancer-binding protein and ATP to maintain an open RNAP complex during transcription (Kustu *et al.*, 1991; Wedel & Kustu, 1995). RpoN recognizes unusually close promoter boxes, –12 and –24 (Zhao *et al.*, 2010). Regulons controlled by sigma factors, including RpoS, are dependent on cellular induction of the sigma factor and the activity of accessory transcription factors for expression.

1.3 RpoS regulon expression

1.3.1 Induction of RpoS

The RpoS regulon, and therefore genes necessary for stationary phase and stress survival, is induced when RpoS protein levels in the cell are high. RpoS abundance is controlled by the transcription and translation of *rpoS*, as well as RpoS proteolysis (Lange

& Hengge-Aronis, 1994). RpoS is undetectable by quantitative immunoblot in exponential phase but reaches over 1,600 molecules per cell in stationary phase (Piper *et al.*, 2009). When entering stationary phase in rich media, *rpoS* transcription increases five-fold and *rpoS* translation increases up to 30-fold. RpoS protein is also more stable in stationary phase, with a half-life of 1.4 min in exponential phase and 10.5 min in stationary phase (Lange & Hengge-Aronis, 1994). Similarly in stress, intracellular RpoS levels increase (Jishage *et al.*, 1996), such as through a 14-fold induction of *rpoS* translation in hyperosmotic conditions (Lange & Hengge-Aronis, 1994).

Factors affecting these three stages of RpoS expression are numerous (Dong *et al.*, 2008a). Some negative regulators of *rpoS* transcription in exponential phase are 3'-5'-cyclic AMP-cAMP response protein (cAMP-CRP) and Fis. A Δ*cya* mutant, which is unable to produce cyclic AMP (cAMP), has high RpoS abundance in exponential phase, and the addition of cAMP reduces *rpoS* transcription (Lange & Hengge-Aronis, 1991; Lange & Hengge-Aronis, 1994). As well, a Δ*fis* mutant of the closely related *Salmonella enterica* has nine-fold more expression from the *rpoS* promoter (Hirsch & Elliott, 2005b). Fis blocks transcription by binding to the *rpoS* promoter (Hirsch & Elliott, 2005a). Examples of positive regulators of *rpoS* transcription are guanosine 3’, 5’-bispyrophosphate (ppGpp) and polyphosphate (Dong *et al.*, 2008a). As for *rpoS* translation, the small non-coding RNAs (sRNA), DsrA and RprA, bind the 5’ region of *rpoS* mRNA, disrupting a hairpin loop and allowing for translation (Majdalani *et al.*, 2001; Majdalani *et al.*, 1998), while a histone-like protein, H-NS, reduces translation by an unknown mechanism (Barth *et al.*, 1995; Battesti *et al.*, 2011). Lastly, in proteolysis, the response regulator RssB interacts with RpoS and guides it to ClpXP for degradation in exponential phase (Becker *et al.*, 1999). Clearly, regulation of RpoS abundance, and therefore its regulon, is a complex response to many regulatory signals.

In stress conditions, there are several regulatory mechanisms triggering RpoS induction (Battesti *et al*., 2011). Under normal conditions, the antitoxin, MqsA, represses *rpoS* transcription by binding to a palindromic sequence in the *rpoS* promoter. Upon oxidative stress, MqsA is degraded by Lon protease, which allows for expression of *rpoS* (Wang *et al.*, 2011). As well, prevention of RpoS proteolysis is a key factor in RpoS regulon induction during stress (Battesti *et al*., 2011). In particular, RssB is inhibited by IraP during phosphate starvation (Bougdour *et al.*, 2006), IraM during magnesium starvation, and IraD following DNA damage (Bougdour *et al.*, 2008). With RpoS induction, *E. coli* has increased resistance to stress and survival in stationary phase.

1.3.2 RpoS function in stationary phase and stress response

RpoS regulates up to 10% of the *E. coli* genome (Patten *et al*., 2004; Weber *et al*., 2005) and, due to its significant global impact on gene expression, is considered to be a second vegetative sigma factor during non-optimal growth conditions (Weber *et al*., 2005). Examples of key RpoS-dependent genes important for stress resistance are *katE* and *sodC* for oxidative response and *otsBA* for temperature shift. One of two catalase genes in *E. coli*, *katE*, is highly dependent on RpoS for expression and functions to degrade hydrogen peroxide into water and oxygen (Schellhorn & Hassan, 1988). Similarly, a superoxide dismutase (CuZnSOD, encoded by *sodC*) that dismutates reactive superoxide to hydrogen peroxide and oxygen is also highly RpoS-dependent, as *sodC* transcription is over 30-fold less abundant in an *rpoS* mutant culture grown aerobically in stationary phase (Gort *et al.*, 1999). After temperature shift, RpoS induces *otsBA*, which encode trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, necessary for trehalose synthesis (Hengge-Aronis *et al.*, 1991). Trehalose is a thermoprotectant, and as such, an *otsA* mutant loses significant viability compared to a wild type control at 4°C (Kandror *et al.*, 2002).

Although typically recognized as a general stress and stationary phase regulator, RpoS also has a regulatory role in exponential phase in the absence of stress (Dong *et al.*, 2008b; Rahman *et al.*, 2006). Microarray analysis indicates that almost 300 genes are regulated by RpoS in exponential phase (Dong *et al.*, 2008b). Of highly RpoS-dependent genes (expression ratio $rpoS^{+}$:$rpoS^{-} \geq 4$), only 12 of 75 are commonly regulated by RpoS in both stationary phase and exponential phase. This indicates that RpoS regulon function in exponential phase is largely distinct from its function in stationary phase. In exponential phase, RpoS positively regulates genes important in carbon transport, protein folding and iron acquisition (Dong *et al.*, 2008b). Crl also stimulates expression of some RpoS regulon genes in exponential phase (Dong *et al.*, 2008b), consistent with its differential effect on regulation when RpoS concentrations are low (Typas *et al.*, 2007a).

Negative regulation by RpoS has a large impact on global *E. coli* gene expression, since an equivalent number of genes are down-regulated as are up-regulated in early stationary phase (Patten *et al.*, 2004). Negatively regulated genes include those responsible for flagellar biosynthesis and energy metabolism (genes that encode for tricarboxylic acid (TCA) cycle enzymes) (Patten *et al*., 2004; Rahman *et al*., 2006). RpoS likely functions indirectly as a negative regulator due to sigma factor competition for limited core RNAP. There are approximately 2,500 core RNAP molecules per cell and a surplus of sigma factors, which includes ~7,000 RpoD molecules per cell (Piper *et al*., 2009). Evidence that supports the sigma factor competition model includes higher expression of the RpoD-dependent universal stress protein gene, *uspB*, in an *rpoS* mutant or in a strain with RpoD overproduction (Farewell *et al.*, 1998). As well, RpoS-dependent gene expression decreases when RpoD is overproduced (Farewell *et al.*, 1998). Because the maximal intracellular level of RpoS is only 30% that of RpoD (Jishage *et al*., 1996; Piper *et al*., 2009) and RpoS has the weakest affinity to core RNAP among the other six *E. coli* sigma factors (Maeda *et al*., 2000), accessory transcription factors, in addition to increased RpoS levels, aid RpoS in competing with other sigma factors for core RNAP. For example, anti-sigma factor Rsd (*r*egulator of *s*igma *D*) roughly doubles in abundance from exponential phase to stationary phase and sequesters a high portion of RpoD in stationary phase, allowing for more RpoS-core RNAP binding (Piper *et al*., 2009). As well, the alarmone ppGpp provides greater competitive advantage to RpoS, RpoE, and RpoN over RpoD for core RNAP, and RpoS is less efficient at binding in stationary phase in the absence of ppGpp (Jishage *et al*., 2002; Sze & Shingler, 1999). Considering the large number of genes positively or negatively regulated by RpoS, the loss of RpoS by mutation has a profound effect on phenotype.

1.4 Mutations in *rpoS*

Loss of RpoS function mutations are selected under conditions that favour expression of genes negatively regulated by RpoS, and therefore, selection of *rpoS* mutations may serve as a molecular switch mechanism for rapid environmental adaptation (Chen *et al*., 2004; Patten *et al*., 2004). In a phosphate-limited chemostat, *E. coli* acquires mutations that result in a decrease in RpoS protein levels (Wang *et al*., 2010). As well, *rpoS* polymorphisms are present in laboratory and pathogenic *E. coli* (Atlung *et al*., 2002; Dong *et al*., 2009) and in species of Salmonellae (Jordan *et al*., 1999). While an *rpoS* mutant is impaired in stationary phase growth (Lange & Hengge-Aronis, 1991) and stress resistance (Hengge-Aronis *et al*., 1993; Kabir *et al*., 2004; Small *et al*., 1994), a mutant is better able to utilize poor carbon sources (Chen *et al*., 2004; King *et al*., 2006). Mutations that are selected in *rpoS* occur in one of four functional regions.

1.4.1 RpoS functional regions

The functional regions of RpoS control promoter recognition, core RNA polymerase attachment, or transcription efficiency. The least conserved regions across sigma 70 factors are regions 1 and 3, which are important for maintaining an open complex formation during transcription (Wilson & Dombroski, 1997) and affinity to core RNAP (Zhou *et al*., 1992), respectively.

Region 2 of RpoS, the most similar to that of RpoD, is subdivided into regions 2.1, 2.2, 2.3, 2.4, and 2.5 (Lonetto *et al*., 1992). Mutations within region 2 of *rpoS* result in defects of DNA melting and RNAP isomerization (Lee & Gralla, 2003). Region 2.5 is specifically implicated in recognizing the C(–13) and T(–14) of RpoS-dependent promoters, a sequence that confers RpoS specificity (Becker & Hengge-Aronis, 2001). The most characterized of the subregions, region 2.4, recognizes the –10 promoter regions of dependent genes (Gross *et al*., 1996; Waldburger *et al*., 1990).

Region 4 of RpoS is subdivided into regions 4.1 and 4.2. Region 4.2 recognizes the –35 promoter sequence of RpoS-dependent genes (Helmann & Chamberlin, 1988). While the –35 motif is not required for recognition of all RpoS-dependent promoters (Colland *et al*., 1999; Hiratsu *et al*., 1995), RpoS recognizes the same –35 motif as that of RpoD (Gaal *et al*., 2001) and is important for recognition of other promoters (Rosenthal *et al.*, 2006). The importance of functional region 4 for RpoS recognition is uncertain.

1.4.2 Environmental selection of RpoS mutants

Strains with non-functional RpoS due to acquired *rpoS* mutations are present in pathogenic (Dong *et al.*, 2009) and in laboratory *E. coli* populations (Atlung *et al.*, 2002). Because RpoS has a global regulatory effect during stress and stationary phase, environmental selection of deleterious mutations in the *rpoS* gene has significant implications for cell survival. In particular, RpoS mutants are more susceptible to stress conditions but can metabolize a greater number of substrates (King *et al.*, 2004), likely through induction of TCA cycle enzymes that are negatively regulated by RpoS (Patten *et al.*, 2004). As mentioned previously, an increase in TCA cycle enzymes in an RpoS mutant is likely through sigma factor competition, where loss of RpoS allows for other sigma factors, such as growth-related RpoD, to bind to limited core RNAP (Farewell *et al.*, 1998; Maeda *et al.*, 2000). Therefore, *rpoS* mutational selection is the result of antagonistic pleiotropy, where loss of RpoS confers an advantage when grown on poor carbon sources and a disadvantage in stress (Ferenci, 2008; Nystrom, 2004).

Mutations in *rpoS* are selected when *E. coli* is grown with succinate as the sole carbon source. Compared to wild type, RpoS mutants have enhanced growth on succinate minimal media and are visible as larger colonies (Chen *et al.*, 2004). Subjecting *E. coli* to other stresses, such as long-term growth (Zambrano *et al.*, 1993) and limited nutrient conditions (King *et al.*, 2006), also selects for *rpoS* mutations. The effect of selective forces within natural environments upon the acquisition of *rpoS* mutations in environmental *E. coli* populations remains to be determined. Importantly, *E. coli* isolates in the environment are likely subject to both *rpoS*$^{+}$ and *rpo*S^{-} selection conditions, such as osmotic shock by release into an aquatic environment or by dehydration (Garfield & Walker, 2008; Munro *et al.*, 1995) and nutrient deprivation (Ferenci & Spira, 2007), respectively. Could adaptation through environmental selection of *rpoS* mutations and modulation of RpoS regulon composition be a contributing factor to the evolutionary development of this regulon?

1.5 Thesis overview

Regulons provide coordinated gene expression for cell growth and for responding to the environment. Due to the prevalence of a core gene set in regulons (Erill *et al.*, 2007; Perez *et al*., 2009) and because sigma factors are more conserved than other transcription factors (Perez-Rueda *et al*., 2009), it is likely that some regulon members co-evolve with their regulating sigma factor with vertical inheritance. The RpoS regulon is a useful model in the study of regulon evolution and adaptation because it has been extensively researched in proteobacteria (Patten *et al*., 2004; Schuster *et al*., 2004) and a spirochaete (Caimano *et al*., 2007) and is subject to environmental selection, even within one species (Chen *et al*., 2004; Dong *et al*., 2009). As well, although RpoS mutants have been identified in laboratory (Atlung *et al*., 2002) and pathogenic (Dong *et al*., 2009) *E. coli*, selection for RpoS and its cognate regulon in environmental *E. coli* populations that are subject to diverse selective pressures is not known.

For this thesis, the RpoS regulon of *E. coli* was used as a model system to study regulon evolution and adaptation in the natural environment. It was hypothesized that regulons, in particular RpoS and RpoS regulon composition, are modulated according to bacterial niche and environmental pressures. The three following chapters address the evolution of the RpoS regulon, selection of RpoS in environmental *E. coli* isolates, and, for comparison, the conservation of oxidative stress regulons. Several questions were investigated, including what species contain an RpoS homolog, how conserved is the RpoS regulon, and what effect does the environment have on RpoS adaptation? These chapters shed light on RpoS regulon divergence in bacteria of disparate niches and the flexibility of the RpoS regulon, even among strains of the same species. Although strictly avoided, some repetition of introductory and discussion material occurs in Chapters 2-4 as part of stand-alone publications. Some references are repeated, since separate reference lists are provided with each published article. References for the introduction and discussion are amalgamated into one list at the end of the discussion.

CHAPTER 2:

Evolution of the RpoS regulon: Origin of RpoS and the conservation of RpoS-dependent regulation in bacteria

adapted from

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2.1 Preface

This chapter is adapted from an article published in Journal of Molecular Evolution to adhere to thesis format requirements. Two figures, originally presented as supplementary Figures 1 and 2 in the published article, are now within the main body of this chapter as Figures 2.2 and 2.3. Figure 2.6 is also modified from the published article to include *E. coli* K12 RpoD in the sequence alignment. S. M. Chiang performed all experiments from year 2008-2010 and is the primary author of the article.

2.2 Abstract

The RpoS sigma factor in proteobacteria regulates genes in stationary phase and in response to stress. Although of conserved function, the RpoS regulon may have different gene composition across species due to high genomic diversity and to known environmental conditions that select for RpoS mutants. In this study, the distribution of RpoS homologs in prokaryotes and the differential dependence of regulon members on RpoS for expression in two γ-proteobacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were examined. Using a maximum-likelihood phylogeny and reciprocal best hits analysis, we show that the RpoS sigma factor is conserved within γ-, β-, and δ-proteobacteria. Annotated RpoS of *Borrelia* and the enteric RpoS are postulated to have separate evolutionary origins. To determine the conservation of RpoS-dependent gene expression across species, reciprocal best hits analysis was used to identify orthologs of the *E. coli* RpoS regulon in the RpoS regulon of *P. aeruginosa*. Of the 186 RpoS-dependent genes of *E. coli*, fifty proteins have an ortholog within the *P. aeruginosa* genome. Twelve genes of the fifty orthologs are RpoS-dependent in both species, and at least four genes are regulated by RpoS in other γ-proteobacteria. Despite RpoS conservation in γ-, β-, and δ-proteobacteria, RpoS regulon composition is subject to modification between species. Environmental selection for RpoS mutants likely contributes to the evolutionary divergence and specialization of the RpoS regulon within different bacterial genomes.

Key Words: RpoS, regulon, evolution, proteobacteria, sigma factor, *Escherichia coli*, *Pseudomonas aeruginosa*

2.3 Introduction

Development of gene regulatory systems is a key aspect of bacterial evolution, given that gene regulation impacts phenotype (Perez and Groisman 2009a) and becomes increasingly complex with genome size (Maslov et al. 2009; Vicente and Mingorance 2008). Regulons are major regulatory systems that provide a controlled gene expression response to environmental conditions. In some regulons, regulation of a subset of genes, typically with roles in housekeeping tasks (Erill et al. 2007), are conserved under the same regulator. In the SOS regulon, three DNA polymerases (PolB, DinB, UmuC) experience selective pressure to maintain function within natural populations of *Escherichia coli* (Bjedov et al. 2003), and similar selective pressure on DNA repair polymerases is suggested in all three domains of life (Bjedov et al. 2003; Ohmori et al. 2001). Regulons, however, are also characteristically plastic across species (Lozada-Chavez et al. 2006). For example, the PhoPQ regulon contains over 200 highly regulated genes in both *E. coli* (Minagawa et al. 2003) and *Salmonella typhimurium* (Monsieurs et al. 2005), of which only 13 operons are in common (Monsieurs et al. 2005). These highly plastic bacterial regulons show evidence of lineage-specific modifications (Liu and Ochman 2007). Secondary flagellar genes in proteobacteria exhibit considerable genomic rearrangement, and the genes within γ- and β-proteobacterial flagellar operons are largely dissociated in ε- and α-proteobacteria (Liu and Ochman 2007). Selective pressure appears to maintain essential core genes within regulons, as well as allow for plasticity and for lineage-specific changes in regulon composition between species.

Regulons that contain genes of nonessential function, such as the stress response RpoS regulon, may be important for adaptation or survival of the cell in nature. Without housekeeping genes, the selective pressure to maintain regulon composition may be minimal. This study examines the evolution of the RpoS regulon, which can be rapidly lost under environmental pressure (King et al. 2006; Zambrano et al. 1993), as a model for nonessential regulon development. RpoS controls the expression of genes that enhance cell survival under stress conditions and during entry into stationary phase in many proteobacteria (see reviews Hengge-Aronis 2000; Loewen and Hengge-Aronis 1994). RpoS also controls the expression of virulence genes in the *Borrelia* (Caimano et al. 2004), *Salmonella* (Kowarz et al. 1994), and *Yersinia* genera (Iriarte et al. 1995), as well as aids in *Legionella* replication in macrophages (for review on role of RpoS in pathogens, see Dong and Schellhorn 2010). In *E. coli* and *Pseudomonas aeruginosa*, over 100 and 700 genes are positively regulated by RpoS in early stationary phase, respectively (Patten et al. 2004; Schuster et al. 2004). Despite the large size and importance of the RpoS regulon in stress survival and virulence, loss of RpoS increases cells' ability to scavenge for limited nutrients (Chen et al. 2004; Ferenci and Spira 2007; King et al. 2006). In fact, pathogenic *E. coli*, that tolerate environments where RpoS function enhances survival such as acidic pH of the stomach (Foster 2004), can readily lose RpoS function through mutation (Dong et al. 2009a). The *rpoS* gene is highly polymorphic even within strains of one species (Dong et al. 2009a; Jordan et al. 1999).

The RpoS sigma factor belongs to the sigma 70 family, widely present in bacterial species (Lonetto et al. 1992; Potvin et al. 2008). In phylogenetic analyses, sigma factors cluster according to structure and physiological role, such as clustering of essential primary sigma factors (Wosten 1998). RpoS has high amino acid similarity to the primary sigma factor of *E. coli*, RpoD (Mulvey and Loewen 1989; Wosten 1998). Other sigma 70 family sigma factors of *E. coli* and γ-proteobacteria include RpoH (heat-shock sigma), RpoF (flagellar sigma), RpoE and FecI (extracytoplasmic function sigmas) (Wosten 1998). Distribution of RpoS homologs in prokaryotes is primarily within γ- and β-proteobacteria (Gruber and Bryant 1997; Wosten 1998), but RpoS has also been reported in δ-proteobacteria (Nunez et al. 2004) and spirochaetes (Elias et al. 2000), as well as an RpoS-like sigma factor in Bacteroidetes (Vingadassalom et al. 2005). Incorporation of stress-related and stationary-phase genes into the RpoS regulon of these diverse bacterial species has not been examined.

In this study, we examined if (1) RpoS has co-evolved with regulon members through speciation as indicated by the presence of a core regulon, or, alternatively, (2) the RpoS regulon has little or no regulon conservation between species, which would infer a low selective pressure to maintain composition. To understand the evolution of the RpoS sigma factor, we identify the distribution of RpoS homologs in bacteria and archaea. As well, the evolution of regulon composition is examined through comparison of *E. coli* and *Pseudomonas aeruginosa* RpoS regulons. Conserved RpoS-regulated members in *E. coli* and *P. aeruginosa* may indicate selective pressure on particular physiological functions of the regulon during speciation, as well as help to illustrate how nonessential stress regulons undergo diversification and selection.

2.4 Methods

Identification of prokaryote classes with RpoS homologs. *Phylogenetic analyses*. Homologs to the *E. coli* K12 RpoS (NP_417221) sequence were searched for using Basic Local Alignment Search Tool for protein-protein alignment (BLASTp) (Altschul et al. 1990) with an *E*-value threshold of 1e-04 and BLASTp defaults [BLASTp defaults: word size 3; scoring matrix BLOSUM62 (Henikoff and Henikoff 1992); gap costs–existence 11, extension 1] against 41 diverse prokaryote genera, which serve as a nonredundant set spanning all available fully sequenced genomes (Table 2.1). Each of the 41 genomes, available from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/) (July 2009), was independently searched. All protein sequences with an *E*-value $\leq$ 1e-04 were retrieved. Sequences were aligned using Clustal X (Larkin et al. 2007), and a maximum-likelihood phylogeny was constructed using Tree-Puzzle 5.2 (Schmidt et al. 2002) with the quartet puzzling algorithm (Strimmer and von Haeseler 1996). To test the integrity of branches, 50,000 puzzling steps were computed for QP values. The protein alignment was visualized as a phylogeny with the use of MEGA 4.0 (Tamura et al. 2007), and RpoS homologs were identified as a distinct cluster.

Reciprocal best hits analyses. To ensure that all possible RpoS homologs were evaluated, we employed a reciprocal BLAST search (Altenhoff and Dessimoz 2009; Moreno-Hagelsieb and Latimer 2008) to identify any homologs not present in the 41 genomes employed in phylogeny construction. An analysis of *E*-value threshold versus number of hits to *E. coli* RpoS (NP_417221) against NCBI's RefSeq database (Pruitt et al. 2007) was performed. Number of reciprocal best hits to *E. coli* RpoS was then determined to ascertain an appropriate *E*-value that would minimize false-positive RpoS homologs and ensure that no RpoS homologs of distantly related species or horizontally transferred RpoS homologs were excluded. Protein sequences from all hits in the RefSeq database were retrieved at each given *E*-value threshold (*E*-values: 1e-70, 1e-60, 1e-50, 1e-40, 1e-04, 1e04). These sequences were then reciprocally BLAST with the same *E*-value parameter against the *E. coli* K12 genome (NC_000913) using a BLAST standalone package (Altschul et al. 1990; Camacho et al. 2009). Best hits to the *E. coli* genome were retrieved, and reciprocal best hits to RpoS were determined.

RpoS and RpoD distribution in prokaryotes. RpoD homologs were identified in the 41 genera (Table 2.1) using a neighbor-joining tree. Sequences were retrieved by searching *E. coli* RpoD (NP_417539) against each of the 41 genomes using BLASTp with an *E*-value threshold of 1e-04, as done for RpoS homolog identification by phylogeny. The NJ tree was constructed and bootstrapped 1000× by Clustal X (Larkin et al. 2007), and the phylogeny was visualized using MEGA 4.0 (Tamura et al. 2007). RpoD homologs were identified from other sigma factors as a cluster.

RpoS and RpoD homolog distribution in prokaryotes were compared against a 16S rRNA phylogeny of the 41 distinct, representative microbial genomes. Ribosomal RNA sequences for each species were retrieved from the Ribosomal Database Project website (Cole et al. 2007) and aligned using Clustal X (Larkin et al. 2007). The aligned proteins were visualized as a phylogeny with the use of MEGA 4.0 (Tamura et al. 2007).

Comparison of *E. coli* and *P. aeruginosa* RpoS regulons. A reciprocal best hits approach was used to identify ortholog members of the *E. coli* RpoS regulon in *P. aeruginosa* (Altenhoff and Dessimoz 2009; Moreno-Hagelsieb and Latimer 2008). All *E. coli* K12 (NC_000913) and *P. aeruginosa* PA01 (NC_002516) proteins were BLAST against each other (*E*-value $\leq$ 1e-04). Reciprocal best hits in *E. coli* and *P. aeruginosa* were considered orthologs. Some proteins were second or third best hits, and in these cases, we searched for synteny of the gene region using BioCyc (Karp et al. 2005).

2.5 Results

RpoS homologs are confined in γ-, β-, and δ-proteobacterial classes. RpoS homologs have been identified in species of γ- and β-proteobacteria (Hales and Shuman 1999; Kowarz et al. 1994; Subsin et al. 2003), as well as the δ–proteobacterium *Geobacter* (Nunez et al. 2004) and the spirochaete *Borrelia* (Elias et al. 2000). The presence of RpoS in distantly related species suggests an early evolutionary origin and broad distribution of RpoS homologs in bacteria. We examined the distribution of RpoS homologs in prokaryotes using phylogenetic and reciprocal best hits approaches.

A search for RpoS homologs in 41 diverse, completely sequenced microbial genomes was performed to identify the distribution of RpoS homologs in prokaryotes. The genomes used in the BLAST search were from archaea and bacteria. Bacterial classes consisted of all five proteobacterial classes, actinobacteria, firmicutes, tenericutes, bacteroidetes, chlorobi, spirochaetes, chlamydiae, cyanobacteria, and thermotogae (Table 2.1).

Due to the similarity of RpoS to other sigma factors (Lonetto et al. 1992), BLAST searches alone cannot distinguish between RpoS and other sigma 70 family sigma factor homologs. For this reason, a maximum-likelihood phylogeny tree was constructed of all proteins with an *E*-value $\leq$ 1e-04 to *E. coli* RpoS. In the resulting phylogeny, RpoS homologs clearly segregated as a cluster (Figure 2.1).

The RpoS homolog cluster consisted of bacterial species from the γ-, β-, and δ-proteobacterial classes. Interestingly, no sigma factor of the β-proteobacteria, *Neisseria gonorrhoeae* FA, mapped to the RpoS cluster, although all other species of β-proteobacteria examined possessed an RpoS homolog. Also absent from the RpoS cluster were sigma factors of α- and ε-proteobacteria and the spirochaete, *Borrelia afzelii* ACA-1. For the β-proteobacterium, *Burkholderia ambifaria* AMMD, RpoS is annotated as two separate proteins within the genome, but only one annotated RpoS protein (YP_773648.1; annotated Buramb RpoS1 on Figure 2.1) was found in the RpoS sigma factor cluster.

The species *Acidithiobacillus ferrooxidans* ATCC 23270 possesses two RpoS homologs according to the maximum-likelihood phylogeny (Figure 2.1; annotated as Acifer P1, Acifer P2). RpoS is most similar to the essential, primary sigma factor RpoD, and therefore, a duplicate RpoS homolog could potentially have arisen through other RpoD duplications. However, separate BLAST searches with RpoS (NP_417221) and RpoD (NP_417539) from *E. coli* K12 as queries against *A. ferrooxidans* revealed that both RpoS homologs were closer to RpoS than RpoD in similarity [alignments using SIM (Huang and Miller 1991) corresponded with BLAST similarity results], and a separate RpoD homolog was identified. The multiple *A. ferrooxidans* RpoS homologs identified may be tentatively explained as a result of an RpoS duplication event, resulting in two similar RpoS proteins within *A. ferrooxidans*.

Because RpoS homologs of distantly related species or those that have been acquired by horizontal transfer might be excluded by the use of 41 representative genomes and by the use of a stringent *E*-value threshold, an additional reciprocal best hits analysis was performed on all proteins in NCBI RefSeq database (Pruitt et al. 2007) with a range of *E*-values (January 11, 2010; Figure 2.2). At a low *E*-value ≤ 1e-70, all reciprocal best hits to RpoS belonged to γ- and β-proteobacterial classes, with the exception of one protein (ZP_01449025) in α-proteobacterium, *Rhodobacterales bacterium* HTCC2255 (Appendix A). With an increase in *E*-value to 1e-04 from 1e-70, the number of reciprocal best hits to *E. coli* RpoS increased minimally from 351 to 488 but resulted in a large decrease of 97% to 7% reciprocal best hits to total number of hits. Reciprocal best hits to RpoS at *E*-value ≤ 1e-04 primarily belonged to the γ-, β-, and δ-proteobacterial classes (89% of reciprocal best hits; Appendix B), which largely supports the phylogenetic determination of RpoS distribution. About 11% of the reciprocal best hits were from other bacterial classes, including α-proteobacteria, Deferribacteres, ζ-proteobacteria, Verrucomicrobiae, Opitutae, Cyanobacteria, ε-proteobacteria, Clostridia, Sphingobacteria, and Planctomycetacia. Many of these reciprocal best hits were to more divergent sigma 70 family sigma factors and are likely false-positive homologs.

Only four reciprocal best hits of the other bacterial groups were annotated as RpoS homologs, and these belong to three bacterial classes: α-proteobacteria (as with *E*-value 1e-70, ZP_01449025 of *Rhodobacterales bacterium* HTCC2255), ζ-proteobacteria (ZP_01451363 of *Mariprofundus ferrooxydans* PV-1), and planctomycetes (ZP_01856592 of *Planctomyces maris* DSM 8797 and ZP_04428852 of *Planctomyces limnophilus* DSM 3776). The α-proteobacterium, *Rhodobacterales bacterium* HTCC2255, may contain contaminating sequences (GenBank AATR00000000.1), and the reciprocal best hit to RpoS has similarity and significance (Score = 412 and *E*-value = 3e-113) close to γ-proteobacteria, suggesting that α-proteobacteria do not possess true RpoS homologs. As well, the ζ-proteobacterial class, neutrophilic Fe-oxidizing bacteria, has only recently been identified (Emerson et al. 2007). Maximum-likelihood phylogeny predicts the ζ-proteobacterial class as closely related to α- and γ-/β-proteobacteria (Emerson et al. 2007), and the presence of RpoS homologs in ζ-proteobacteria may be more robustly examined as more sequences become available. Finally, planctomycetes are a distinct bacterial group from proteobacteria (Fuerst 1995; Jenkins and Fuerst 2001). Although horizontal gene transfer cannot be ruled out, reciprocal best hits of *Planctomyces maris* and *Planctomyces limnophilus* to RpoS possessed less than 80% query coverage and *E*-values less than 2e-55, comparable to an *E. coli* RpoS alignment to RpoD (79% query coverage and *E*-value 3e-55). The reciprocal best hits analysis, combined with phylogenetic analysis (Figure 2.1), provides a comprehensive approach to identifying the distribution of RpoS homologs in prokaryotes.

Comparison of RpoS and RpoD distribution. Due to the high sequence similarity between *rpoS* and *rpoD* (Mulvey and Loewen 1989) and because RpoD is the essential growth-related sigma factor, *rpoS* likely arose from an *rpoD* duplication event. We compared the distribution of RpoS and RpoD homologs to determine a putative point of origin for RpoS.

As in the determination of RpoS homolog distribution, a similarity and phylogeny-based approach was used to identify RpoD homologs with the use of a neighbor-joining tree (Figure 2.3). RpoD homologs formed a cluster, with the exception of a group of type II sigma-70 family sigma factors in the cyanobacteria, *Prochlorococcus* and *Synechococcus*, and several sigma factors in the actinobacteria, *Streptomyces* and *Mycobacterium*. Previous studies have shown the presence of more than one RpoD homolog in cyanobacteria (Tanaka et al. 1992) and actinobacteria (Tanaka et al. 1988).

Multiple RpoD homologs were previously identified in cyanobacteria, including *Synechococcus* (Gruber and Gross 2003; Tanaka et al. 1992). RpoD1 of *Synechococcus* is a primary sigma factor, highly similar to *E. coli* K12 RpoD (Asayama et al. 1996). The other RpoD homologs in cyanobacteria are alternative sigma factors that are dispensable for growth (Goto-Seki et al. 1999), some of which have a role during carbon and nitrogen starvation (Caslake et al. 1997).

Actinobacteria have a complex multicellular life cycle, which includes production of secondary metabolites (Flardh and Buttner 2009). Multiple sigma factors may be important for appropriate gene expression during morphological changes in life cycle. *Streptomyces*, for example, has over 63 sigma factors (Gruber and Gross 2003). The actinobacteria *Streptomyces* and *Mycobacterium* used in this study have multiple RpoD homologs, which is apparent in our BLAST search. Only HrdB and Sigma A of *Streptomyces* and *Mycobacterium*, respectively, are essential for growth (Buttner et al. 1990; Gruber and Gross 2003).

Surprisingly, RpoS of δ-proteobacteria, *Geobacter bemidjiensis* and *Pelobacter carbinolicus*, were found to be closer in lineage to RpoD than *Borrelia afzelii* RpoD. Possibly, the high level of recombination (Casjens et al. 2000) experienced within the *Borrelia* genome has led to a more diverged RpoD sequence (see Discussion).

Finally, to better compare the distribution of RpoS and RpoD homologs, we constructed a phylogeny of the 41 genera using 16S rRNA and compared RpoS and RpoD homologs by percent identity to *E. coli* K12 RpoS and RpoD (Figure 2.4). Our analyses indicate that RpoD is highly conserved throughout the bacterial domain, while RpoS is present only within proteobacteria, which corresponds to RpoD duplication prior to the divergence of proteobacteria. The high conservation of RpoD is not surprising, since it is essential (Lonetto et al. 1992; Paget and Helmann 2003).

Proteobacterial RpoS is not homologous to annotated *Borrelia* RpoS. The annotated *Borrelia burgdorferi* RpoS homolog is important for osmotic stress resistance in stationary phase and extreme pH, but it is not important for survival during temperature shift, serum starvation, and exposure to reactive oxygen species (Caimano et al. 2004; Elias et al. 2000). Unlike *E. coli*, RpoS of *Borrelia* is not a general stress response sigma factor, although it controls the expression of several lipoproteins (e.g., *ospC*, *dbpBA*, *ospF*) and is important in virulence (Eggers et al. 2004; Hubner et al. 2001; Yang et al. 2005). Also unique to *Borrelia*, RpoS expression is controlled by RpoN (Hubner et al. 2001; Smith et al. 2007).

Not surprising, the sequence of the *Borrelia* RpoS is considerably divergent from RpoS homologs found in proteobacteria. Compared to the well-studied *E. coli* K12 RpoS, *B. burgdorferi* RpoS has only 33.5% identity. In the maximum-likelihood tree, *Borrelia afzelii* RpoS does not cluster with RpoS of proteobacteria (Figure 2.1). Furthermore, the genomic regions surrounding the *rpoS* gene in *B. burgdorferi* have no significant gene similarity to the surrounding regions of *rpoS* in the proteobacteria (Figure 2.5). It is important to mention that low synteny in the *rpoS* gene region may be expected for highly diverged bacterial species and the *rpoS-mutS* region is highly polymorphic

(Herbelin et al. 2000; Kotewicz et al. 2003), yet combined results of phylogenetic clustering, RpoS function, RpoS similarity, genomic synteny and RpoS reciprocal best hits indicate *Borrelia* RpoS to be distinct from proteobacterial RpoS.

Conservation of RpoS regulon members. The RpoS regulon has a similar stationary-phase and stress-related function in many proteobacterial species (Schuster et al. 2004; Subsin et al. 2003; Tanaka and Takahashi 1994; Yildiz and Schoolnik 1998). The similarity in function may be the result of the co-evolution of a core set of regulon genes with the RpoS sigma factor. The presence of core regulon genes is evident in other regulons, such as the SOS regulon (Bjedov et al. 2003), yet the RpoS regulon is not essential and may, therefore, be subject to minimal selective pressure. To investigate the possible co-evolution of the RpoS sigma factor with expression-dependent genes, we compared the *E. coli* K12 and *P. aeruginosa* PA01 RpoS regulons. The RpoS regulon is well-defined in *E. coli* (Hengge-Aronis 2000; Patten et al. 2004; Weber et al. 2005) and, as such, has been used as a standard of comparison for the identification of RpoS homologs and of RpoS regulons in other bacterial species (Caimano et al. 2004; Schuster et al. 2004). The RpoS regulon of *P. aeruginosa* is also well-characterized by microarray analysis (Schuster et al. 2004) and was used in this study to evaluate the conservation of RpoS regulation of *E. coli* and *P. aeruginosa* genes.

Due to the large divergence of the *E. coli* and *P. aeruginosa* genomes, we used a reciprocal best hits approach to identify orthologs of *E. coli* RpoS regulon genes in *P. aeruginosa*. The proteins in *E. coli* (NC_000913) were BLAST against the proteins in *P. aeruginosa* PA01 (NC_002516) (Altschul et al. 1990) using an *E*-value threshold of 1e-

04, and reciprocal best hits to the 186 members of the *E. coli* RpoS regulon (Patten et al. 2004) were identified in *P. aeruginosa*. Overall, 50 proteins that are RpoS-dependent in *E. coli* were found to have orthologs in *P. aeruginosa*. Of these 50 orthologous proteins, only eleven regulatory units (twelve genes) were similarly RpoS-dependent in both species (Table 2.2).

Five of the 12 conserved orthologs are expressed during stress. In particular, bacterial lipocalin, a large mechanosensitive channel and an unknown protein, encoded by *blc*, *mscL* and *ygaU* respectively, are induced during osmotic stress (Bishop 2000; Stokes et al. 2003; Weber et al. 2006). Bacterial lipocalin functions to transfer or store proteins that maintain the cell membrane (Campanacci et al. 2006), and the large mechanosensitive channel (Sukharev et al. 1994), MscL, responds to pressure on the lipid bilayer of the cell membrane to maintain structural integrity during stationary phase (Stokes et al. 2003). Disruption of the *mscL* gene results in increased sensitivity to hypoosmotic conditions (Stokes et al. 2003). Other stress-induced orthologs are the phosphate-starvation-induced gene, *psiF* (Metcalf et al. 1990), and bacterioferritin, Bfr. Bacterioferritin is likely important in protection against oxidative damage from iron-induced radicals (Bou-Abdallah et al. 2002).

Many of the conserved RpoS-dependent orthologs between *E. coli* and *P. aeruginosa* are of unknown function. One operon, *yeaGH*, identified as RpoS-regulated in both species, is also induced during nitrogen limitation (Zimmer et al. 2000). In the presence of Mn^{2+}, the YeaG protein demonstrates autokinase activity (Tagourti et al.

2008). Although the YeaG protein is well-conserved in bacteria and archaea (Tagourti et al. 2008), the exact functions of YeaG and YeaH remain unknown.

Four conserved orthologs (*ycgB*, *yeaG*, *yohC*, *ygaU*) between *E. coli* and *P. aeruginosa* are also regulated by RpoS in *Salmonella enterica* serovar Typhimurium (Ibanez-Ruiz et al. 2000; Kenyon et al. 2007). Because the expression of these genes is dependent on RpoS in at least three γ-proteobacteria, many of these orthologs may be RpoS-regulated in a wide range of RpoS-containing species, although further research would be necessary to confirm this hypothesis.

The RpoS regulons of *E. coli* and *P. aeruginosa* have little apparent overlap, possibly due to low selective pressure for the co-evolution of RpoS with RpoS-regulated genes. The 12 conserved RpoS-regulated orthologs are maintained independently of RpoS, since several of these proteins have evolutionary origins prior to RpoS (e.g., *bfr*, *yeaG*) (Grossman et al. 1992; Tagourti et al. 2008). Functional determination of the orthologs with unknown function may provide insight into the evolution of RpoS regulation.

2.6 Discussion

The sigma 70 family of sigma factors is present in a broad range of prokaryotes as the promoter recognition subunit of RNA polymerase (Gruber and Gross 2003; Lonetto et al. 1992). Sigma factors recognize promoter sequences of genes and, thereby, regulate the expression of distinct gene sets to elicit a coordinated functional response. Development of stress-response regulons may be important for bacterial species to cope with adverse environmental conditions. In particular, the RpoS sigma factor regulates stress-related and stationary-phase genes in many proteobacteria (Patten et al. 2004; Schuster et al. 2004).

Due to the sequence similarity of sigma 70 family sigma factors, RpoS homologs are more difficult to distinguish from other sigma factors using a simple percent identity or BLAST scores criteria. A BLAST and phylogeny-based approach for the identification of RpoS homologs combines sequence similarity and amino acid alignment to identify RpoS in prokaryotes. Alternatively, conserved insertion and deletions (indels) can be used for phylogenetic inference of bacterial species and identification of protein homologs (Gao et al. 2009). An alignment of RpoS sequences from Burkholderiales and Enterobacteriales revealed one conserved indel in RpoS region 3 (Figure 2.6). Indels were also identified in Vibrionales and Pseudomonadales (data not shown). Conserved indels, such as found in RpoS homologs, may indicate changes in biological and physiological characteristics of a protein and may be used to determine evolutionary branching of bacteria (Gupta 1998). Because a universally conserved indel in RpoS could not be identified, indels were not used for identification of RpoS homologs in this study.

Importantly, however, the identification of RpoS indels within bacterial classes is not inconsistent with the determination of RpoS distribution by phylogeny and is a further indication of species-specific modification of RpoS.

Using a maximum-likelihood phylogeny, a segregated cluster of RpoS homologs identified RpoS as present in species of γ-, β-, and δ-proteobacteria (Figure 2.1). *Acidithiobacillus ferrooxidans* has duplicate RpoS homologs that were likely the result of RpoS duplication. The duplicate RpoS homologs of *A. ferrooxidans* share 52% identity (94% alignment coverage). Comparatively, the RpoD homolog has 40% identity to the RpoS homolog, and therefore, RpoS duplication, as opposed to a second RpoD duplication, is more likely.

The use of the phylogeny approach helps with protein annotation, as is the case for RpoS homologs of *Burkholderia ambifaria* and of δ-proteobacteria. It is apparent from the RpoS cluster (Figure 2.1) that *B. ambifaria* contains only one RpoS homolog, despite annotation of two homologs within the genome. As well, the RpoS homologs of δ-proteobacteria are currently annotated as simply RNA polymerases, although they are more accurately described as RpoS sigma factors.

Interestingly, RpoS is present in only three of the four β-proteobacteria used in this study. The loss of RpoS within *E. coli* by environmental selection of mutants has been previously reported (Dong et al. 2009a; King et al. 2006). It is possible that RpoS was similarly lost in other species, such as the β-proteobacterium, *Neisseria gonorrhoeae*, which no longer carries an RpoS sigma factor. However, the *nlpD* gene (GeneID:3281140) region of *Neisseria gonorrhoeae* aligned with *E. coli nlpD* gene

(GeneID:947011) region does not identify a downstream homologous *rpoS* gene in *Neisseria* (data not shown). *Neisseria* undergoes frequent transformation, as it remains competent in all growth stages (Barlow 2009). Natural transformation, a mechanism for genome modification, has contributed to this species' resistance to multiple antibiotics (Barlow 2009) and may have further altered genomic regions encoding regulatory proteins. In addition to the lack of an identifiable RpoS homolog (this study), *Neisseria* lack an HrcA homolog, a general stress response regulator in other β-proteobacteria (Permina and Gelfand 2003). There are also no identifiable HrcA recognition sites in *Neisseria* (Permina and Gelfand 2003). Instead, *Neisseria* utilize RpoH, a heat-shock sigma factor, to regulate stress genes (Gunesekere et al. 2006). Whether through mutation or transformation-faciliated genome modification, the *Neisseria* genome has unique differences from other β-proteobacterial species.

In addition to *Neisseria*, RpoS may have been lost from α- and ε-proteobacterial species. This is consistent with the evolutionary development of species revealed by indel comparison (Gupta 2000) (Figure 2.7). Alternatively, the *rpoS* gene may have arisen prior to δ-, β, and γ-proteobacterial divergence. A supertree model formed from 730 trees of orthologous gene families across 45 prokaryote and eukaryote species indicates that δ-, β-, and γ-proteobacteria have more recent evolutionary origins than ε-, and even α-proteobacteria (Daubin et al. 2002). The lack of strong selection for RpoS in bacteria increases the complexity of determining RpoS origin, but we can propose that RpoS arose prior to the divergence of δ-, β, and γ-proteobacteria. Important to note, however, RpoS of γ- and β-proteobacteria have greater similarity than to RpoS of δ-proteobacteria (data not shown). A separate *rpoD* duplication within δ-proteobacteria from γ- and β-proteobacteria can also account for a lack of RpoS homologs in α- and ε-proteobacteria.

Due to the similarity of RpoS to RpoD (Mulvey and Loewen 1989), the origin of RpoS likely occurred as a duplication of the *rpoD* gene prior to the divergence of proteobacteria (this study). Other duplication events of sigma 70 family sigma factors outside proteobacteria, resulting in RpoS-like sigma factors, is excluded from this study, yet other duplication events may explain the annotation of RpoS in the spirochaete *Borrelia afzelii*. The RpoS homolog in *B. burgdorferi* was identified by sequence similarity to other RpoS homologs and by enhanced resistance to osmotic stress in stationary phase (Elias et al. 2000; Fraser et al. 1997). With the criteria used in this study however, *Borrelia* does not possess an RpoS homolog.

Borrelia is a highly adaptable organism that survives a wide range of host conditions. Its genome consists of a 910-kb linear chromosome and 21 extrachromosomal plasmids (Casjens et al. 2000). The plasmids likely provide an adaptive advantage to the cell (Pal and Fikrig 2003). Due to the importance of adaptation and the high number of plasmids, the *Borrelia* genome is subject to frequent recombination and duplication (Casjens et al. 2000). *Borrelia* may have experienced a distinct RpoD duplication event from other species and subsequent loss of region 1 that resulted in an RpoS-like protein. The functional differences between *Borrelia* RpoS-like protein and RpoS of proteobacteria are more clearly explained when the two regulatory factors are understood as arising from separate evolutionary duplications. This would suggest that the regulatory network RpoN-RpoS in *Borrelia* is unique to the *Borrelia* genus. We propose a separate evolutionary origin for *Borrelia* RpoS from proteobacterial RpoS that corresponds with known evolutionary lineages (Figure 2.7) (Gupta 2000). With the exception of RpoD region 1, RpoD and RpoS are well conserved, with 42.7% identity of regions 2-4 between *E. coli* RpoD and RpoS. Putatively, a separate duplication of RpoD in *Borrelia* from proteobacteria, followed by the subsequent loss of region 1, may explain the RpoS-like sigma factor in *Borrelia* (Figure 2.7). Furthermore, a separate duplication event indicates a possible selective advantage to an RpoS-like sigma factor in bacterial adaptation.

It is important to note that changes in promoter recognition sites, in addition to changes in RpoS, may be an integral factor in the evolution of RpoS regulation. Changes in promoter regions alter RpoS recognition (Miksch et al. 2005) and are likely a substantial means for modification of regulon composition. RpoS promoter regions are similar to those of RpoD, but RpoS promoters have unique features, such as a characteristic C(-13) residue, that provide selective RpoS recognition (Typas et al. 2007a). RpoS tolerates promoter deviations in the -35 sequence (Typas et al. 2007b), as well as in the -10/-35 spacer region (Typas and Hengge 2006). Because of promoter deviations, co-evolution of RpoS with RpoS-dependent promoters is difficult to determine. However, experimental determination of *E. coli* and *P. aeruginosa* RpoS-dependent promoters shows interspecies uniformity in the -10 sequence (Schuster et al. 2004; Typas et al. 2007a). Further analysis is necessary to better determine co-evolution of RpoS with dependent promoters.

In addition to RpoS distribution, the conservation of RpoS regulon members during speciation may indicate the presence of a core regulon. Unlike the RpoS sigma factor however, RpoS-dependent genes are not well conserved, even within the γ-proteobacterial species, as evident from the direct comparison of *E. coli* and *P. aeruginosa* RpoS regulons. Using independent microarray analyses (Patten et al. 2004; Schuster et al. 2004) to comprehensively identify the RpoS regulon and reciprocal best hits analyses to identify orthologs (this study), *P. aeruginosa* was found to share 50 orthologs of the 186 genes that are within the *E. coli* RpoS regulon. Of the 50 orthologs, only twelve genes are regulated by RpoS in both species (Table 2.2). Because only 12/50 orthologs are RpoS-regulated, RpoS regulon genes appear to be lost/gained within the regulon despite the conservation of the gene and the RpoS sigma factor. The RpoS sigma factor is frequently lost due to environmental selection (Chen et al. 2004; Dong et al. 2009a; Zambrano et al. 1993), which has likely contributed to many species-specific modifications of the regulon within proteobacteria. Low conservation of bacterial regulons has been previously reported for the PhoPQ regulon of *E. coli*, *Salmonella enterica* serovar Typhimurium and *Yersinia pestis* (Monsieurs et al. 2005; Perez and Groisman 2009b). As well unlike the SOS regulon important for DNA-damage repair (Bjedov et al. 2003), the RpoS regulon does not include essential genes. Considering the analysis of the RpoS regulon in this study, nonessential stress regulons that can be lost through environmental selection (Chen et al. 2004; Dong et al. 2009a) may be subject to frequent modifications within individual species.

Horizontal gene transfer between bacteria contributes to regulatory diversity (Cotter and DiRita 2000; Maslov et al. 2009; Reid and Abratt 2005). The low conservation of RpoS regulon composition, even between bacteria of γ-proteobacteria, suggests a possible role for horizontal gene transfer in RpoS regulon modification. A toolbox model of evolution, where whole functional modules are laterally transferred between species (Maslov et al. 2009), may allow for rapid remodeling of RpoS-regulated transcription. Other regulons and regulatory factors, such as the PhoP regulon of Enterobacteriaceae (Perez and Groisman 2009a; Perez and Groisman 2009b) and virulence factors of *Salmonella* and *Vibrio* (Cotter and DiRita 2000), have been modified through horizontal gene transfer, and future work on RpoS regulon member evolution will be integral in determining the extent to which horizontal transfer has also modified RpoS regulon composition.

Interestingly despite the low number of shared RpoS-regulated genes, the RpoS regulons of *P. aeruginosa* and *E. coli* are similarly induced in stationary phase and important in stress-response (Hengge-Aronis 2000; Jorgensen et al. 1999). The functional similarity of the RpoS regulon in proteobacterial species is probably not the result of a conserved core regulon during speciation, but indeed, regulon members are likely selected by species-specific environmental determinants. It is likely that the nonessential nature of the RpoS regulon and the selection for loss of RpoS in some environments (Chen et al. 2004; Dong et al. 2009b) has allowed for many specific environmental adaptations. Investigation into functional gene groups between species may help to elucidate how the RpoS regulon maintains a similar function despite high genomic diversity between species.

2.7 Conclusions

The evolution of the RpoS regulon consists of the origin and distribution of RpoS in microbes and the recruitment of genes into the regulon. Using a maximum-likelihood phylogeny, we determined that the RpoS sigma factor arose prior to the divergence of proteobacteria, and annotation of RpoS in other classes, such as spirochaetes, may be the result of separate duplication events of sigma 70 family sigma factors. In addition to RpoS protein, our study compares the *E. coli* RpoS regulon with the *P. aeruginosa* RpoS regulon. Although 50 orthologs of the *E. coli* RpoS regulon were found in *P. aeruginosa*, only a small number of orthologs were also RpoS-dependent in *P. aeruginosa*. Given that bacterial regulons can be highly variable between species (Liu and Ochman 2007; Monsieurs et al. 2005), it is not surprising that the RpoS regulon has many species-specific differences. Modification of regulon composition may be important in species adaptation to environmental stress.

In this study, we examined how a specific, well-characterized stress regulon evolves. However, additional research is needed to fully understand how such stress regulons as the RpoS regulon have evolved in a broad phylogenetic group of medically relevant bacteria. In particular, RpoS is present in many enterobacteria and is known to enhance bacterial pathogenesis (Dong et al. 2009b; Dong and Schellhorn 2010), and so this study provides a base for further investigation into environmental selection of stress regulons. In addition to understanding the conditions of regulon selection, future studies, using the RpoS regulon as a robust model, will examine the development and diversification of paralogous genes under similar gene regulation. Several outstanding issues remain to be investigated, including identification of the underlying selective mechanisms that recruit laterally transferred genes into existing regulons and how, in some cases, gene duplication and subsequent regulatory divergence, in the case of paralogous genes, contribute to specific regulon responses.

2.8 Acknowledgements

This study was supported by research grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Canadian Institutes of Health Research (CIHR) to H.E.S. S.C. was supported by an Ontario Graduate Scholarship. We thank R.A. Morton for help with the reciprocal best hits analysis comparing RpoS regulons, R.S. Gupta for help in the indel search, and A. Pasha for help with programming to identify RpoS homologs as reciprocal best hits. We further thank R.A. Morton, R.S. Gupta, and J. Dushoff for informative discussions on phylogeny construction, and T. Dong, C. Joyce, S. Sathiasothy, J. Wasniewski, and R. Yu for comments on the manuscript and technical assistance.

Table 2.1. Diverse microbial species used to determine the distribution of RpoS and RpoD homologs.

Species	Domain	Class
Aeropyrum pernix K1	Archaea	Crenarchaeota
Sulfolobus acidocaldarius DSM 639	Archaea	Crenarchaeota
Pyrobaculum aerophilum str. IM2	Archaea	Crenarchaeota
Methanococcus aeolicus Nankai-3	Archaea	Euryarchaeota
Methanosarcina acetivorans C2A	Archaea	Euryarchaeota
Pyrococcus abyssi GE5	Archaea	Euryarchaeota
Thermoplasma acidophilum DSM 1728	Archaea	Euryarchaeota
Mycobacterium abscessus	Bacteria	Actinobacteria
Streptomyces avermitilis MA-4680	Bacteria	Actinobacteria
Azorhizobium caulinodans ORS 571	Bacteria	Alpha-proteobacteria
Brucella abortus S19	Bacteria	Alpha-proteobacteria
Mesorhizobium loti MAFF303099	Bacteria	Alpha-proteobacteria
Agrobacterium tumefaciens str. C58	Bacteria	Alpha-proteobacteria
Ehrlichia canis str. Jake	Bacteria	Alpha-proteobacteria
Rickettsia akari str. Hartford	Bacteria	Alpha-proteobacteria
Bacteroides fragilis NCTC 9343	Bacteria	Bacteroidetes
Burkholderia ambifaria AMMD	Bacteria	Beta-proteobacteria
Ralstonia eutropha H16	Bacteria	Beta-proteobacteria
Neisseria gonorrhoeae FA 1090	Bacteria	Beta-proteobacteria
Janthinobacterium sp. Marseille	Bacteria	Beta-proteobacteria
Chlamydia muridarum Nigg	Bacteria	Chlamydiae
Chlorobium chlorochromatii CaD3	Bacteria	Chlorobi
Prochlorococcus marinus str. AS9601	Bacteria	Cyanobacteria
Synechococcus elongatus PCC 6301	Bacteria	Cyanobacteria
Geobacter bemidjiensis Bem	Bacteria	Delta-proteobacteria
Pelobacter carbinolicus DSM 2380	Bacteria	Delta-proteobacteria
Campylobacter concisus 13826	Bacteria	Epsilon-proteobacteria
Helicobacter acinonychis str. Sheeba	Bacteria	Epsilon-proteobacteria
Bacillus amyloliquefaciens FZB42	Bacteria	Firmicutes
Listeria innocua Clip11262	Bacteria	Firmicutes
Staphylococcus aureus RF122	Bacteria	Firmicutes
Lactobacillus acidophilus NCFM	Bacteria	Firmicutes
Streptococcus agalactiae 2603V/R	Bacteria	Firmicutes
Escherichia coli str. K-12 substr. MG1655	Bacteria	Gamma-proteobacteria
Acidithiobacillus ferrooxidans ATCC 23270	Bacteria	Gamma-proteobacteria
Legionella pneumophila str. Corby	Bacteria	Gamma-proteobacteria
Pseudomonas aeruginosa LESB58	Bacteria	Gamma-proteobacteria
Vibrio cholerae O1 biovar El Tor str. N16961	Bacteria	Gamma-proteobacteria
Borrelia afzelii ACA-1	Bacteria	Spirochaetes
Mycoplasma agalactiae PG2	Bacteria	Tenericutes
Thermotoga lettingae TMO	Bacteria	Thermotogae

Table 2.2. Identified orthologs of the 186 *E. coli* K12 RpoS regulon genes in *P. aeruginosa* PA01 determined by reciprocal best hits.

E. coli K12			*P. aeruginosa* PAO1		Function
Blattner	Gene	RpoS-dependence[a]	Locus	RpoS-dependence[b]	
b0384	*psiF*	3.4	PA4874	7.5	Phosphate-starvation-induced (Metcalf et al. 1990); unknown function
b0837	*yliI*	2.0	PA1112	2.0	Aldose sugar dehydrogenase (Southall et al. 2006); associated with outer membrane (Marani et al. 2006)
b1188	*ycgB*	5.1	PA0586	6.2	Putative interaction with *yeaGH* (Andres et al. 2009); unknown function
b1783	*yeaG*	16.7	PA0588	4.7	Serine protein kinase (Tagourti et al. 2008); putative nitrogen metabolism function (Andres et al. 2009)
b1784	*yeaH*	3.1	PA0587	6.3	Putative nitrogen metabolism function (Andres et al. 2009)
b1795	*yeaQ*	3.5	PA5424	4.0	Downregulated in low acetate (Lee et al. 2003)
b2080	*yegP*	6.4	PA0329	4.2	Unknown function
b2135	*yohC*	5.3	PA1190	3.3	Membrane protein (Daley et al. 2005)
b2665	*ygaU*	7.3	PA5178	1.8	Osmotic-stress-induced (Weber et al. 2006); unknown function
b3291	*mscL*	2.2	PA4614	3.1	Mechanosensitive channel transporter (Oakley et al. 1999)
b3336	*bfr*	33.5	PA3251	7.9	Iron transport (Andrews et al. 2003)
b4149	*blc*	3.0	PA5107	1.9	Putative protein storage/transport for membrane maintenance (Campanacci et al. 2006)

The RpoS-dependence according to independent microarray analyses is shown ([a]Patten et al. 2004; [b]Schuster et al. 2004). Function is taken from studies using *E. coli*.

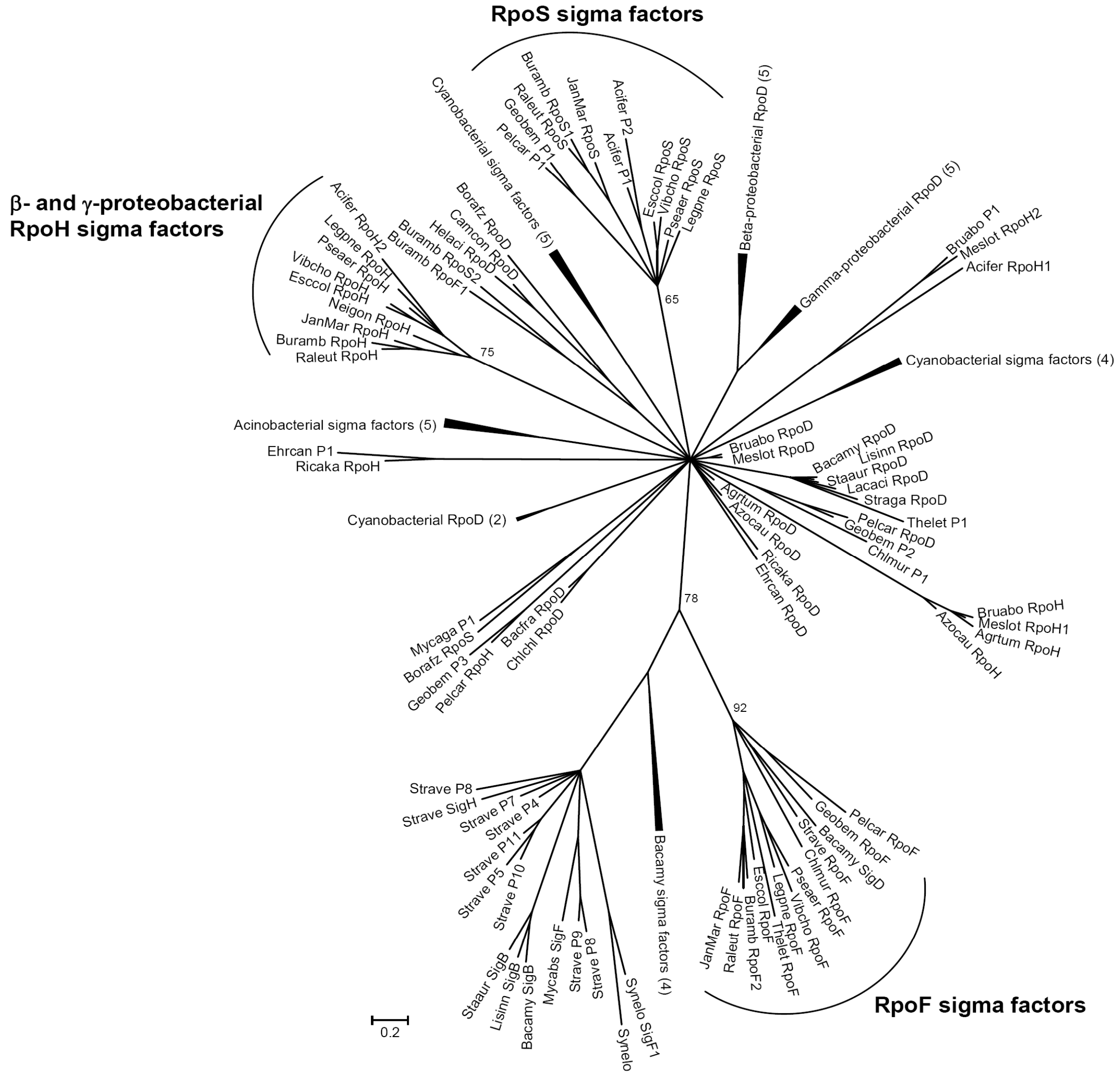


Figure 2.1. Unrooted, maximum-likelihood phylogeny of sigma 70 family sigma factors from 41 diverse genera. Phylogeny was constructed by Tree-Puzzle (Schmidt et al. 2002) with 50,000 quartet puzzling steps (Strimmer and von Haeseler 1996), and quartet puzzling reliability values (percentage) are indicated on select branches. RNA polymerase sigma factors without annotation are labelled as "P," and multiple sigma factors of the same annotation are given consecutive numbers. The numbers of proteins within collapsed branches are provided in brackets. The scale bar represents 0.2 amino acid substitutions per site. Sigma 70 family sigma factors used in this phylogeny are: *Escherichia coli* (Esccol) RpoS (NP_417221), RpoD (NP_417539), RpoH (NP_290009), RpoF (NP_288383); *Vibrio cholerae* (Vibcho) RpoS (NP_230185), RpoD (NP_230168), RpoH (NP_229808), RpoF (NP_231698); *Pseudomonas aeruginosa* (Pseaer) RpoS

(NP_252312), RpoD (NP_249267), RpoH (NP_249067), RpoF (NP_250146), *Legionella pneumophila* (Legpne) RpoS (YP_001250033), RpoD (YP_001251110), RpoH (YP_001249805), RpoF (YP_001250533); *Acidithiobacillus ferrooxidans* (Acifer) P1 (YP_002219091), P2 (YP_002219943), RpoD (YP_002426728), RpoH1 (YP_002220399), RpoH2 (YP_002220778); *Janthinobacterium sp.* Marseille (JanMar) RpoS (YP_001353824), RpoD (YP_001352183), RpoH (YP_001354832), RpoF (YP_001353773); *Burkholderia ambifaria* (Buramb) RpoS1 (YP_773648), RpoS2 (YP_775431), RpoD (YP_775146), RpoH (YP_774765), RpoF1 (YP_778037), RpoF2 (YP_772080); *Ralstonia eutropha* (Raleut) RpoS (YP_726836), RpoD (YP_727172), P1 (YP_726126), RpoH (YP_724873), RpoF (YP_728422); *Geobacter bemidjiensis* (Geobem) P1 (YP_002139485), P2 (YP_002140482), P3 (YP_002137397), RpoF (YP_002140552*); Pelobacter carbinolicus* (Pelcar) RpoD (YP_358084), RpoH (YP_357791), RpoF (YP_356578); *Neisseria gonorrhoeae* (Neigon) RpoD (YP_208094), RpoH (YP_207448); *Azorhizobium caulinodans* (Azocau) RpoD (YP_001526559), RpoH (YP_001527169); *Brucella abortus* (Bruabo) RpoD (YP_414866), RpoH (YP_222319), P1 (YP_001935614); *Mesorhizobium loti* (Meslot) RpoD (NP_103805), RpoH1 (NP_104782), RpoH2 (NP_104874); *Agrobacterium tumefaciens* (Agrtum) RpoD (NP_355127), RpoH (NP_355397); *Ehrlichia canis* (Ehrcan) RpoD (YP_302957), P1 (YP_303026); *Rickettsia akari* (Ricaka) RpoD (YP_001494064), RpoH (YP_001493264); *Helicobacter acinonychis* (Helaci) RpoD (YP_665236), P1 (YP_356846); *Campylobacter concisus* (Camcon) RpoD (YP_001467098); *Bacillus amyloliquefaciens* (Bacamy) RpoD (YP_001421942), SigE (YP_00142110), SigG (YP_001421110), SigF (YP_001421748), SigK (YP_001421995), SigB (YP_001420137), SigD (YP_00141225); *Listeria innocua* (Lisinn) RpoD (NP_470827), SigB (NP_470235); *Staphylococcus aureus* (Staaur) RpoD (NP_372085), SigB (YP_041514); *Lactobacillus acidophilus* (Lacaci) RpoD (YP_194063); *Streptococcus agalactiae* (Straga) RpoD (NP_688423); *Borrelia afzelii* (Borafz) RpoS (ZP_03435453), RpoD (ZP_03435385); *Mycoplasma agalactiae* (Mycaga) P1 (YP_001256407); *Thermotoga lettingae* (Thelet) P1 (YP_001471591), RpoF (YP_001470247); *Bacteroides fragilis* (Bacfra) RpoD (YP_100026), *Chlamydia muridarum* (Chlmur) P1 (NP_297278), RpoF (NP_296710); *Chlorobium chlorochromatii* (Chlchl) RpoD (YP_378703); *Mycobacterium abscessus* (Mycabs) P1 (YP_001703741), RpoD (YP_001703741), SigF (YP_001703246); *Streptomyces avermitilis* (Strave) P1 (NP_826856), P2 (NP_823620), P3 (NP_824871), P4 (NP_825363), P5 (NP_822326), P6 (NP_825362), P7 (NP_824667), P8 (NP_828576), P9 (NP_823049), P10 (NP_822262), P11 (NP_821916), SigH (NP_824189), SigF (NP_823806); *Prochlorococcus marinus* (Promar) P1 (YP_001009878), P2 (YP_001010229), P3 (YP_398287), P4 (YP_001009026), RpoD (YP_001008946); *Synechococcus elongatus* (Synelo) RpoD (YP_171589), RpoD2 (YP_173055), RpoD4 (YP_171663), RpoD6 (YP_170725), SigC (YP_172955), SigD (YP_171567), SigF1 (YP_173205), SigF2 (YP_173019).

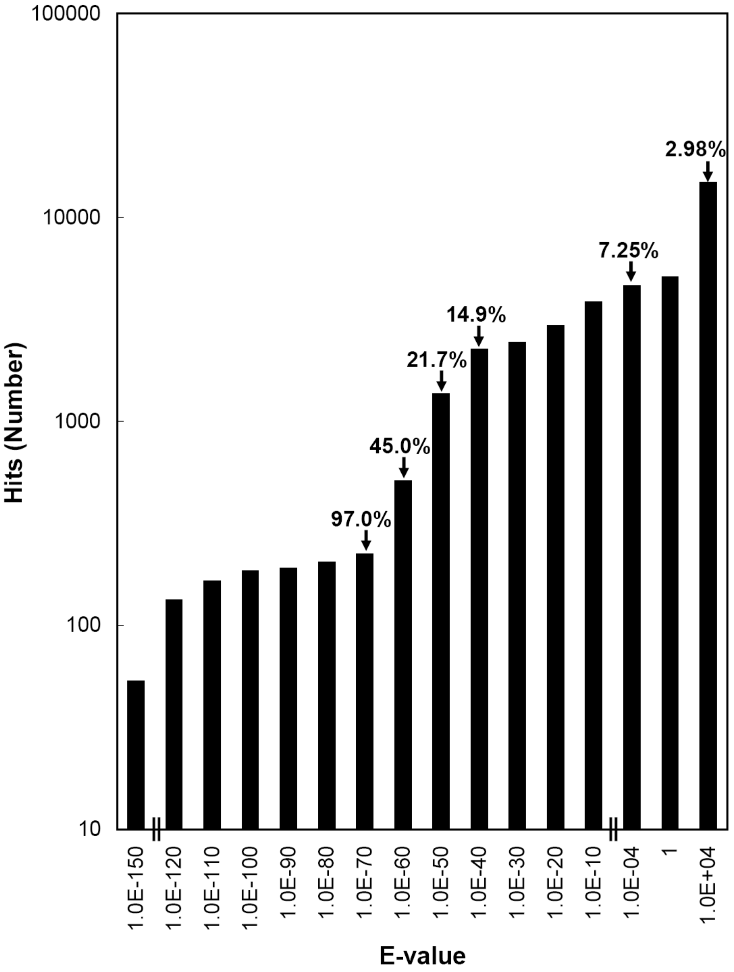


Figure 2.2. Number of hits of *E. coli* RpoS (NP_417221) against NCBI's RefSeq database (Pruitt et al. 2007) compared to *E*-value threshold. Percentages of reciprocal best hits to the total number of hits at some *E*-values are indicated by arrows.

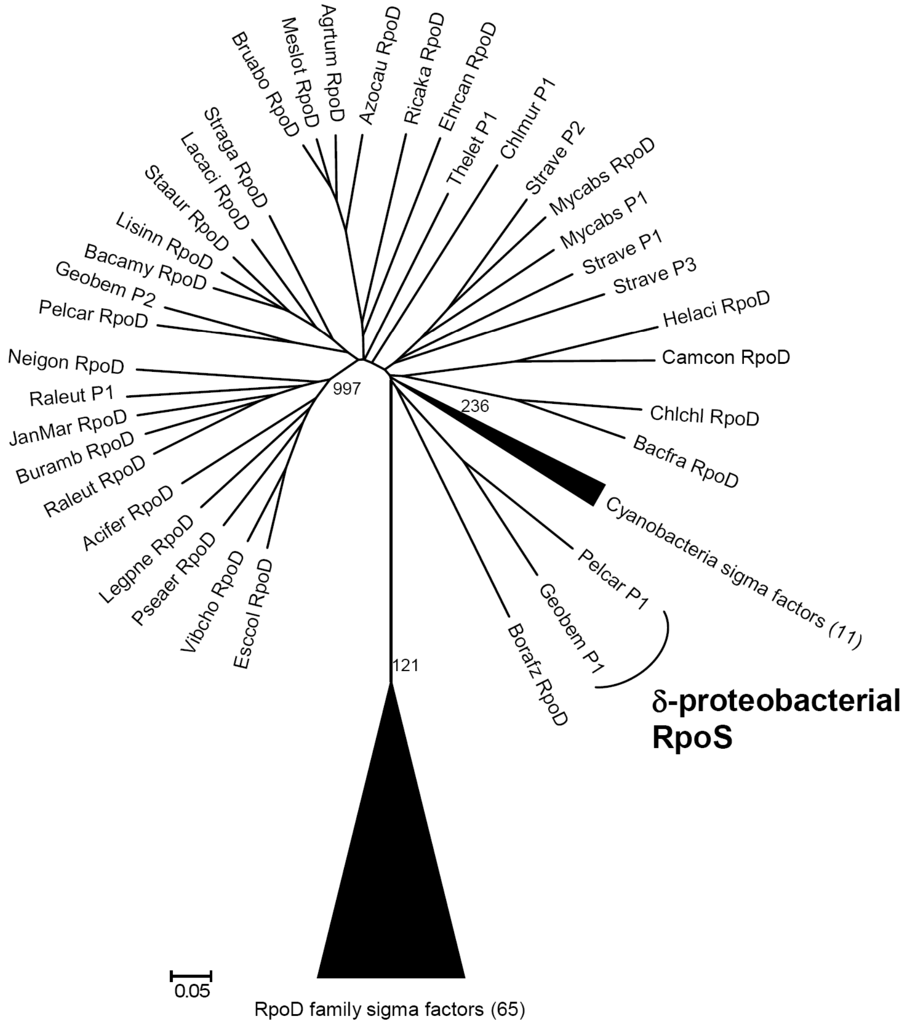


Figure 2.3. Unrooted, neighbor-joining phylogeny of sigma 70 family sigma factors from 41 diverse genera. Phylogeny was constructed by Clustal X (Larkin et al. 2007) with 1000× bootstrap replications. Bootstrap values are indicated on select branches. The numbers of proteins within collapsed branches are provided in brackets. The scale bar represents 0.05 amino acid substitutions per site. Sigma 70 family sigma factors used in this phylogeny are as in Figure 2.1.

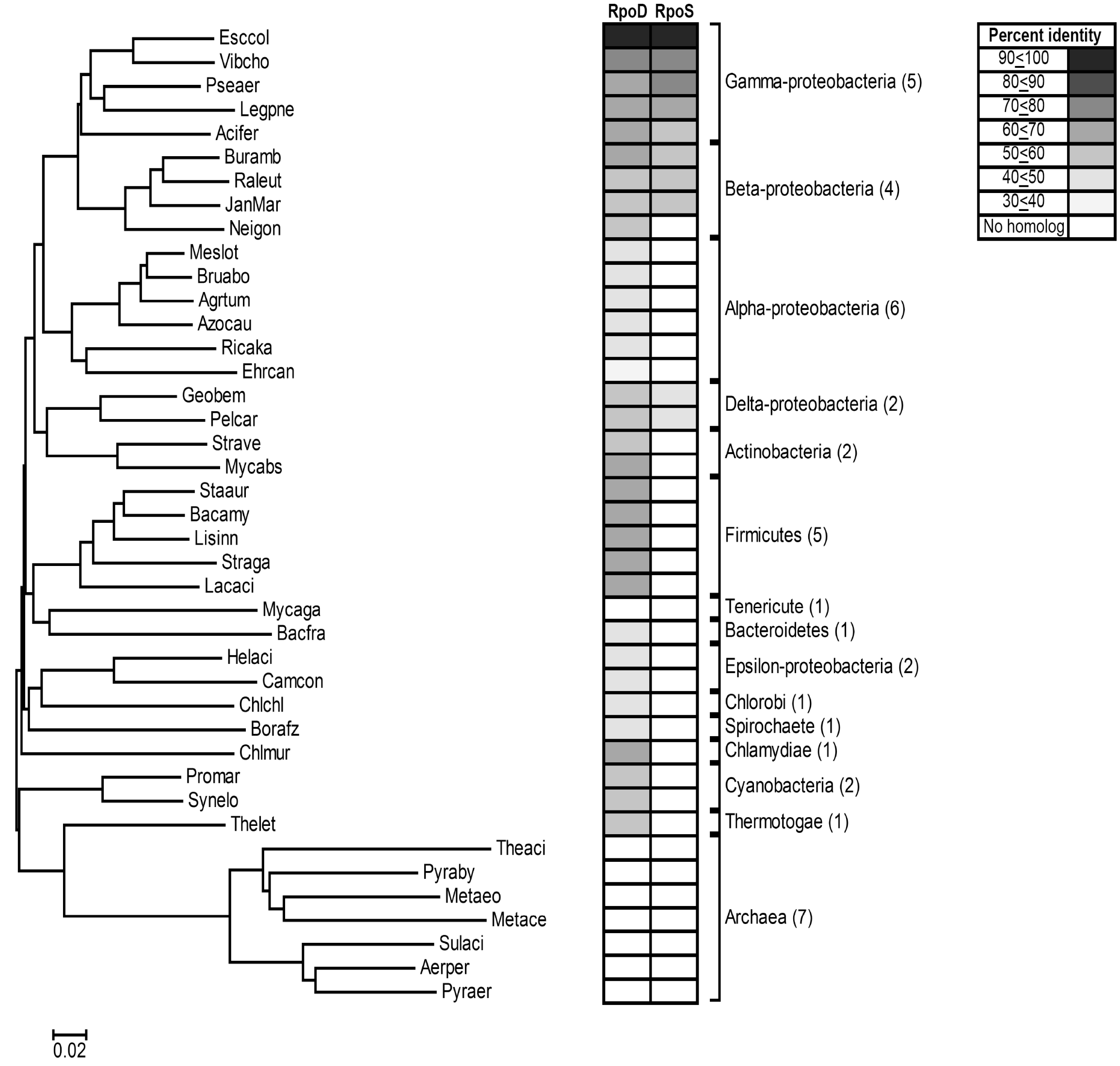


Figure 2.4. Comparison of RpoD and RpoS homolog distributions among 41 diverse microbial genera. The phylogeny was constructed with the use of 16S rRNA sequences (Cole et al. 2007) with the neighbor-joining method. RpoD and RpoS homolog percent identity to *E. coli* K12 RpoD and RpoS sequences are indicated by color. RpoS homologs are conserved within γ-, β-, and δ-proteobacteria, while RpoD homologs are widely conserved in the bacterial domain. The scale bar represents 0.02 nucleotide substitutions per site.

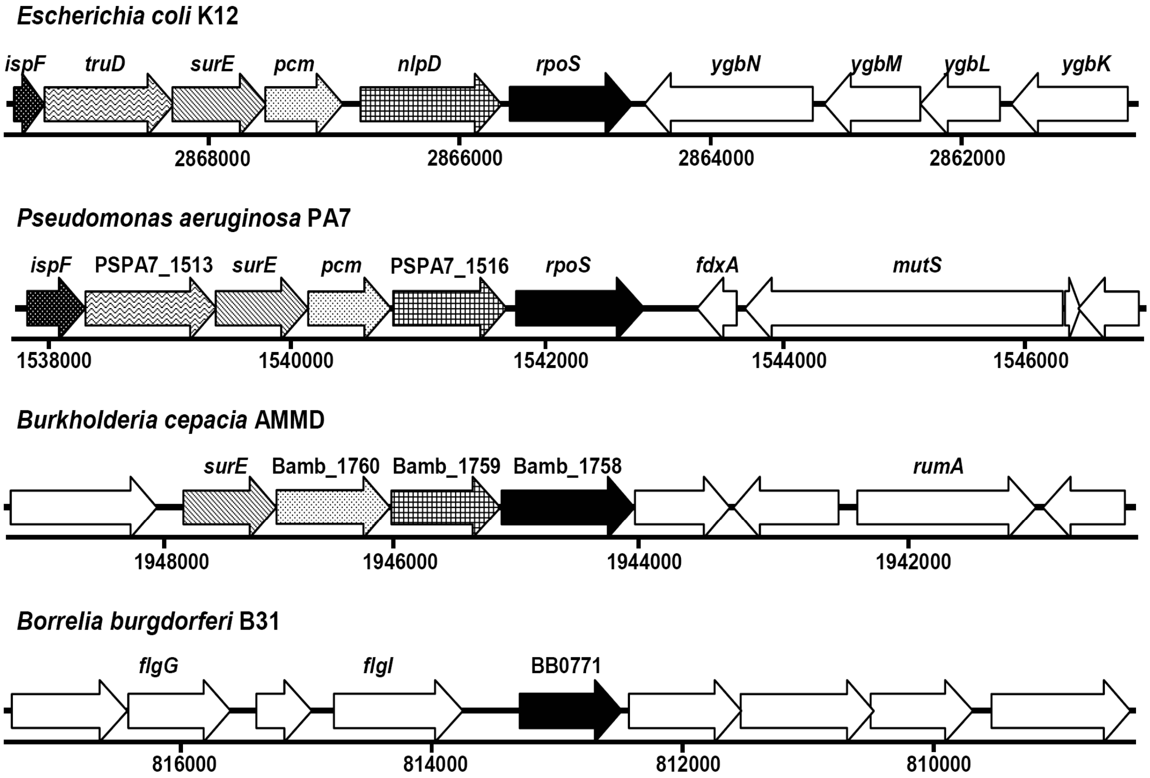
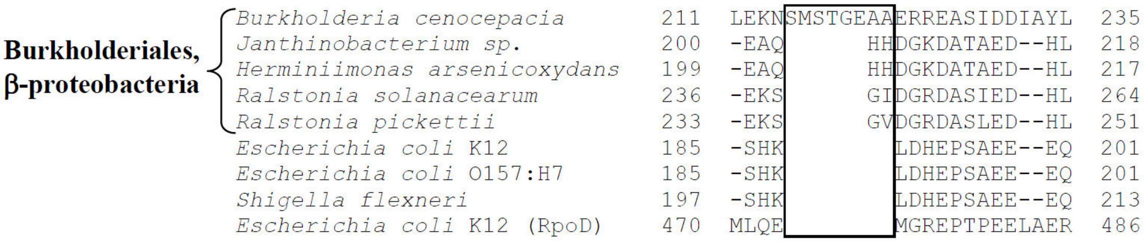


Figure 2.5. Multigenome alignment of the regions surrounding *rpoS* in *Borrelia burgdorferi*, *Burkholderia cepacia*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Homologs are indicated by pattern. The region around *rpoS* is not conserved in *B. burgdorferi* compared to the proteobacteria. Genome locations are shown.



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Burkholderia cenocepacia         211  LEKNSMSTGEAAERREASIDDIAYL  235
Janthinobacterium sp.            200  -EAQ       HHDGKDATAED--HL  218
Herminiimonas arsenicoxydans     199  -EAQ       HHDGKDATAED--HL  217
Ralstonia solanacearum           236  -EKS       GIDGRDASIED--HL  264
Ralstonia pickettii              233  -EKS       GVDGRDASLED--HL  251
Escherichia coli K12             185  -SHK        LDHEPSAEE--EQ  201
Escherichia coli O157:H7         185  -SHK        LDHEPSAEE--EQ  201
Shigella flexneri                197  -SHK        LDHEPSAEE--EQ  213
Escherichia coli K12 (RpoD)      470  MLQE        MGREPTPEELAER  486
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Figure 2.6. Conserved indel within the RpoS amino acid sequence of Burkholderiales. RpoS from the Enterobacteriales (*E. coli* K12, *E. coli* O157:H7, and *Shigella flexneri*) and RpoD from *E. coli* K12 are aligned with Burkholderiales for comparison. Amino acid position is shown.

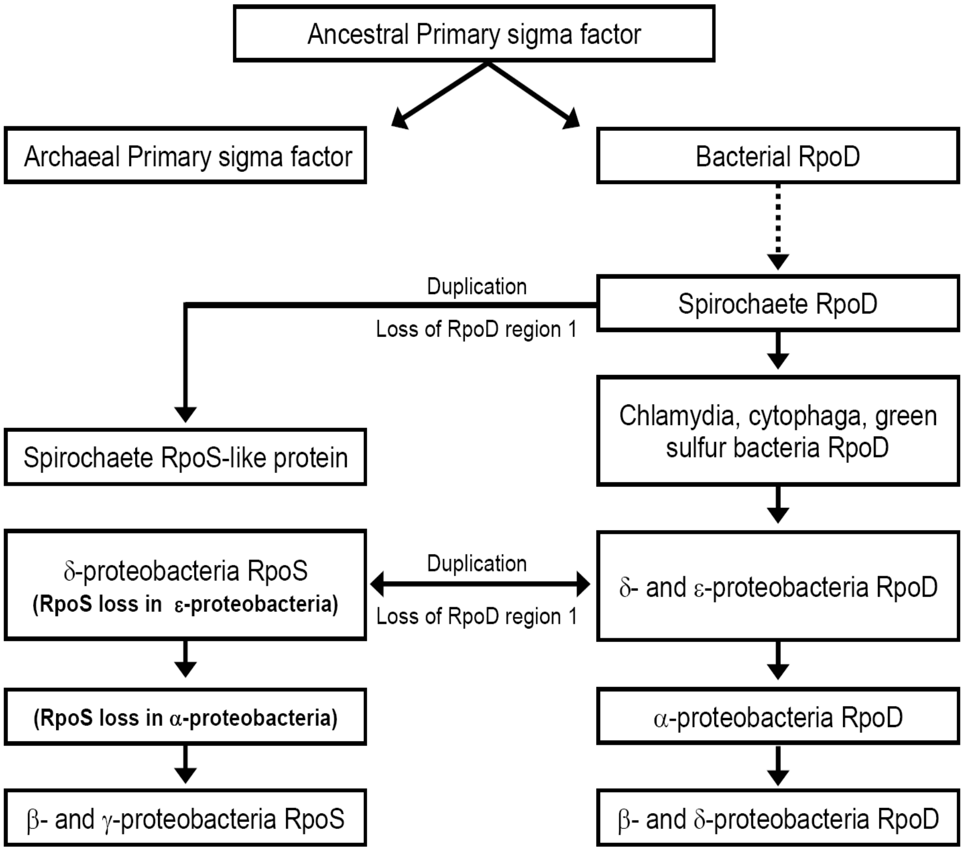

Figure 2.7. Proposed model of RpoS evolution using evolutionary lineages determined by indel comparison. RpoS originated prior to the divergence of proteobacteria, which was later lost in α- and ε-proteobacteria, as indicated by brackets. *Borrelia* RpoS may have a separate evolutionary origin. Refer to “Discussion” and Gupta (2000) for more details.

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CHAPTER 3:

Phenotypic diversity caused by differential RpoS activity among environmental *Escherichia coli* isolates

adapted from

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3.1 Preface

This chapter is adapted from an article published in Applied and Environmental Microbiology to adhere to thesis format requirements. One figure and one table, originally presented as supplementary Figure 1 and supplementary Table 3 in the published article, are now within the main body of this chapter as Figure 3.1 and Table 3.4. An additional figure, Figure 3.2, originally presented as “data not shown,” is now added. S. M. Chiang performed all experiments from year 2008-2011 and is the primary author of the article. T. Dong helped in the initial screening for RpoS mutants among environmental *E. coli* isolates, and T. A. Edge provided environmental *E. coli* isolates and source details.

3.2 Abstract

Enteric bacteria deposited into the environment by animal hosts are subject to diverse selective pressures. These pressures may act on phenotypic differences in bacterial populations and select adaptive mutations for survival in stress. As a model to study phenotypic diversity in environmental bacteria, we examined mutations of the stress response sigma factor, RpoS, in environmental *Escherichia coli* isolates. A total of 2,040 isolates from urban beaches and nearby fecal pollution sources on Lake Ontario (Canada) were screened for RpoS function by examining growth on succinate and catalase activity, two RpoS-dependent phenotypes. The *rpoS* sequence was determined for 45 isolates, including all candidate RpoS mutants, and of these, six isolates were confirmed as mutants with the complete loss of RpoS function. Similarly to laboratory strains, the RpoS expression of these environmental isolates was stationary phase dependent. However, the expression of RpoS regulon members KatE and AppA had differing levels of expression in several environmental isolates compared to those in laboratory strains. Furthermore, after plating *rpoS*$^+$ isolates on succinate, RpoS mutants could be readily selected from environmental *E. coli*. Naturally isolated and succinate-selected RpoS mutants had lower generation times on poor carbon sources and lower stress resistance than their *rpoS*$^+$ isogenic parental strains. These results show that RpoS mutants are present in the environment (with a frequency of 0.003 among isolates) and that, similarly to laboratory and pathogenic strains, growth on poor carbon sources selects for *rpoS* mutations in environmental *E. coli*. RpoS selection may be an important determinant of phenotypic diversification and, hence, the survival of *E. coli* in the environment.

3.3 Introduction

The fecal bacterial contamination of fresh waters is a recurring issue for public beaches surrounding the Great Lakes of North America (57, 79). Direct fecal deposits (20, 78), as well as input from streams (60) and land runoffs (5), often result in high levels of bacteria in both water and sediment. Populations of *Escherichia coli* can persist for long periods outside a host organism and possibly multiply in soil (34). Although fecal deposits are implicated as the primary source of *E. coli* distribution, the environment likely selects for some stress-tolerant *E. coli* strains (4, 35). These "naturalized" *E. coli* populations add a newly recognized complexity to the contamination of fresh waters (11, 34, 80). The persistence and possible multiplication of *E. coli* in the environment may allow for the selection of adaptive mutations comparable to that of long-term laboratory *E. coli* cultures (82, 84). In particular for environmental *E. coli* populations, the selection of adaptive mutations may be central for the survival of cells in adverse conditions (24).

E. coli isolated from different hosts can possess high genotypic diversity (63). Spontaneous mutations are estimated to occur at 5.4×10^{-10} mutations per base pair per replication (19), and the selection of some mutations by unique environmental conditions results in niche-specific adaptation (37, 74). The mutation rate itself is dependent on the environment and likely is important in evolution (7, 29). For laboratory *E. coli* K-12, adaptive mutations are frequently fixed in populations during starvation in long-term batch cultures (28, 84, 85). One such mutation is within the stationary-phase sigma factor gene *rpoS* (13). The loss of RpoS, however, reduces mutagenesis in aging colonies (7) and the variability of adaptation in an evolving *E. coli* population (71). As seen with the

Lac system, RpoS is required for point mutations and gene amplification in stationary phase (52), likely in part due to the RpoS regulation of error-prone DNA polymerase IV (47). Greater evolutionary potential can result in a more enduring population, despite a possible short-term disadvantage in competitive fitness (81).

RpoS (σ^S), which is present in *E. coli* and many proteobacteria (14), regulates stationary-phase and stress-related genes (51, 64). Interestingly, RpoS mutants have shorter doubling times on poor carbon sources and in long-term culture (13, 61). The fixation of *rpoS* mutations in a population provides enhanced fitness in nutrient-poor conditions but reduced stress resistance (21, 42, 62), as in low pH (70) or osmotic upshift (31). *E. coli* growth in a glucose-limited chemostat environment selects for multiple *rpoS* genotype subpopulations (54), which can lead to divergence under environmental selection (24). The *rpoS* gene can further be regarded as polymorphic (39, 55, 61), and this polymorphism influences the trade-off between self preservation and nutritional competence (SPANC) (22, 26). Phenotypic diversity observed in clinical isolates was attributable, at least in part, to distinct RpoS levels among isolates and its effect on SPANC (49). RpoS mutants have been identified among laboratory (36, 75) and pathogenic (17) *E. coli* strains, and the ancestral *rpoS* sequence of laboratory *E. coli* K-12 likely possessed an amber mutation at codon 33 (2). Nine of 31 tested *E. coli* strains from the ECOR collection, a standard reference collection of *E. coli* from hosts (63), carried deleterious *rpoS* mutations (25). We have previously found that poor carbon sources, such as succinate, readily select for the loss of RpoS function in both laboratory (13) and pathogenic strains (17) at a frequency of 10^{-8} mutants per cell plated. By extension, stressful environmental conditions, such as poor carbon and nutrient sources, may select for RpoS mutants in environmental *E. coli* populations.

Adaptive mutations within the *rpoS* gene are selected in laboratory and pathogenic *E. coli* strains in nutrient-poor conditions or during growth on nonpreferred carbon sources (13, 17, 61, 84). Because *E. coli* can be found in a wide range of environments (10, 34, 56), including putative poor carbon environments, we postulated that RpoS mutants are present, and may be selected, in environmental *E. coli* populations. Recent work using the ECOR strains found RpoS mutants in 29% of natural isolates (25). In this study, the frequency of RpoS mutants among environmental *E. coli* isolates was determined, as well as the ability to select for RpoS mutants from environmental *E. coli* on a poor carbon source. Differential RpoS expression is examined as a mechanism for generating phenotypic diversity in environmental *E. coli*. The RpoS sigma factor is a useful model because it is well-studied, is known to be lost by selection in nutrient-poor conditions and, as a global regulator has phenotypic effects that can be readily assessed in the laboratory.

3.4 Materials and Methods

Bacterial strains. Laboratory *Escherichia coli* K-12 strains used in this study were MG1655 and the precise *rpoS* deletion of MG1655, which was constructed previously (64). A total of 2,040 environmental *E. coli* isolates were collected from urban beaches (water and sand samples) and nearby fecal pollution sources (wastewater effluents and animal fecal droppings) in the cities of Hamilton and Toronto on Lake Ontario (Canada). The cities of Hamilton and Toronto, Ontario, are large urban centers along the shoreline at the western end of Lake Ontario. The area has a temperate climate, with summer temperatures reaching more than 30°C at times, and ice cover in the nearshore areas during the winter months. *E. coli* isolates were obtained from water and sand samples at Bayfront Park and Burlington Beaches around Hamilton ($43^{o}15'$ N, $79^{o}51'$ W), and at Kew and Centre Island Beaches in Toronto ($43^{o}40'$ N, $79^{o}24'$ W). *E. coli* isolates from wastewater effluent samples were obtained from the final effluents of sewage treatment plants in Hamilton and Toronto. *E. coli* isolates were obtained from fresh fecal droppings of dogs (*Canis lupus familiaris*) and cats (*Felis catus*) at Hamilton and Toronto animal shelters. Isolates also were obtained from fresh fecal droppings of Canada Goose (*Branta canadensis*), gulls (*Larus delawarensis*), and Mallard ducks (*Anas platyrhynchos*) around Hamilton and Toronto beaches. The *E. coli* isolates were obtained by previously described methods (13), and their environmental sources are summarized in Table 3.1. A full table of individual isolates is provided in Appendix C.

Media and growth conditions. Environmental isolates were replica plated into sterile 96-well microplates containing 200 μl/well of Luria-Bertani (LB) medium (58).

The microplates were incubated overnight without shaking at 37°C. Prior to experiments, isolates were replica plated from the microplates containing LB medium into sterile microplates containing 200 μl/well of 1× M9 salts (58) for the purpose of minimizing nutrient carryover from the LB-rich media. The isolates in 1× M9 salts were used immediately in replica plate experiments onto solid medium. For testing growth on tricarboxylic acid (TCA) intermediates and weak acids, isolates were replicated onto 0.5% (wt/vol) glucose, 1% succinate, 1% fumarate, 1% citrate, 1% α-ketoglutarate, 1% acetate, 1% (vol/vol) lactate, 0.5% formate, or 0.5% proprionate M9 minimal media (pH 7) (65). For sequencing and immunoblot analyses, isolates were streaked onto solid LB medium and incubated overnight at 37°C. Single colony isolates were used in all experiments. For calculating generation times, environmental isolates were grown at 37°C with shaking to an optical density at 600 nm (OD_{600}) of ~1 in 0.5% (wt/vol) glucose M9 minimal medium and subcultured to an OD_{600} of ~0.03 in 0.5% glucose, 1% succinate, or 1% fumarate M9 minimal medium (pH 7). Growth was monitored spectrophotometrically (OD_{600}), and the generation time was calculated (17).

RpoS-dependent phenotype tests. *(i) Growth on succinate minimal medium.* Environmental isolates were replica plated onto 1% succinate M9 minimal medium (65) and incubated overnight at 37°C. Isolates were observed for growth after 24 and 48 h of incubation. Isolate patches with substantial growth after 24 h at 37°C were considered to grow well on succinate (designated Suc^{++}). As a control for nonselective growth on minimal medium, isolates were replica plated onto 0.5% glucose M9 minimal medium (65).

(ii) Catalase test. Environmental isolates were replica plated onto solid LB medium without antibiotics. Plates were incubated overnight at 37°C and tested for the presence of catalase by the addition of 5 μl of 30% hydrogen peroxide (H_2O_2) onto the patches. Isolates were recorded as catalase positive or negative, where catalase-negative strains had a severe lag in bubbling time after the addition of H_2O_2 (13).

Selection for loss of RpoS activity on succinate minimal media. Selection for the loss of RpoS activity by growth with succinate as the sole carbon source was performed by growing single colonies of environmental isolates in LB medium at 37°C and 200 rpm overnight. Cultures were washed by centrifugation in 1× M9 salts, and cells ($\sim10^9$) were plated on succinate minimal plates (13). After 48 to 72 h of incubation at 37°C, fast-growing mutants (Suc^{++}) could be observed. Single Suc^{++} mutant colonies then were serially streaked onto LB solid medium for purity. Suc^{++} mutants were tested for catalase activity and succinate growth as described above. The loss of RpoS activity in selected mutants was confirmed by the sequencing of the *rpoS* gene.

PCR amplification and sequencing of *rpoS* and *fliA* genes. Whole-colony PCR amplicons of *rpoS* and *fliA* genes from several isolates were sequenced. Single colonies were picked and boiled in 10 μl of sterile deionized/distilled water (ddH_2O) at 95°C for 5 min. A 2-μl aliquot then was transferred to the PCR reagent mix. The primers used for *rpoS* and *fliA* open reading frame (ORF) amplification are listed in Table 3.2 (synthesized by MOBIX Laboratory, McMaster University, Hamilton, Ontario, Canada). PCR was performed with Fermentas *Pfu* DNA polymerase (Fermentas, Inc., Burlington, Ontario, Canada). PCR conditions consisted of an initial denaturation step at 95°C for 2 min, 30 cycles of 30 s at 95°C, 30 s at 58°C, and 2.5 min at 72°C, and a terminal extension step at 72°C for 5 min. All PCR products were purified using a NucleoSpin Extract II kit (Machery-Nagel GmbH & Co., Inc., Bethlehem, PA) and visualized on a 1% agarose gel for quantification prior to sequencing. Samples were sequenced by MOBIX Laboratory and analyzed with Sequence Scanner, version 1.0 (Applied Biosystems, Inc., Streetsville, Ontario, Canada). Sequencing was performed on both strands of the PCR product.

Analyses of *rpoS* and *fliA* sequences in environmental *E. coli* isolates. The *rpoS* and *fliA* ORF sequences were edited using Sequence Scanner, version 1.0 (Applied Biosystems, Inc., Streetsville, Ontario, Canada) and aligned with Clustal X (IUB DNA weight matrix) (46). The *rpoS* sequences were compared to the consensus sequence, which is a composite sequence composed of the most frequent (modal) base at each nucleotide position among isolate and laboratory K-12 *rpoS* sequences. The *rpoS* sequences were constructed into a dendogram using the neighbor-joining method (46). The dendogram was bootstrapped 1,000 times, and it was visualized with MEGA 4.0 (73), as previously described (14). The *fliA* sequences were compared to the laboratory K-12 *fliA* sequence.

Motility assay. Individual colonies of bacteria were stabbed into 200 μl of 0.15% LB agar in a sterile 96-well microplate. The microplate was incubated at 37°C overnight without shaking prior to observation.

Native PAGE analysis of catalase activity. Overnight *E. coli* cultures in LB liquid medium were sampled and centrifuged at 4,000 × *g* for 15 min. Samples were washed with 50 mM potassium phosphate buffer (pH 7.0) and sonicated (17). Five micrograms of protein was separated on 10% nondenaturing polyacrylamide gels and stained for catalase using horseradish peroxidase and diaminobenzidine (30).

Immunoblot analyses of RpoS protein levels. Overnight *E. coli* cultures in LB liquid medium were subcultured 1:10,000 into 50 ml of LB and incubated at 37^{o}C with shanking at 200 rpm. Culture samples were taken at exponential phase (OD$_{600}$ of 0.3), early stationary phase (OD$_{600}$ of 1.5), and 24 h after subculture. Chloramphenicol was immediately added to the samples to a final concentration of 150 μg/ml to stop protein synthesis, and samples were centrifuged at 12,000 × *g* for 5 min. The supernatant was removed by pipette, and pellets were resuspended in SDS loading buffer (125 mM Tris-Cl, pH 6.8; 2.5% β-mercaptoethanol; 8.7% glycerol; 1% SDS; 0.01% bromophenol blue) for a final cell concentration equivalent to an OD$_{600}$ of 1.0. Resuspended pellets were placed in boiling water for 5 min.

Ten microliters of protein samples was resolved on 10% SDS polyacrylamide stacking gels. A second gel was stained for protein with 0.1% Coomassie blue dye to ensure equal protein loading. Resolved proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Inc., Billerica, MA), and the membrane was incubated for 1 h at room temperature in 5% milk made in TBS-T (87 mM NaCl; 10 mM Tris-Cl, pH 8; 0.05% Tween 20). The blocking buffer was replaced with a 1:10,000 dilution of primary antibody (anti-σ^{s} antibody; Neoclone, Inc., Madison, WI; or anti-AppA; a gift from C.W. Forsberg, University of Guelph), and the membrane was left overnight with gentle shaking at 4^{o}C. After washing with TBS-T, the membrane then was placed for 1 h at room temperature with shaking in a 1:3,000 dilution of the secondary anti-mouse antibody for the σ^s membrane or anti-rabbit antibody for the AppA membrane (Bio-Rad Laboratories, Inc., Mississauga, Ontario, Canada). After washing again with TBS-T, the secondary antibody fluorophore was activated in ECL staining solution (1:1 detection reagent mixture; Amersham GE Healthcare, Inc., Baie d'Urfe, Quebec, Canada) for 1 min prior to exposure on Amersham Hyperfilm ECL for 10 s to 1 min.

Survival assays. *(i) Acid resistance.* Overnight cultures of environmental isolates in LB were subcultured to ~10^5 cells into LB medium adjusted to pH 2.5 with hydrochloric acid. Cultures then were incubated at 37^{o}C with shanking at 200 rpm for 2 h and serially plated onto LB plates to determine the final CFU/ml (76). Percent survival was calculated as (final CFU/ml)/(initial CFU/ml) × 100.

(ii) H_2O_2 resistance. Overnight cultures of environmental isolates in LB were washed with 0.9% NaCl and subcultured to ~10^7 cells into LB medium containing 15 mM H_2O_2. Cultures then were incubated at 37^{o}C with shaking at 200 rpm, and CFU/ml was determined over time by serially plating onto LB plates (18, 45). The percent survival was calculated as (final CFU/ml)/(initial CFU/ml) × 100.

Nucleotide sequence accession numbers. Sequences were deposited at GenBank (3) with accession numbers JN191237 to JN191281 for *rpoS*, JN191282 to JN191289 for *fliA*, and JN191290 to JN191292 for succinate-selected RpoS mutants.

3.5 Results

Environmental *E. coli* isolates. A collection of 2,040 diverse environmental *E. coli* isolates from urban beaches and nearby fecal pollution sources in the cities of Hamilton and Toronto were used for this study. These isolates were collected between April and September 2004 and between May and June 2005 from beach water, foreshore beach sand, sewage treatment plant final effluent, untreated sewage from combined sewer overflow storage tanks, and fresh fecal droppings from dogs, cats, gulls, ducks, and Canada Goose (Table 3.1). These isolates were used as a representative collection of *E. coli* isolated from environmental sources outside a host organism.

Screen for RpoS activity using two RpoS-dependent phenotypes, growth on succinate and catalase activity. RpoS activity of *E. coli* isolates were assessed using two RpoS-dependent phenotypes, growth on succinate and catalase activity, to identify the prevalence of environmental RpoS mutants.

RpoS mutants of laboratory *E. coli* exhibit better growth on poor carbon than wild-type cells (13, 41). After replica plating environmental *E. coli* isolates onto media with succinate as the sole carbon source, 93 isolates had significant growth after 24 h of incubation (Suc^{++}), which is similar to that of an *rpoS* mutant control. Seven Suc^{++} isolates also were catalase negative, as determined by a catalase test. The Suc^{++} isolates were from beach water (45/971), beach sand (31/617), untreated sewage (6/68), sewage treatment plant final effluent (4/84), and fresh animal fecal droppings (7/300). The largest percentage of Suc^{++} isolates, therefore, was from untreated sewage (8.8%).

RpoS regulates one of two primary catalase genes, *katE*, in laboratory *E. coli* K-12 (67), and consequently an RpoS mutant colony has reduced bubbling compared to that of an RpoS-positive colony with the addition of hydrogen peroxide. Of the 2,040 isolates, 38 isolates were determined to have reduced catalase activity. These isolates were from beach water (14/971), beach sand (11/617), animal fecal droppings (5/300), sewage treatment plant final effluent (5/84), and untreated sewage (3/68). Similarly to the Suc^{++} phenotype, the largest percentages of catalase-negative isolates were from untreated sewage (4.4%) and final effluent (5.6%).

***rpoS* alleles confirm presence of RpoS mutants among environmental *E. coli* isolates.** The *rpoS* genes of 45 environmental *E. coli* isolates, including all isolates that were both Suc^{++} and catalase deficient, as well as representatives of isolates with only one RpoS mutant phenotype, were sequenced to confirm the presence of RpoS mutants. A dendogram of the 45 *rpoS* sequences with the isolate sources can be found in Figure 3.1.

Eight nonsynonymous and 81 distinct synonymous mutations were identified among the isolates (Appendix D). A nonsynonymous mutation at codon 33, where glutamine (CAG) is replaced with glutamic acid (GAG), is characteristic of non-K-12 strains (2, 72), and not surprisingly, it was found in all environmental *E. coli rpoS* sequences determined in this study. Three other nonsynonymous mutations resulted in an amino acid change but yielded a putatively functional RpoS protein, as these mutations had no other notable impact on the RpoS protein. More specifically, isolate ABC01 from beach sand had lysine (AAG) replace glutamic acid (GAG) at codon 122; EKF07 from beach water had tyrosine (TAC) replace asparagine (AAC) at codon 124; and ECH01 from beach water had serine (TCC) replace threonine (ACC) at codon 298. The remaining four nonsynonymous mutations resulted in a nonfunctional RpoS protein. ECE12 isolated from untreated sewage had a 5-bp deletion (nucleotides 255 to 259); BNB03, BNB04, and BNB07 from beach sand had a 1-bp deletion (nucleotide 378); AZB07 from beach sand had a G→A transition (nucleotide 443), resulting in an amber stop codon; and ASC02 from sewage treatment plant final effluent had a 1,329-bp insertion, which has 100% identity to a putative IS10 transposase of *E. coli* O111:H- strain 11128 (NCBI; February 2011) and a characteristic surrounding 9-bp DNA repeat (43) (Table 3.3).

Comparison of mutational frequencies of the *rpoS* and *fliA* genes. Among the environmental *E. coli* isolates, there were 81 distinct synonymous mutations within the *rpoS* gene (Appendix D), for a frequency of 1.8×10^{-3} synonymous mutations per base per isolate. The *rpoS*-*mutS* region of *E. coli*, as in other enteric bacteria, is an area of high genetic variation and putative high recombination (9, 32, 55). Therefore, we chose a gene of length similar to that of *rpoS*, the flagellar sigma factor *fliA*, to determine if the frequency of synonymous mutations in *rpoS* was unique among other genes in environmental *E. coli*.

The *fliA* gene was sequenced for eight isolates of different catalase activity and succinate growth. These isolates were one RpoS mutant (ECE12); two *rpoS*$^+$ (according to *rpoS* sequencing), catalase-negative isolates; three *rpoS*$^+$, Suc^{++} isolates; and two comparison isolates. All isolates carried synonymous mutations from K-12 *fliA*, and one nonsynonymous mutation was identified in ABB10 (Table 3.4). A motility assay in

0.15% LB agar indicated that ABB10 had reduced motility (Figure 3.2, lane 4). Among the eight isolates, 12 synonymous mutations were identified in 720 nucleotides (Table 3.4), for a frequency of 2.1×10^{-3} synonymous mutations per base per isolate. Therefore, the number of synonymous mutations in the *fliA* gene was comparable to that in the *rpoS* gene, with a frequency of 10^{-3} synonymous mutations per base per isolate.

RpoS protein levels in environmental *E. coli* isolates. In laboratory *E. coli*, RpoS protein levels increase during entry into stationary phase (38). For environmental isolates, RpoS expression may be altered through phenotypic diversification and environmental selection. To examine if RpoS in environmental isolates has stationary-phase expression similar to that of laboratory strains, we performed immunoblots on six isolates at exponential phase, early stationary phase, and 24 h after subculture. These isolates were three isolates with a functional RpoS phenotype (ABB02, ECA03, and ECA08) and three isolates with the *rpoS*$^{+}$, Suc^{++} phenotype (ECF09, ECG09, and ECC08). All isolates grew well in rich media (Figure 3.3a).

Like the control *E. coli* K-12 strain, RpoS expression was stationary phase dependent in all environmental *E. coli* isolates tested (Figure 3.3b). The expression of RpoS from early stationary phase to 24 h postinoculation decreased in environmental isolates, although the overall expression of RpoS was isolate dependent. ABB02, ECA03, and ECG09 had more RpoS protein at early stationary phase than the other three isolates. Importantly, RpoS expression was not decreased in *rpoS*$^{+}$, Suc^{++} isolates.

RpoS-dependent protein expression. The RpoS sigma factor regulates a large portion of the *E. coli* genome (44, 64, 77). To examine the effect of RpoS on regulon genes in environmental *E. coli*, the protein levels from two RpoS-dependent genes, *katE* (68) and *appA* (45), were determined (Figure 3.4).

For confirmed RpoS mutants ECE12, AZB07, and BNB03, RpoS protein could not be detected by immunobloting (Figure 3.4, lanes 3 to 5). KatE expression also was low, comparable to that of the K-12Δ*rpoS* strain. For all other isolates tested, RpoS protein levels were similar, with the exception of lower RpoS levels in isolate AZB10 from beach sand (Figure 3.4, lane 6). Isolate ECF11 from bird fecal droppings had minimal KatE expression (Figure 3.4, lane 11), and for the *rpoS*$^+$, catalase-deficient isolates, KatE expression was too low for detection, even though RpoS was present (Figure 3.4, lanes 7 to 10 and 12). As *rpoS*$^+$ environmental isolate controls, isolates ABC10 and ABD09 from animal feces and untreated sewage, respectively, expressed both RpoS and KatE similarly to K-12 (Figure 3.4, lanes 13 and 14).

Despite the presence of RpoS as determined by immunoblotting, KatE expression is low in several isolates, which may be the result of impaired RpoS activity. To determine if RpoS activity is impaired, the levels of a second RpoS-dependent protein, AppA (45), were examined and compared to the levels of KatE and RpoS. As expected, the RpoS mutant isolates had lower AppA expression. The *rpoS*$^+$, catalase-deficient isolates had greater AppA expression than K-12, with the exceptions of AZB10 and ECF11. AZB10, with lower overall RpoS expression, had undetectable levels of AppA (Figure 3.4, lane 6), and ECF11, despite the presence of RpoS, had lower AppA levels (Figure 3.4, lane 11).

Selection of RpoS mutants from environmental *E. coli* on succinate. In this study, we identified six null RpoS mutants in 2,040 environmental isolates (0.3% among isolates). Using laboratory and pathogenic *E. coli*, we have previously reported that RpoS mutants are readily selected on poor carbon sources (13, 17). To determine if RpoS in environmental *E. coli* isolates can be similarly subject to environmental selection, we plated three *rpoS*$^+$ environmental isolates (ECA01, ECD03, and ECF01) on succinate minimal media.

After 2 to 3 days of incubation at 37^{o}C on succinate minimal media, larger colonies (Suc^{++}) were visible at a frequency of approximately 10^{-8}, as seen with laboratory and pathogenic strains (13, 17). One independent mutant from each isolate was tested and confirmed to be an RpoS mutant by sequencing the *rpoS* ORF. Single null mutations were identified within the *rpoS* ORF of each succinate-selected mutant (Table 3.5).

Characterization of RpoS mutants. For environmental *E. coli* outside a host, *rpoS* mutations may be selected under adverse conditions and lead to phenotypic diversity. The loss of RpoS increases the ability of *E. coli* to grow on nonpreferred carbon sources (13, 17, 23) and concomitantly decreases survival in stress (24, 61, 76). Therefore, to examine the RpoS mutant phenotype of environmental *E. coli* populations, naturally isolated RpoS mutant AZB07 and succinate-selected RpoS mutant ECA01Suc^{++} were tested for their abilities to grow on poor carbon sources as well as for their resistance to acidic pH and to oxidative stress.

For growth on the poor carbon sources succinate and fumarate, RpoS mutants had lower generation times than the K-12 control and ECA01Suc^{++} isogenic parental strain, ECA01 (Table 3.6). More specifically, RpoS mutants had approximately 18× faster growth on succinate and 6× faster growth on fumarate. As seen in other studies (13, 17), the difference in generation time was not apparent for growth with the preferred carbon source, glucose.

For stress resistance, RpoS mutants were less resistant to both acidic pH (Figure 3.5a) and exposure to H_2O_2 (Figure 3.5b). Functional RpoS was essential for cells to withstand low pH, as less than 0.0001% of RpoS mutant cells were able to retain viability. Interestingly, while the K-12 control had just 31% survival after 2 h at pH 2.5, ECA01 showed an increase in numbers of CFU/ml, which suggests a strong resistance in this environmental isolate to acidic conditions. During exposure to H_2O_2, RpoS mutants lost viability more rapidly than RpoS-positive cells. These results are in agreement with previous research using laboratory *E. coli* strains (31, 70).

3.6 Discussion

Bacteria adaptation depends on complex regulatory systems that may control the differential expression of hundreds of genes. Though such adaptation has been extensively examined in laboratory strains over the last two decades, the role of adaptation in natural *E. coli* isolates has received little attention. In this study, we examined the RpoS regulon as a model of phenotypic diversity among a large collection of environmental *E. coli* isolates. This represents the first broad survey of RpoS activity in *E. coli* isolates collected from the environment. These strains underwent minimal laboratory handling prior to testing and storage, thus they represent a unique collection that can be examined for natural phenotypic diversity. RpoS loss-of-function mutants previously have been identified in several contexts, including laboratory (2, 36, 75) and pathogenic (17) *E. coli* strains and during long-term culture (25, 61, 83) and growth on poor carbon sources (13), suggesting that such mutants are selected for under some circumstances. Because diverse environmental conditions where *E. coli* can be found may mimic these conditions, we wished to determine the frequency of RpoS mutants in environmental *E. coli* isolates.

Two RpoS-dependent phenotype tests, growth on succinate and catalase activity, were used initially in this study to provide an indirect assessment of RpoS activity, since these can be readily adapted to colony screening. The highest percentage of Suc^{++} isolates and of catalase-negative isolates were found in human fecal sources, which may indicate a favorable selective environment. By screening for these phenotypes among the 2,040 environmental isolates, and the subsequent sequencing of the *rpoS* gene in candidate mutant strains, six isolates were identified to carry deleterious *rpoS* mutations. These six isolates, as well as one *rpoS*$^+$ isolate (EKF07, from beach water), had a consistent RpoS mutant phenotype for both succinate growth and catalase activity. The beach water isolate (EKF07) may have been attenuated by a nonsynonymous mutation at codon 124, where tyrosine (TAC) replaced asparagine (AAC). Considering the spontaneous mutation rate of 5.4×10^{-10} mutations per base pair per replication in *E. coli* (19), the relatively high abundance of RpoS mutants among environmental *E. coli* isolates (0.3%) suggests that certain natural environments favor the selective growth of RpoS loss-of-function mutants.

Thirty-one natural isolates from the ECOR collection, a genotypically diverse set of natural *E. coli* strains from animal and human hosts (63), recently was examined for RpoS expression (25). Interestingly, RpoS mutants were found at a substantially higher frequency (0.29) among the ECOR collection compared to that among the environmental isolates studied here (0.003). Because the ECOR collection was compiled largely based on genotypic diversity (63), the sequence variability of the *rpoS* gene in these strains may not be surprising. ECOR strains with elevated RpoS expression also accumulate *rpoS* mutations under nutrient limitation (25). The long-term storage of the ECOR strains, as well as the diverse hosts from which these strains were isolated, may have contributed to a higher observed frequency of RpoS mutants in the ECOR collection than in the environmental isolates examined in this study.

Of the RpoS mutants identified among the environmental isolates, three RpoS mutants (BNB03, BNB04, and BNB07) taken from the same beach sand sample possessed identical *rpoS* mutations. These beach sand isolates may have resulted from clonal growth in a natural environment, as the multiplication of *E. coli* in sediment has previously been shown to reach a high cell density of 10^5 CFU/g nonsterile soil (34). Alternatively, clones from the same fecal deposit may have persisted in the soil. One naturally isolated RpoS mutant (AZB07) from beach sand also was identified to carry the same G→A transition, resulting in an amber stop codon, as a later succinate-selected RpoS mutant (ECD03Suc^{++}). The selection of this G→A transition in both AZB07 and ECD03Suc^{++} is not surprising, given that G/C-to-A/T transitions in bacteria are more common than A/T to G/C (33, 50). The same *rpoS* mutation in the naturally isolated and the succinate-selected RpoS mutant strains also suggests that growth on succinate can select for mutations that mimic mutations that are naturally selected. Finally, to our knowledge, the presence of a transposon element within the *rpoS* gene rendering a nonfunctional RpoS protein, as was found in isolate ASC02 from sewage treatment plant final effluent, has not been reported previously for environmental *E. coli* strains. Unfortunately using the six RpoS mutants identified, it is not possible to determine a common stimulus that selects for RpoS mutants, as the isolates came from several independent sources.

The *rpoS*-*mutS* region of *E. coli* strains is hypervariable (32) and undergoes frequent recombination (9, 15). The *rpoS* gene is polymorphic in *E. coli* (6, 27), as well as in other gammaproteobacteria (39). Despite the reported high sequence variability of the *rpoS* gene, we found that the frequency of synonymous mutations in the *fliA* gene, encoding another nonessential sigma factor, was comparable to that of the *rpoS* gene. At nucleotide position 519 of *fliA*, all sequenced environmental isolates carried guanosine instead of adenosine (found in the *fliA* K-12 strain), strongly suggesting that guanosine at this position is ancestral. Isolate ABB10 from untreated sewage, which carried a Ser→Asn mutation at codon 176, also had decreased motility, which suggests that this site is functionally important for motility. The high and comparable number of mutations identified in *rpoS* and *fliA* is consistent with the theory that many of these mutations are neutral under natural selection (40). Studies on codon bias, a determinant of synonymous mutation rates in enterobacteria (69), and its effect on selection for *rpoS* mutations in environmental *E. coli* would be of future interest. Putatively, synonymous mutations in *rpoS* accumulate under low environmental selection, while under strong selection, such as succinate minimal media, single *rpoS* null mutations become fixed in *E. coli* populations.

As noted above, RpoS regulates the expression of the catalase gene, *katE* (67), and using the catalase test (see Materials and Methods), environmental isolates were screened for RpoS activity. A total of 38 isolates showed reduced bubbling, indicating lower catalase expression, yet only six of these were RpoS mutants. Previous studies have found that mutations in the *rpoS* allele result in intermediate bubbling (84). In two *rpoS*$^+$, catalase-negative isolates, ECH01 and EKF07, threonine is replaced with serine (amino acid 198) and asparagine is replaced with tyrosine (amino acid 124), respectively (Appendix D), which indicates the functional importance of these amino acids at these sites. However, the remaining *rpoS*$^+$, catalase-negative isolates possessed only synonymous *rpoS* mutations. Environmental *E. coli* strains may have adaptive modifications in the expression of regulon genes that are independent of those found in

RpoS. RpoS regulon composition, compared between species, is diverse and largely species specific (14, 66), but the extent of RpoS regulon plasticity between strains of the same species is not known. By examining the expression of a second RpoS-dependent protein, AppA (45), and the expression of RpoS in several *rpoS*$^+$, catalase-negative isolates, it was determined that only one isolate (AZB10 from beach sand; Figure 3.4, lane 6) had reduced RpoS expression, resulting in lower regulon expression. The remaining *rpoS*$^+$, catalase-negative isolates exhibited little to no KatE expression, although RpoS and RpoS-dependent AppA were present (Figure 3.4, lanes 7 to 12). This indicates that the expression of even prototypical RpoS regulon members is strongly influenced by factors in environmental *E. coli* isolates. Mutations to RpoS posttranslational factors, such as to Crl (8), also may contribute to differential regulon expression. Interestingly, the AppA expression of *rpoS*$^+$, catalase-negative isolates, with the exception of isolate AZB10 from beach sand and isolate ECF11 from bird fecal droppings, had higher AppA levels than the laboratory control (Figure 3.4, lanes 7 to 10 and 12). A decrease in the expression of one RpoS regulon member may allow for more transcriptional recruitment of RpoS to other dependent promoters. Additional work on RpoS regulon expression is needed to validate this possibility.

Mutations in *rpoS* can be selected from laboratory and pathogenic *E. coli* strains grown with succinate as the sole carbon source (13, 17). In this study, we show that *rpoS* mutants also can be readily selected from environmental *E. coli* when grown on succinate (Table 3.5) and have enhanced growth on poor carbon sources (Table 3.6). Because RpoS mutants were found in 0.3% of isolates, there likely are natural environments that also favor RpoS mutant growth. Of course, it is not clear from this study when *rpoS* mutations accrue, during passage through the gut or after deposition in the environment, and what natural environments select for RpoS mutants. *E. coli* can adapt to carbon source and oxygen availability through changes in metabolism (48), and the loss of RpoS previously has been shown to induce the expression of TCA cycle genes (64). RpoS mutants may have an adaptive advantage during nutrient-limited growth in the natural environment as well as in some host environments, such as in the urinary tract, where the TCA cycle is required for infection *in vivo* (1). On the other hand, fermentation in the gut and the production of short-chain fatty acids, such as acetate, proprionate, and butyrate (16, 53), may select for isolates with functional RpoS protein, as RpoS is important in acid resistance (Figure 3.5b). The environmental isolates used in this study exhibit differential abilities to utilize carbon sources, as growth on a range of TCA cycle intermediates or on weak acids present in the gut is strain dependent (data not shown). Metabolic demands on *E. coli* in the host (1, 12, 59) and in the environment may be important determinants of RpoS selection. Further study is required to identify natural conditions that select for *rpoS* mutations and diverse metabolic capabilities of environmental *E. coli*.

In conclusion, RpoS is an alternative sigma factor that is known to be subject to environmental selection (13, 41). As such, mutations in the *rpoS* gene lead to phenotypic diversity in environmental *E. coli* isolates. Previous work has focused on *rpoS* mutations in laboratory and pathogenic *E. coli* (13, 17), yet the diverse conditions that environmental *E. coli* experience may have high selective pressures. Indeed, environmental RpoS mutants were determined to exist at a frequency as high as 0.003, and RpoS mutants from environmental *E. coli* isolates could be selected when grown on a poor carbon source. The natural selection of RpoS mutants outside a host may, therefore, be an important determinant in environmental *E. coli* adaptation and survival.

3.7 Acknowledgements

We thank Stephen Hill for his help with retrieving the 2,040 *E. coli* isolates and Vithooshan Vijayakumaran for immunoblot analysis of ASC02.

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Table 3.1. Summary of source types for the 2,040 environmental *E. coli* isolates used in this study.

Type	No. of isolates
Beach water	971
Beach sand	617
Canada Goose (*Branta canadensis*) dropping	131
Sewage plant final effluent	84
Untreated CSO sewage	68
Dog (*Canis lupus familiaris*) dropping	65
Cat (*Felis catus*) dropping	48
Gull (*Larus delawarensis*) dropping	38
Mallard duck (*Anas platyrhynchos*) dropping	18

Table 3.2. *rpoS* and *fliA* oligonucleotides used in this study for ORF amplification.

Oligonucleotide	ORF	Sequence
ML-08-145	*rpoS*	5’- CAACAAGAAGTGAAGGCGGG -3’
ML-08-4514	*rpoS*	5’- CTTGCATTTTGAAATTCGTTACA -3’
rpoS754	*rpoS*	5’- GATGACGATATGAAGCAGAG -3’
ML-08-4515	*rpoS*	5’- TTAACGACCATTCTCGGTTTTAC -3’
ML-08-5873	*rpoS*	5’- GGTGCAATCTCCAGCCG -3’
ML-08-5874	*rpoS*	5’- GGAGAATCGTGGCTTAGTCAG -3’
rpoSrv834	*rpoS*	5’- TAACATCAAACGAATCGACC -3’
ML-08-3246	*fliA*	5’- ACCTGTAACCCCCAAATAAC -3’
ML-08-3247	*fliA*	5’- CAATGGGTCTGGCTGTG -3’

Table 3.3. Identified mutations in the *rpoS* gene that predict a nonfunctional RpoS protein in environmental *E. coli* isolates.

Isolate	Source type	Type of mutation	Effect of mutation on amino acid sequence	Location (nucleotides)	*rpoS* accession no.
ECE12	Untreated combined sewer overflow	5-bp deletion	Frameshift, short protein	255-259	JN191271
BNB03[a]	Beach sand	1-bp deletion	Frameshift, short protein	378	JN191252
AZB07	Beach sand	G→A transition	TGG→TAG (amber stop codon)	443	JN191250
ASC02	Sewage treatment plant final effluent	1,329-bp insertion (IS*10* transposase) and 9-bp duplication	Short protein	776	JN191249

[a] Isolates BNB04 (JN191253) and BNB07 (JN191254), from beach sand, possessed the same *rpoS* mutation as isolate BNB03.

Table 3.4. Mutations within the *fliA* gene of eight environmental *E.coli* isolates compared to *fliA* of *E. coli* K-12.

Isolate	Succinate growth (+/-)[a]	Catalase activity (+/-)[a]	Nucleotide position													*fliA* accession no.
			225	339	345	471	504	516	519	526-528	555	603	633	678	684	
K-12	-	+	C	C	C	C	C	A	A	AGT (Ser)	C	C	C	C	T	NC_000913
ECA01	-	+	-	-	T	-	-	-	G	---	T	-	-	-	C	JN191284
ECF01	-	+	-	T	-	T	-	G	G	---	T	-	-	-	C	JN191288
ABB10	-	-	-	-	-	-	-	-	G	AAT (Asn)	-	T	T	-	-	JN191282
ECF11	-	-	A	-	-	-	-	-	G	---	-	T	T	T		JN191289
ECE12	+	-	-	T	-	-	-	-	G	---	-	-	-	-	C	JN191287
ABC03	+	+	-	-	-	-	T	-	G	---	-	-	-	-	C	JN191283
ECA04	+	+	-	-	T	-	-	-	G	---	T	-	-	-	C	JN191285
ECB02	+	+	-	-	T	-	-	-	G	---	T	-	-	-	C	JN191286

[a] Ability to grow on succinate (Suc^{++}) and catalase expression are indicated.

Table 3.5. Mutations within the *rpoS* gene of succinate-selected mutants of environmental isolates.

Succinate mutant	Selection source	Type of mutation	Effect of mutation on amino acid sequence	Location (amino acids)	*rpoS* accession no.
ECA01Suc^{++}	ECA01	96-bp deletion	32-amino-acid deletion	54-85	JN191290
ECD03Suc^{++}	ECD03	G→A transition	TGG→TAG (amber stop codon)	148	JN191291
ECF01Suc^{++}	ECF01	Adenosine insertion	Frameshift, short protein	32	JN191292

Table 3.6. Growth of environmental *E. coli* RpoS mutants on glucose or on the poor carbon source succinate or fumarate.

Substrate	Generation time (min) of strain[a]				
	K-12	K-12Δ*rpoS*	ECA01	ECA01Suc^{++}	AZB07
Glucose	93.0 ± 2.1	85.1 ± 3.5	79.5 ± 3.8	78.1 ± 8.8	76.3 ± 1.0
Succinate	1,347.1 ± 62.9	141.9 ± 5.1	2,403.0 ± 105.2	90.8 ± 5.9	75.4 ± 2.7
Fumarate	832.1 ± 100.2	129.6 ± 13.3	537.4 ± 68.5	81.1 ± 7.5	118.9 ± 1.2

[a] ECA01Suc^{++} is a succinate-selected RpoS mutant from ECA01, and AZB07 is an RpoS mutant isolated from beach sand. Values are mean ± standard errors of the means, where $n = 3$.

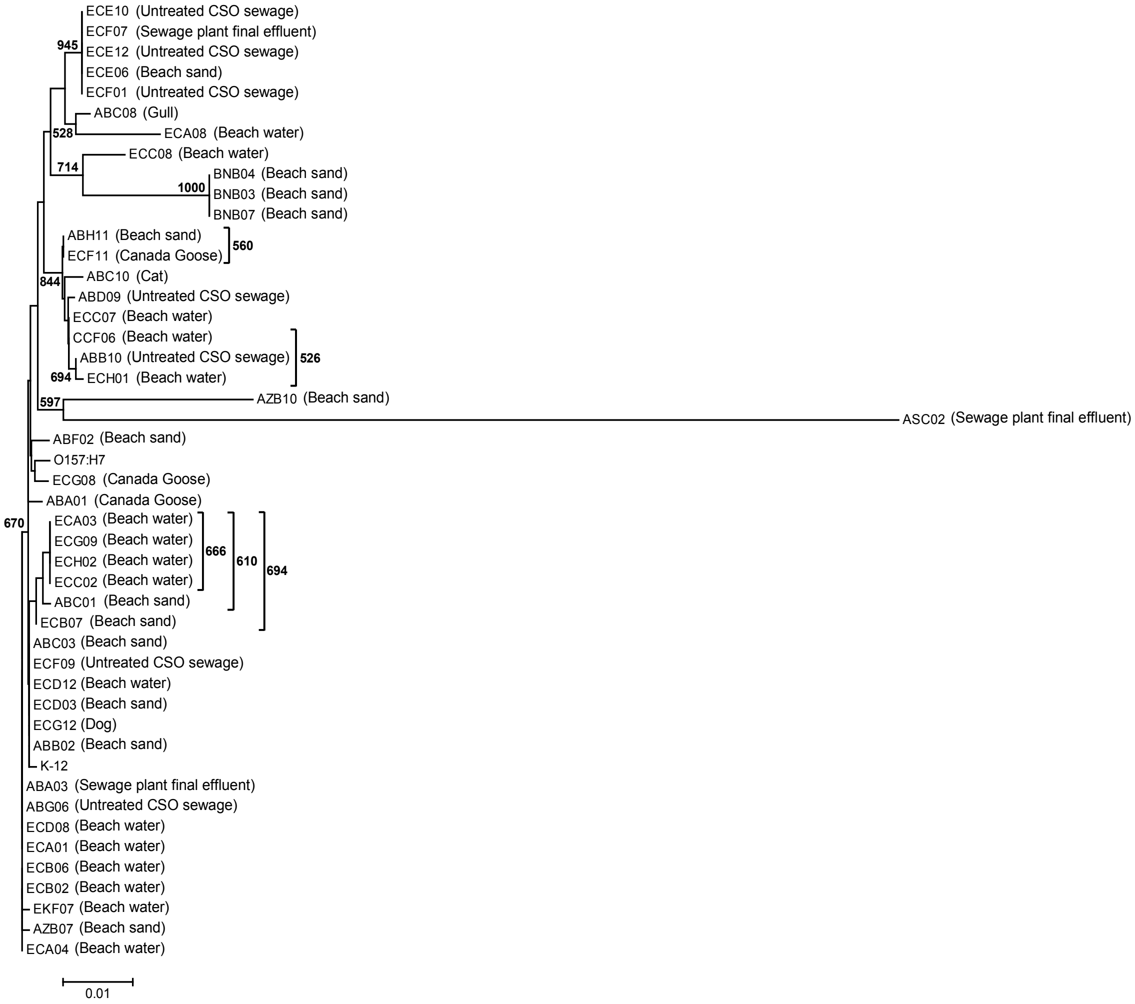

Figure 3.1. Neighbor-joining dendogram showing genotypic diversity of 45 environmental *E. coli* isolates, as well as *E. coli* K-12 (NC_000913) and *E. coli* O157:H7 (NC_002695) for comparison, based on *rpoS* sequences. Source of isolate is provided in brackets. Bootstrap values are indicated for branches ≥ 500 out of 1000, and for short branches, bootstrap values are listed to the right of the clade. Scale bar represents 0.01 nucleotide substitutions per site.

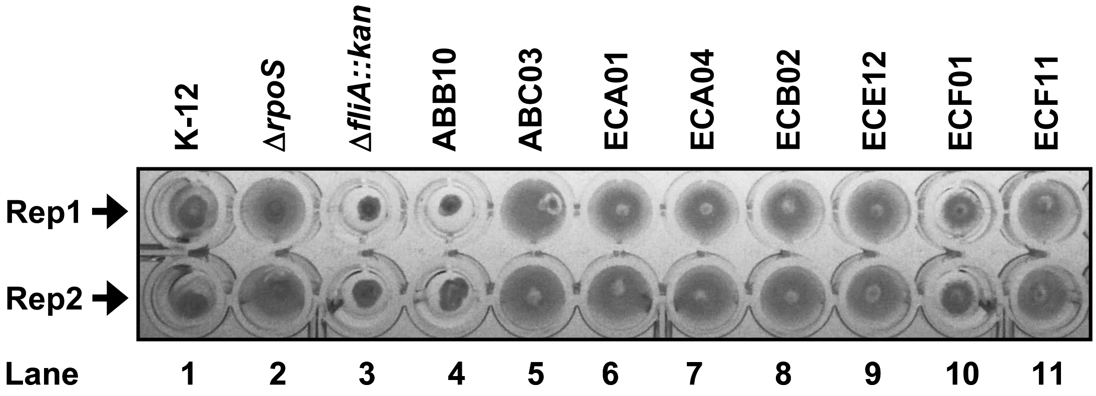

Figure 3.2. Motility of eight environmental *E. coli* isolates compared to laboratory controls. Isolates were stabbed into 200 μl of 0.15% LB agar in a sterile 96-well microplate and incubated at 37°C overnight. Rep1 and Rep2 indicate two biological replications.

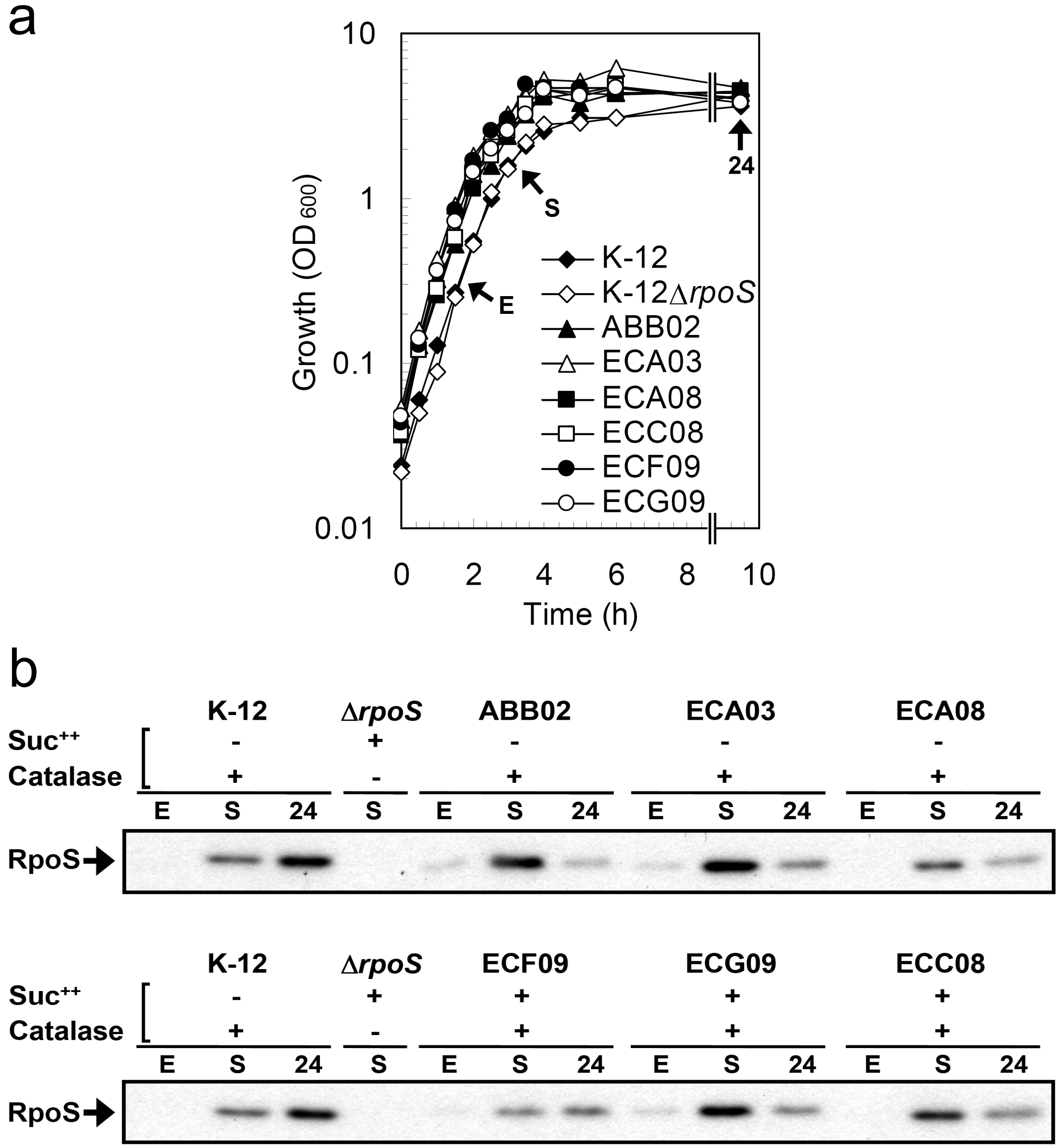

Figure 3.3. Growth and RpoS expression of environmental isolates in rich media. (a) Growth of environmental isolates in rich media. ABB02 from beach sand, ECA03 from beach water, and ECA08 from beach water have RpoS-positive catalase and succinate growth phenotypes. ECF09 from untreated combined sewer overflow sewage, ECG09 from beach water, and ECC08 from beach water have RpoS-negative, Suc^{++} phenotype but are catalase positive. Samplings indicated as exponential phase (E), early stationary phase (S), and 24 h postinoculation (24). (b) Immunoblot detection of RpoS protein levels in environmental *E. coli* isolates.

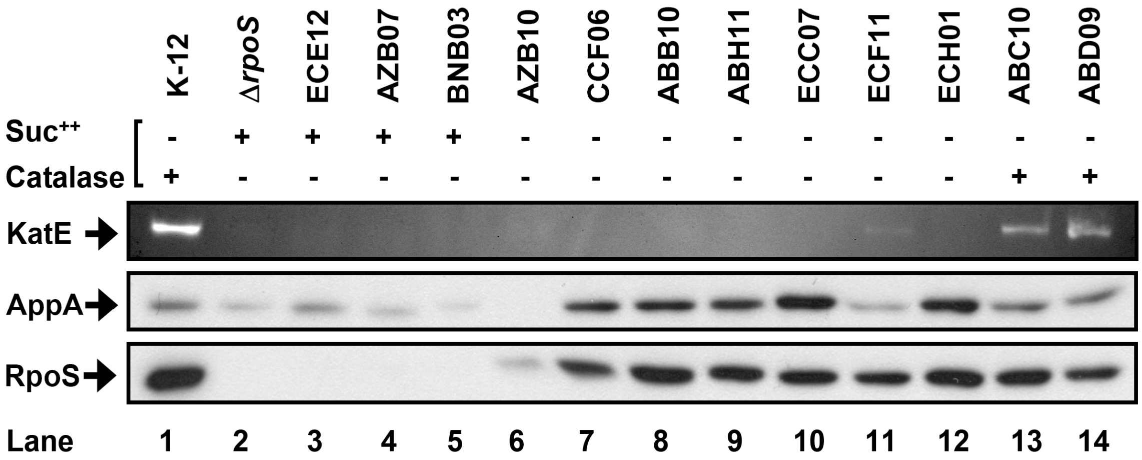

Figure 3.4. RpoS-dependent KatE and AppA levels compared to those of RpoS in catalase-deficient environmental *E. coli* isolates in stationary phase. The upper gel shows 5 µg of protein run on nature PAGE and stained for catalase. The middle and lower panels show 4 µg of protein run on SDS-PAGE, and AppA and RpoS proteins were detected by immunoblotting. Isolates ECE12, AZB07, and BNB03 are confirmed RpoS mutants. Isolates ABC10 and ABD09 are catalase positive.

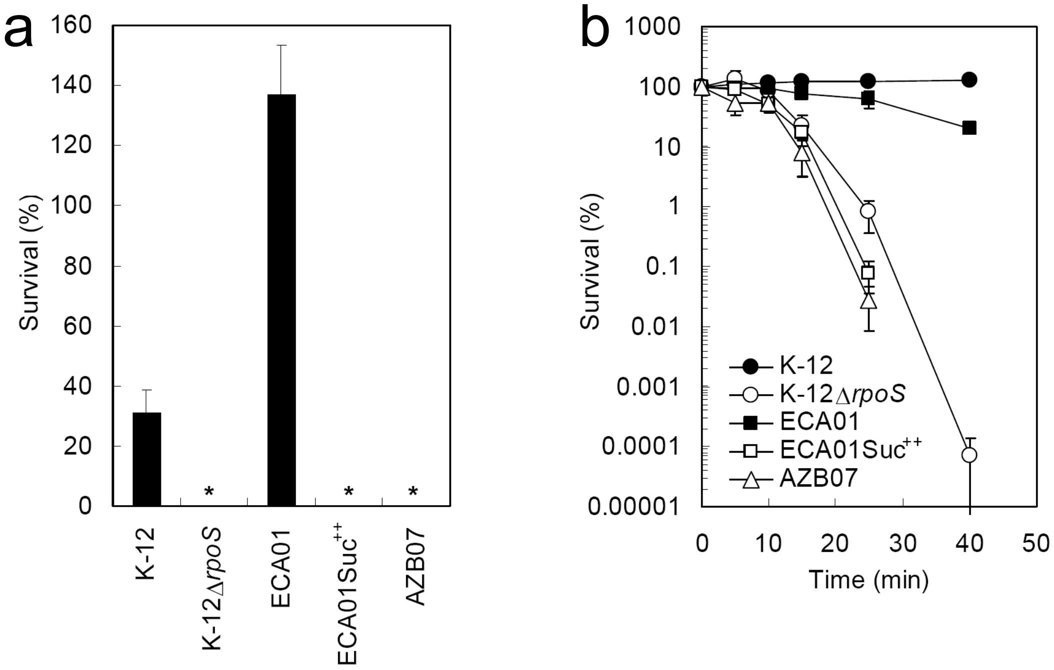

Figure 3.5. Stress resistance capabilities for RpoS mutants of environmental *E. coli*. (a) Survival of environmental isolates in acidic conditions. LB medium (pH 2.5) was inoculated with ~10^5 cells and incubated at 37°C with shaking for 2 h. Percent survival was calculated as (final CFU/ml)/ (initial CFU/ml) × 100, and values of <0.0001% are indicated with an asterisk. ECA01Suc^{++} is a succinate-selected RpoS mutant from ECA01, and AZB07 is an RpoS mutant isolated from beach sand. The experiment was done in triplicate, and values represent means ± standard errors of the means. (b) Survival of environmental isolates in oxidative stress. LB medium with 15 mM H_2O_2 was inoculated with ~10^7 cells, and the percent survival was calculated over time. At 40 min, ECA01Suc^{++} and AZB07 had <0.0001% survival. ECA01Suc^{++} is a succinate-selected RpoS mutant from ECA01, and AZB07 is an RpoS mutant isolated from beach sand. Experiments were done in triplicate, and values represent means ± SEM.

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CHAPTER 4:

Regulators of oxidative stress response genes in *E. coli* and their functional conservation in bacteria

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4.1 Preface

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4.2 Abstract

Oxidative stress, through the production of reactive oxygen species, is a natural consequence of aerobic metabolism. *Escherichia coli* has several major regulators activated during oxidative stress, including OxyR, SoxRS, and RpoS. OxyR and SoxR undergo conformation changes when oxidized in the presence of hydrogen peroxide and superoxide radicals, respectively, and subsequently control the expression of cognate genes. In contrast, the RpoS regulon is induced by an increase in RpoS levels. Current knowledge regarding the activation and function of these regulators and their dependent genes in *E. coli* during oxidative stress forms the scope of this review. Despite the enormous genomic diversity of bacteria, oxidative stress response regulators in *E. coli* are functionally conserved in a wide range of bacterial groups, possibly reflecting positive selection of these regulators. SoxRS and RpoS homologs are present and respond to oxidative stress in *Proteobacteria*, and OxyR homologs are present and function in H_2O_2 resistance in a range of bacteria, from gammaproteobacteria to *Actinobacteria*. Bacteria have developed complex, adapted gene regulatory responses to oxidative stress, perhaps due to the prevalence of reactive oxygen species produced endogenously through metabolism or due to the necessity of aerotolerance mechanisms in anaerobic bacteria exposed to oxygen.

4.3 Oxidative stress in bacteria

Bacteria, under aerobic conditions, experience oxidative stress through the formation of reactive oxygen species (ROS) that can damage several cellular sites, including iron-sulfur clusters, cysteine and methionine protein residues, and DNA [1, 2]. ROS are an inevitable by-product of oxygen exposure and utilization. For example, superoxide radicals ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are produced when flavin cofactors, such as found in NADH dehydrogenase II of the respiratory chain, autooxidize and react with O_2 molecules (Eq. (1)) [3], and the highly reactive hydroxyl radical ($^{\bullet}OH$) is formed when hydrogen peroxide reacts with unincorporated ferrous iron by Fenton chemistry (Eq. (2)) [4]. Reactive nitric oxide, an intermediate in denitrification (Eq. (3)) [5], is another example of a common reactive species in bacteria. Therefore, oxidative stress responses have necessarily evolved to ensure survival of organisms in aerobic conditions.

$$O_2 + e^- \xrightarrow{\text{FAD}} \mathbf{O_2}^{\cdot -} \qquad \text{equation 1}$$

$$\text{Fe(II)} + H_2O_2 \rightarrow \text{Fe(III)} + OH^- + {}^{\cdot}\mathbf{OH} \qquad \text{equation 2}$$

$$NO_3^- \rightarrow NO_2^- \rightarrow \mathbf{NO}^{\cdot} \rightarrow N_2O \rightarrow N_2 \qquad \text{equation 3}$$

The gene regulatory response of *Escherichia coli* to superoxide and peroxide stress is largely mediated through the induction of superoxide dismutases (SOD) and catalases, respectively. Superoxide is dismutated to form hydrogen peroxide (H_2O_2) by superoxide dismutase activity (Eq. (4)) [6]. *E. coli* has three superoxide dismutases, namely MnSOD (*sodA*), FeSOD (*sodB*), and CuZnSOD (*sodC*). Catalases, part of the peroxidase family of enzymes, subsequently degrade H_2O_2 into H_2O and O_2 (Eq. (5)). *E. coli* has two catalases, hydroperoxidase I (HPI) and hydroperoxidase II (HPII), encoded by *katG* and *katE*.

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{\text{SOD}} \mathbf{H_2O_2} + O_2 \qquad \text{equation 4}$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \qquad \text{equation 5}$$

E. coli superoxide dismutase and catalase genes are members of two major oxidative stress regulons, the OxyR and SoxRS regulons, as well as the RpoS general stress regulon. Current understanding on the induction of these regulons during oxidative stress will be discussed here. As well although *E. coli* is often used as the model system, these regulons have been studied in a range of bacterial phyla, from *Actinobacteria* to *Proteobacteria*. Evolutionarily speaking, bacteria experience extensive modifications in genomic content through gene loss/gain, gene duplication, and horizontal transfer [7], which allows adaptation of oxidative stress responses within species. This review will also focus on how regulators of oxidative stress discovered in *E. coli* and *Salmonella enterica* function in other bacterial species and confer an adapted regulatory response.

4.4 Oxidative stress regulons in *E. coli*

4.4.1 OxyR, principal regulator for hydrogen peroxide detoxification

OxyR, a 34 kDa protein that forms a homotetramer [8], is a homolog of the LysR family of transcriptional regulators in *E. coli* [9, 10], and characteristic of this protein family [11], OxyR negatively regulates expression from its encoding gene, *oxyR*, and positively regulates an adjacent small RNA gene, *oxyS* [12]. OxyR controls a regulon of almost 40 genes, which protect the cell from hydrogen peroxide toxicity (Table 4.1). Consequently, *oxyR* mutants are hypersensitive to H_2O_2, and constitutive expression of the OxyR regulon due to dominant mutations in *oxyR*, such as found in mutants *oxyR1* of *S. enterica* and *oxyR2* of *E. coli*, increases resistance [13]. OxyR also has a role in protecting against heat stress [13], near-UV [14], singlet oxygen [15], lipid peroxidation-mediated cell damage [16], and neutrophil-mediated killing [17].

Transcriptional induction of dependent promoters occurs when OxyR is converted to its oxidized state [18] (Figure 4.1a). In this state, OxyR recognizes four ATAG elements with 10 bp spacing. The spacing of these elements allows for the four subunits of OxyR to bind to four adjacent major grooves on one side of the DNA helix. Reduced OxyR, on the other hand, binds to just two major grooves [19]. A comparison of oxidized and reduced OxyR by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry revealed that, in the oxidized OxyR readout, (a) two peaks corresponding to conserved Cys199 and Cys208 are absent and (b) a new peak corresponding to the sum of these two cysteines joined by a disulfide bond is present [20]. Therefore, in the presence of H_2O_2, the sulfur residue of Cys199 is oxidized to sulfenic acid and forms a reversible disulfide bond with Cys208 [20]. As a result, oxidized and reduced forms of the OxyR regulatory domain have significant structural differences [21]. Oxidized OxyR binds co-operatively with RNA polymerase to positively regulate transcription of dependent promoters [22], while reduced OxyR negatively regulates other genes, such as *agn43* [23] and *stiA* of *S. enterica* [24]. In the absence of oxidative stress, OxyR-dependent glutathione reductase (*gorA*) and glutaredoxin (*grxA*) return OxyR to its reduced form [20].

In *E. coli* and *S. enterica*, 28 genes are induced by OxyR, 10 genes are repressed, and one operon of *S. enterica* phage P22, *gtrABC*, can be induced or repressed, depending on the binding location of OxyR to the *gtrABC* regulatory region (Table 4.1). In *oxyR* mutants, mutations that suppress hydrogen peroxide sensitivity result in elevated levels of OxyR regulon members, hydroperoxidase I (encoded by *katG*) and alkyl hydroperoxide reductase (Ahp, encoded by *ahpCF*) [25]. Catalase and Ahp confer resistance to exogenous H_2O_2 [13] and homeostatic regulation of endogenous H_2O_2 [26]. Intracellular hydrogen peroxide, normally maintained at ~0.2 μM, may reach as high as 1 μM concentration in *E. coli oxyR* mutants during exponential growth [26]. Although KatG activity is inversely correlated with intracellular H_2O_2 levels, deletion of *katG* results in only slightly elevated H_2O_2 levels in exponential phase and does not fully account for the high H_2O_2 concentration in *oxyR* mutants [26]. Catalase mutants in dilute concentrations are equally sensitive to H_2O_2 compared to wild type [27]. Ahp scavenges H_2O_2 in catalase mutants to the same degree as wildtype [28]. As revealed by H_2O_2 decomposition measurements, Ahp is kinetically more efficient than catalase at scavenging H_2O_2, but Ahp activity is saturated at relatively low H_2O_2 levels (10^{-5} M) [28]. Because *katG* is induced by OxyR activation in *ahp* mutants [28], KatG, which has both catalase and peroxidase activity [29], may compensate for Ahp activity when Ahp is saturated.

4.4.2 Functional conservation of the OxyR regulon in *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*

Since its initial characterization, the function of OxyR and its cognate regulon have been examined in a wide range of *Proteobacteria* (a large group containing many important animal and plant pathogens) as well as in the phyla *Bacteroidetes* and *Actinobacteria*. The *Proteobacteria* consists of five classes based on 16S rRNA sequence similarity. Beginning with the most recently diverged, these are the gammaproteobacteria (includes *E. coli* and *S. enteric*a), betaproteobacteria, alphaproteobacteria, deltaproteobacteria, and epsilonproteobacteria. To determine the extent of functional conservation, relevant studies on the OxyR regulon in species of the five *Proteobacteria* classes and other phyla are considered here.

In most gammaproteobacteria, catalase regulation by OxyR is conserved, such as *katA* and *katY* of *Yersinia* sp. [30] and *hktE* (homolog of *E. coli katE*) of *Haemophilus* sp. [31] (Figure 4.2). Likewise, expression of alkyl hydroperoxide reductase (*ahpCF*) homologs is commonly under OxyR control [32, 33]. While OxyR of *E. coli* controls the expression of genes primarily by binding to promoters in its oxidized state, more complex forms of regulation have been found in other gammaproteobacterial species. For example, OxyR of *Xanthomonas campestris* regulates the same *ahpC* promoter in both oxidized and reduced forms, where the oxidized form induces and the reduced form represses *ahpC* expression [34]. One exception to OxyR functional conservation in gammaproteobacteria is OxyR of *Legionella pneumophila*, denoted $\mathrm{OxyR_{Lp}}$. $\mathrm{OxyR_{Lp}}$ represses one of two alkyl hydroperoxide reductases (*ahpC2D*), even in the presence of H_2O_2, and weakly binds prototypical OxyR-regulated promoters of *E. coli*. Instead of an oxidative stress response regulator, $\mathrm{OxyR_{Lp}}$ is thought to regulate the transition of *L. pneumophila* from a vegetative state to a dormant cyst-like form [35].

As is the case with other regulatory systems [36], OxyR regulon composition varies among different classes of *Proteobacteria*. In betaproteobacteria, *Neisseria gonorrhoeae* OxyR, with 37% identity to *E. coli* OxyR [37], regulates only three genes, *gor* (glutathione reductase), *prx* (peroxiredoxin), and *katA* [38]. Despite the small size of the *Neisseria* OxyR regulon, the OxyR crystal structure of *Neisseria meningitidis* suggests that OxyR is activated through the formation of the same disulphide bond between C199 and C208, similar to *E. coli* OxyR [39]. In alphaproteobacteria, OxyR regulation of catalase is species-specific. For example, the catalase gene, *katA*, of *Sinorhizobium meliloti* is dually regulated by OxyR [40], but *katG* of *Bradyrhizobium japonicum* is OxyR-independent [41]. Lastly in delta- and epsilon-proteobacteria, OxyR homologs have not been identified [42, 43]. Instead, *ahpC* is repressed by a Fur homolog, PerR [42, 43]. PerR and OxyR have evolved independently [44], and homologs of both OxyR and PerR are not typically found in the same bacterial species [45]. A functional PerR protein is not present in *E. coli*.

OxyR is important for aerotolerance in the anaerobic phylum *Bacteroidetes* [46-48]. OxyR of *Bacteroides fragilis* has 40% identity to OxyR of aerobic organisms [49] and regulates an acute response regulon of 13 genes, which remove peroxide species and maintain intracellular redox balance [48]. In *Tannerella forsythia* and *Porphyromonas gingivalis*, OxyR is constitutively active, possibly due to an altered OxyR structure that results in a locked-on, oxidized conformation [46, 47]. As might be expected in these species, prototypical OxyR-controlled genes, such as *ahpC*, require OxyR for expression but are not induced in the presence of H_2O_2 [46, 47].

Deinococcus radiodurans is the only bacterial species known to possess two OxyR homologs, DrOxyR (DR0615) and $OxyR_2$ (DRA0336), both of which are important for resistance to H_2O_2 [50]. Similar to the conserved cysteines of *E. coli*, DrOxyR carries a conserved cysteine at nucleotide 210, which likely senses peroxide but cannot form a disulfide bond [51]. Unlike OxyR in *E. coli*, DrOxyR represses one of two *dps* (*dr2263*) genes in *D. radiodurans* and does not affect the other *dps* gene (*drB0092*), as well as *ahpF*, in oxidative stress conditions. Consistent with a role for OxyR in oxidative stress resistance however, DrOxyR positively regulates the catalase gene, *katE* [51].

In species of *Actinobacteria* that contain a functional OxyR protein, OxyR maintains a role in protection against H_2O_2. For instance, while the catalase gene, *catA*, is not regulated by OxyR in *Streptomyces* sp., overexpression of OxyR provides H_2O_2 resistance, partly by induction of *ahpCD* expression [52]. Similarly in *Mycobacterium marinum*, OxyR is required for induction of *ahpC* during H_2O_2 exposure [53]. Other species of *Mycobacterium*, however, possess non-functional OxyR proteins as a consequence of acquired *oxyR* mutations [54, 55]. Consistent with a non-functional OxyR protein, gene expression in *Mycobacterium tuberculosis* is unaltered by exposure to H_2O_2 [56]. Interestingly, a commonly used antituberculosis agent, isoniazid (INH), acts, at least in part, by the INH-mediated production of reactive oxygen species through peroxidase activity [57]. Among clinical isolates of *Mycobacterium*, AhpC levels are correlated with INH resistance [58], with repression of *ahpC* in INH-sensitive strains *M. tuberculosis* and *Mycobacterium bovis* [59]. Additionally, an *ahpC* mutant of the naturally INH-resistant *M. smegmatis* dramatically increases sensitivity to INH [60]. An oxidative stress mechanism has similarly been reported as a common mechanism of killing by bactericidal antibiotics [61].

OxyR regulon function is conserved in bacteria. Although homologous relationships of regulon members are not explicitly considered in this review, catalases (Figure 4.2) and/or alkyl hydroperoxide reductases are commonly under OxyR control in highly divergent species. Of course, conserved OxyR-dependent regulation does not extend to *E. coli*'s entire OxyR regulon, as *E. coli* has 28 genes induced by OxyR (Table 4.1), while *N. gonorrhoeae* has just three [38]. Environmental niche may play a role in selection of OxyR regulon function. For example, OxyR of *Bacteroidetes*, with a locked-on conformation, likely provides aerotolerance to these anaerobic species that may be frequently exposed to atmospheric oxygen [46]. As well, the existence of two OxyR homologs in *D. radiodurans*, a species with extreme tolerance to oxidative stress [62], may ensure that the cell retains some OxyR-mediated resistance to H_2O_2, even if one homolog is lost through mutation. Perhaps a common OxyR activation mechanism, in particular the formation of a disulfide bond when oxidized by H_2O_2 [20, 39], and a ubiquitous need for H_2O_2 detoxification has ensured the functional conservation of the OxyR regulon in many bacterial species.

4.4.3 SoxRS, principal regulator of the superoxide radical response

SoxRS is a regulator of oxidative stress response genes in *E. coli*, including the manganese-superoxide dismutase gene, *sodA*. The *soxR* and *soxS* genes are adjacent and divergently transcribed in *E. coli* [63]. Proteins encoded by these genes constitute a two-stage regulatory system [64], in which SoxR, when activated, induces the expression of *soxS*, which in turn regulates several genes important for responding to oxidative stress.

SoxR, for superoxide response, in *E. coli* is a regulator of resistance to a superoxide-generating agent, paraquat [65, 66]. Constitutive SoxR expression results in an increased expression of genes with oxidative stress response functions, while mutants carrying non-functional SoxR are highly sensitive to superoxide-generating agents [66]. Other agents that activate SoxR, and therefore the SoxRS regulon, include nitric oxide [67] and high levels of hydrogen peroxide [68]. SoxR, a homolog to the MerR family of proteins that bind Hg^{2+} [69], is a 17 kDa homodimer that requires two [2Fe-2S] clusters for 100-fold induction of *soxS* [70, 71]. While SoxR binds to the *soxS* promoter in both reduced and oxidized forms, only univalent oxidation of the [2Fe-2S] clusters of SoxR activates *soxS* expression and downstream regulon gene expression [70, 72]. Crystal structure analysis shows that the [2Fe-2S] clusters of oxidized SoxR are solvent-exposed, which creates an asymmetrically charged environment and a possible conformational change in SoxR [73]. When the spacing between the -10 and -35 regions of the *soxS* promoter is modified by site-specific deletions, SoxR no longer induces but instead represses *soxS* by impairing RNA polymerase binding, and basal *soxS* expression increases. This, along with the SoxR-*soxS* crystal structure [73], suggests that the spacer of the *soxS* promoter is restructured by oxidized SoxR to induce transcription by RNA polymerase [74]. Following amelioration of oxidative stress, SoxR is reduced by an unidentified NADPH-dependent SoxR reductase [75] and/or through the action of the *rsxABCDGE* (for reducer of SoxR) and *rseC* products [76] (Figure 4.1b).

SoxR is activated, directly or indirectly, by superoxide radicals [66, 77], but the extent of its activation in response to superoxide radicals is not clear [78-80]. Gu and Imlay (2011) recently found that SoxR oxidation (active state) is due to direct oxidation by redox-cycling agents themselves [78], which is in agreement with earlier work showing that SoxR does not directly sense superoxide [81]. SoxR can be oxidized by redox-cycling drugs because (a) SoxR is not active in superoxide dismutase mutants (where superoxide levels are elevated), (b) SoxR activity is not reduced when superoxide dismutase is overexpressed, and (c) SoxR can be activated in the presence of redox-cycling drugs anaerobically [78]. On the other hand, Liochev and Fridovich (1991) found that expression of SoxRS-dependent glucose-6-phosphate dehydrogenase is higher in a control strain compared to *E. coli* overexpressing MnSOD when exposed to paraquat, suggesting that paraquat-generated superoxide, not paraquat itself, triggers SoxR activation [82]. As well, SoxR oxidation by redox-cycling drugs alone does not fully account for expression of the SoxRS regulon in superoxide dismutase mutants in the absence of redox-cycling drugs [79]. A *sodAsodB* double mutant, which has high intracellular superoxide levels, expresses the SoxRS regulon member, fumarase C, when grown aerobically [77]. SoxR may thus be activated by multiple factors. Further studies on the nature of SoxRS activation are needed to elucidate its role in protection against superoxide.

Following oxidized-SoxR mediated transcription of *soxS*, SoxS, in turn, modulates the expression of over 100 genes [83]. Several SoxRS-dependent genes have a role in the oxidative stress response, including *sodA* (MnSOD) for the dismutation of superoxide [6], *nfo* (endonuclease IV) for DNA repair [84], and *yggX* for protection of iron-sulfur proteins [85]. DNase I footprinting and gel shift assays with purified SoxS protein indicate that SoxS binds directly to at least *sodA*, *micF*, *nfo*, *zwf*, and *waaY* promoters [86, 87], and identification of more promoters that are directly bound by SoxS may be expected in future studies. An optimal "soxbox," the DNA consensus sequence that SoxS binds [88], was determined through systematic mutagenesis of *zwf* and *fpr* promoters to be 5'- AnVGCACWWWnKRHCAAAHn -3', where *n* = A, C, G, T; V = A, C, G; W = A, T; K = G, T; R = A, G; and H = A, C, T [89].

4.4.4 Functional conservation of the SoxRS regulon in *Proteobacteria*

Unlike OxyR homologs, SoxR homologs have been identified in gamma- and alpha-proteobacteria, but not in more distantly related phyla, such as *Bacteroidetes* [90] and *Actinobacteria* [56]. The ability to tolerate oxidative stress through increased SoxRS expression is weakly conserved even among *E. coli* strains, as the *soxR* and *soxS* alleles in clinical *E. coli* isolates are polymorphic [91]. Of six clinical *E. coli* isolates that overexpress *soxS*, half carry single point mutations in *soxR*, while the remaining constitutive *soxS*-expressing isolates are due to *soxRS*-independent mechanism(s) [91]. In addition to oxidative stress response, overexpression of SoxRS confers multiple drug resistance [91, 92].

Salmonella and *Klebsiella*, genera of Enterobacteriales in gammaproteobacteria, have similar SoxRS regulon function to *E. coli* SoxRS. Indeed, *E. coli* and *Salmonella enterica* serovar Typhimurium SoxS proteins are 95% identical [93], and *S. enterica* SoxRS regulon includes similar genes, such as *sodA* and *fpr* (NADPH:ferredoxin oxidoreductase with 80% identity to *E. coli fpr*) [94]. Although SoxRS also has a role in multiple drug resistance for *E. coli* and *Klebsiella pneumoniae* [95], it is unclear if SoxRS has the same role in *Salmonella* sp. [96, 97]. Precise knockouts of *soxR* and *soxS* of serovar Typhimurium do not have increased sensitivity to quinolones, fluoroquinolones, phenicols, and tetracycline [96, 98]. However, a constitutive *soxRS* mutant of *S. enterica* serovar Typhimurium ATCC 14028 was found to have greater resistance to quinolones [97], and heightened SoxR expression in a selected mutant of *Salmonella* Virchow had higher efflux activity, consistent with overexpression of efflux pump AcrAB and multiple drug resistance [99]. Therefore, despite the similarity of *soxRS* genomic region to *E. coli*, *Salmonella* SoxRS function in multiple drug resistance is species-specific.

The SoxR homolog in *Vibrio* sp. has 55% amino acid identity to *E. coli* SoxR [100]. When exposed to the superoxide generator menadione, SoxR-dependent genes are induced in *V. harveyi*, including a MnSOD and glucose-6-phosphate dehydrogenase [100]. The SoxR regulon of *Vibrio* sp. also includes *cadBA*, which encode for lysine-cadaverine antiporter and lysine decarboxylase, respectively, and cadaverine, in addition to neutralizing external media, likely scavenges superoxide [101].

In *Pseudomonas aeruginosa*, the *soxR* gene has 62% identity to *E. coli soxR*, yet a *soxS* homolog is absent. The adjacent genomic location of *soxR* in *P. aeruginosa* carries a hypothetical protein, and SoxR similarly binds to the *soxS*-like promoter and initiates transcription [102]. The SoxR regulon of *Pseudomonas* sp. can be induced in a superoxide-independent manner [103] and does not confer enhanced resistance to paraquat or H_2O_2 exposure [104]. Key SoxRS-dependent genes in *E. coli* are not regulated by SoxR in *Pseudomonas putida*, including *fpr* and *sodA* [105]. Instead, genes that are induced by SoxR under superoxide stress are either efflux pump genes, such as *mexGHI-opmD*, or of unknown function [104]. SoxR of *Pseudomonas* sp. likely functions in quorum sensing [103, 104] and biofilm formation [106]. Indeed, the *mexGHI-opmD* operon may have a specific role in quorum sensing homeostasis, since disruption of this efflux pump by either *mexI* or *opmD* mutations results in reduced production of acylhomoserine lactone and in quorum sensing-impaired phenotypes [107].

Agrobacterium tumefaciens of alphaproteobacteria also contains a SoxR homolog, which is activated by superoxide stress. Like *E. coli*, *A. tumefaciens soxR* mutants are more sensitive to superoxide generators, and SoxR directly regulates a manganese-iron superoxide dismutase, encoded by atu5152 [108]. Similar to the dual function regulation of OxyR, SoxR in a reduced form further acts to repress atu5152 [108]. As well, *A.*

tumefaciens carries three iron-containing superoxide dismutases: SodBI, SodBII, and SodBIII. While SodBI is expressed throughout growth and SodBIII is induced in stationary phase, SodBII is induced by SoxR in stationary phase [109], which suggests SoxR to be an important superoxide regulator in *A. tumefaciens*.

In *Bacteroidetes*, the anaerobic bacterium, *Porphyromonas gingivalis*, contains no *soxR* or *soxS* homologs [110]. In this species, *sod* is instead regulated by OxyR [90]. Because superoxide dismutase activity is essential for *P. gingivalis* aerotolerance [111], it has been suggested that superoxide radicals are formed when *P. gingivalis* is exposed to atmospheric oxygen and that OxyR can be subsequently triggered by the intracellular redox status, not peroxide, to induce *sod* [90]. This hypothesis, although speculative at this time, justifies OxyR regulation of *sod*. In *Actinobacteria*, *M. tuberculosis* SoxRS also responds differently from *E. coli* SoxRS to superoxide stress. Exposure of *M. tuberculosis* to menadione results in induction of seven genes, six of which are heat shock proteins [56].

The function of the SoxRS regulon in superoxide stress response is moderately conserved. SoxRS regulates superoxide dismutases in many gammaproteobacteria (*E. coli*, *Salmonella* sp., *K. pneumoniae*, and *Vibrio* sp.) and in at least one alphaproteobacterium (*A. tumefaciens*). In *Pseudomonas* sp. of the gammaproteobacteria, however, SoxRS does not regulate a superoxide stress response [104]. Interestingly, SoxRS function may be more diverse than response to superoxide stress alone, as SoxRS has a role in regulation of efflux pump genes in *Pseudomonas* sp. [104], *Salmonella* Virchow [99], and *K. pneumoniae* [95]. SoxRS may have evolved initially as a regulator of efflux pump genes and, only more recently in gammaproteobacteria, acquired a role in superoxide stress response. This regulatory diversification suggests that the SoxRS regulon, as compared to the OxyR regulon, may be under weaker selective pressure and is more readily adapted to environment.

4.4.5 RpoS, general stress response regulator

RpoS is a stationary phase and general stress response regulator [112]. RpoS, for RNA polymerase of stationary phase [113], is a 38 kDa protein that belongs to the sigma 70 factor family in bacteria [114].

Several of ~200 genes regulated by RpoS respond to oxidative stress [115, 116], and indeed, *rpoS* mutants are more susceptible to the lethal effects of exogenous H_2O_2 [117]. Unlike OxyR and SoxR that are activated by conformational changes due to oxidization, the induction of RpoS leads to an increased recruitment of RNA polymerase to RpoS-recognized promoters [118, 119]. Entering stationary phase, RpoS levels increase substantially, from below detection levels in exponential phase to over 1600 molecules per cell in stationary phase [120], and this increase in RpoS concentration, and the aid of additional transcription factors [120, 121], allows for RpoS to better compete with other sigma factors for core RNA polymerase and drive expression of stationary phase and stress-related genes [119]. It is important to note that low levels of RpoS in fast-growing cells also act as a regulator of gene expression but that the RpoS-regulated genes of low (exponential phase; no stress) and of high (stationary phase; stress) RpoS abundance is substantially different, with only 16% of RpoS-dependent genes in stationary phase also regulated by RpoS in exponential phase [122].

Recently, a toxin-antitoxin system, MqsR-MqsA, was determined to regulate *rpoS* levels during oxidative stress [123]. Mutants of *mqsRA* with an MqsA-expression plasmid have 4.5-fold less *rpoS* mRNA compared to a control strain when exposed to H_2O_2. It was determined that, by mutating a palindromic sequence in the *rpoS* promoter, MqsA no longer repressed expression from the promoter. Therefore, under normal growth conditions, MqsA binds to the palindromic sequence, 5'-**ACCT**TGC**AGGT**-3', preventing *rpoS* transcription [123]. Under oxidative stress however, Lon protease expression increases 8-fold [124], degrading MqsA and allowing for transcription [123].

RpoS regulated genes important for oxidative stress resistance include *dps* (DNA-binding protein), *katE* (hydroperoxidase II), *xthA* (exonuclease III) [125], and *sodC* (CuZnSOD) [126]. While *katE* contributes little to hydrogen peroxide resistance during exponential growth, it is the main form of catalase during stationary phase [26]. This suggests that OxyR activity decreases with a concomitant increase in RpoS activity, which would reduce unnecessary energy expenditure when nutrients are low and growth has slowed. As well, tight control of these overlapping regulons prevents redundant regulation of shared genes [12], since thirteen genes are regulated in common by OxyR and RpoS [127-130] (Table 4.1). In part, modulation of regulon expression occurs at the regulator level, where RpoS and OxyR, as well as SoxRS, control expression of the other. For instance, OxyR negatively regulates *rpoS* translation through OxyS binding of Hfq [12, 131]. For SoxRS, RpoS is thought to be a positive regulator of *soxS* expression

[132]. This regulation, however, was not confirmed in a time series microarray experiment [83], suggesting that more research is needed in this area.

The RpoS sigma factor is found in gamma-, beta- and delta-proteobacteria [36]. RpoS regulon composition has considerable species-specific modifications, as even closely related species, *E. coli* and *Pseudomonas aeruginosa*, have only 12 of 50 orthologous genes regulated in common by RpoS [36]. Still, in gammaproteobacteria, RpoS regulation of catalase is largely conserved, such as *katG* in *Vibrio vulnificus* [133] and *catB* in *P. putida* [134]. RpoS is also essential for maintaining a lower frequency of DNA mutations in long-term starved populations of *P. putida* by inducing catalase and superoxide dismutase expression [135]. For *Legionella pneumophila*, unlike other gammaproteobacteria, resistance to oxidative stress is not mediated by RpoS, despite a stationary phase-induced stress resistance and RpoS-dependent survival in protozoa [136]. In betaproteobacteria, RpoS mutants are more susceptible to oxidative stress [137, 138]. RpoS positively regulates *oxyR* [139], as it does in *E. coli* [140], and both RpoS and OxyR of *Burkholderia pseudomallei* independently induce *dpsA* during oxidative stress [139, 141]. Lastly, *Geobacter sulfurreducens*, a deltaproteobacterium, requires RpoS in stationary phase and during oxygen exposure [142]. Transcriptomic investigation reveals that RpoS regulates oxidative stress response genes, including those that encode for ferrodoxin, rubredoxin, desulfoferredoxin ferrous iron-binding protein, and superoxide dismutase [143].

An RpoS-like protein is also present in the spirochaete, *Borrelia* sp. [144]. Based on maximum-likelihood phylogeny and reciprocal best hits analysis, *Borrelia* "*rpoS*" is of distinct evolutionary origin to *rpoS* of *Proteobacteria* [36]. The RpoS-like sigma factor [36] does not have a general stress response function and is not required for oxidative stress resistance [145]. RpoS may play a peripheral role in oxidative homeostasis, however, as BosR, for *B*<u>*B*</u>*orrelia* <u>o</u>xidative <u>s</u>tress <u>r</u>egulator [146], likely interacts with the RpoS-RpoN-Rrp2 regulatory pathway and BosR mutants are more sensitive to H_2O_2 [147].

4.5 <u>'Fur'-ther regulators of oxidative stress response</u>

Free intracellular iron is easily oxidized by H_2O_2 to form the reactive hydroxyl radical via the Fenton reaction (Eq. (2)) [1, 4]. Therefore, homeostatic control of free intracellular iron levels is important for minimizing oxidative stress. The Fur repressor is the principal regulator of iron homeostasis and is activated by binding with Fe(II), as well as Fe(III), *in vivo* [148]. Subsequently, Fur binds to DNA at a Fur box to repress iron acquisition genes [149]. At least 36 operons are known to be modulated by Fe(II)-Fur complex in *E. coli* K-12 [150]. In the absence of iron, Fur is inactivated, which leads to de-repression of the regulon. The SoxRS regulon gene, *sodA* (MnSOD), is negatively regulated by Fur [151]. MnSOD repression in the presence of excess iron may help to reduce the flux of hydroxyl radicals, as both SOD, through dismutation of H_2O_2, and iron, through the Fenton reaction, would contribute to hydroxyl radical abundance [152]. The surprising positive regulation by Fur of the iron-containing superoxide dismutase gene, *sodB*, is now understood to be through Fur-mediated repression of a small RNA, RyhB [151, 153]. RyhB directly regulates 18 operons, with functions in not only oxidative stress through the induction of *sodB*, but also in the TCA cycle, glycolysis, iron-sulfur cluster formation, and respiration [154]. Fur also positively affects HPI activity, which may be mediated through RyhB activity [155], although this result was not corroborated in RyhB-microarray experiments [154].

Iron homeostasis and oxidative stress response are connected through regulatory interactions [156]. OxyR and SoxRS, with OxyR binding to the *fur* promoter and SoxS binding to the upstream *fldA* promoter, induce Fur expression [157]. Fur activity is diminished in H_2O_2-containing environments, and OxyR induction of Fur may help alleviate the loss of Fur activity by oxidation [158]. As well, Fur activity is also reduced by NO, possibly through nitrosylation of Fe(II), and this inactivation allows for the expression of NO-detoxifying flavohemoglobin gene, *hmp* [159]. Regulation by RpoS and Fur are also linked, as activities of both proteins are modulated by Crl [160]. Fur binding sequences are present in many Gram-negative and some Gram-positive bacteria [161, 162]. The Fur family of proteins regulates homeostasis of other metals, such as Zur that regulates zinc transport [163].

Other regulators are induced by oxidative stress, including NorR for mitigation of NO [164] and IscR for formation of Fe-S clusters [165]. Under nitrosative stress, the mononuclear non-haem iron center of NorR binds NO, stimulating ATPase activity and transcription via RpoN of *norVW* [166, 167]. NorV and NorW function in NO reduction and detoxification [164]. NorR functional regulation of enzymes for NO detoxification is likely conserved in gammaproteobacteria, including *Pseudomonas* and *Vibrio* [168]. IscR regulates at least 20 operons, including Fe-S cluster assembly operons *sufABCDSE* [169] and *iscRSUA* [170]. In the presence of H_2O_2, the [2Fe-2S] cluster of IscR is oxidized, which leads to de-repression of the *isc* promoter and induction of the *suf* promoter [171]. OxyR and IscR equally induce the *sufA* operon in oxidative stress [171]. IscR binding sites have been identified in gamma- and beta-proteobacteria, as well as the alphaproteobacterium *Rickettsia* [169].

4.6 Concluding remarks

Oxidative stress responses coordinated by specific regulators ensure bacterial survival during episodic exposure to exogenous ROS or to ROS generated as a consequence of normal respiration. Oxidative stress regulators, such as OxyR and RpoS, can modulate expression of one another and, in some circumstances, control expression of the same regulon members. OxyR, present in *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, is likely of an earlier evolutionary origin than SoxRS and RpoS, which are present in *Proteobacteria* alone. It is remarkable that, despite the large genomic diversity of bacteria and the presence of other oxidative stress regulators in bacteria, such as PerR, *E. coli* regulators are functionally conserved in many bacterial species of diverse niches. Even in anaerobic species of *Bacteroidetes* where catalase homologs are absent, OxyR is nonetheless important for H_2O_2 tolerance [46, 47]. As well, although SoxRS functions as a regulator of efflux pump gene expression in *P. aeruginosa* [104], SoxRS commonly regulates superoxide dismutase [94, 100, 108]. SoxRS may have evolved originally as a regulator of efflux pump genes and later adapted as a superoxide response regulator. Because modification to transcriptional regulators is a rapid means of adaptation, as opposed to mutational selection of individual target genes, it is interesting that oxidative stress regulons of *E. coli* are conserved on a functional level in many bacterial species. Regulators of oxidative stress response genes may experience some level of positive selection due to the need for ROS detoxification in oxygen-rich environments.

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Table 4.1. Operons regulated by OxyR in *S. enterica* and *E. coli* K-12.

Operon	Function[a]	Reference[b]	Member of RpoS regulon[c]
Positive regulation by OxyR			
ahpCF	Alkyl hydroperoxide reductase	[13, 172]	
dps	Fe-binding and storage protein	[173]	x
dsbG	Periplasmic disulfide isomerase/thiol-disulphide oxidase	[174]	x
f497	Unknown	[130]	x
fpg	Formamidopyrimidine	[175]	
fur	DNA-binding transcriptional dual regulator	[157]	
gorA	Glutathione oxidoreductase	[13]	x
grxA	Glutaredoxin 1	[176]	
hcp	Hybrid-cluster [4Fe-2S-2O] protein in anaerobic terminal reductases	[177]	
hemH	Ferrochelatase	[165]	x
katG	Catalase/hydroperoxidase I	[13, 172]	x
oxyS	Regulatory sRNA	[12]	
mntH	Manganese/divalent cation transporter	[178]	
rcsC	Hybrid sensory kinase in two-component regulatory system with RcsB and YojN	[130]	
sufABCDSE	Fe-S cluster assembly protein; SufBCD complex; selenocysteine lyase; sulfur acceptor protein	[165]	x
trxB	Thioredoxin reductase	[175]	
trxC	Thioredoxin 2	[179]	
yaaA	Conserved protein	[165]	
yaiA	Predicted protein	[165]	x
ybjM	Predicted inner membrane protein	[165]	
yhjA	Predicted cytochrome *c* peroxidase	[180]	
yljA	Regulatory protein for ClpA substrate specificity	[165]	
Negative regulation by OxyR			
agn43	Antigen 43 (Ag43) phase-variable biofilm formation autotransporter	[181]	
fhuF	Ferric iron reductase involved in ferric hydroximate transport	[174]	
oxyR	DNA-binding transcriptional dual regulator	[9, 182]	
mom (ΦMu)	DNA modification	[182]	
stiA	Putative fimbrial subunit	[24]	
yaeH	Conserved protein	[165]	

Operon	Function[a]	Reference[b]	Member of RpoS regulon[c]
ydcH	Predicted protein	[165]	
ydeN	Conserved protein	[165]	
ytfK	Conserved protein	[165]	
uxuA	Mannonate hydrolase	[165]	
Other regulation by OxyR			
gtrABC[d] (ΦP22)	Bactoprenol-linked glucose translocase; bactoprenol glucosyl transferase; glucosyltransferase	[183]	
yfdI [e]	Predicted inner membrane protein	[174]	

[a] Function obtained for *E. coli* K-12 genes from GenProtEC (http://genprotec.mbl.edu/) (accessed October 13, 2011) [184], for *S. enterica* genes (*stiA* and *gtrABC*) from BioCyc (http://biocyc.org/) [185], and for *mom* gene from reference [186].

[b] References that identify operon under OxyR control.

[c] See references [127-130].

[d] Positive or negative OxyR regulation, depending on which of two regulatory regions OxyR binds.

[e] Unknown OxyR effect.

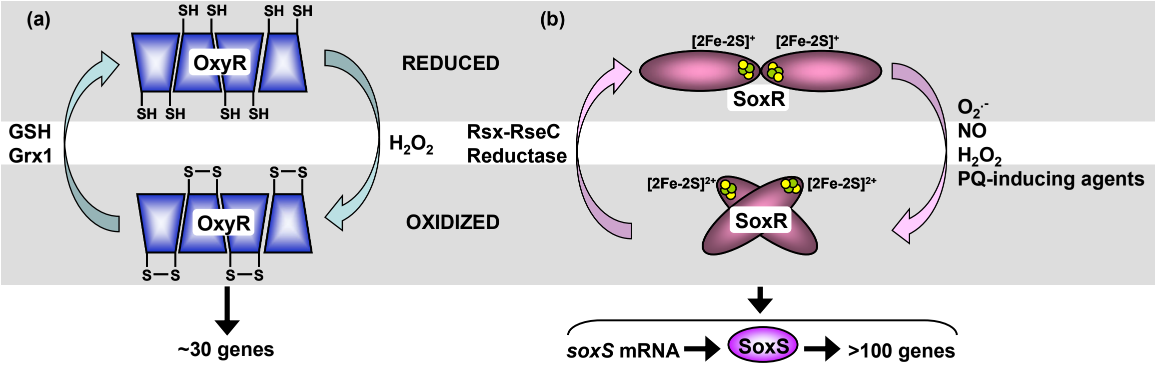
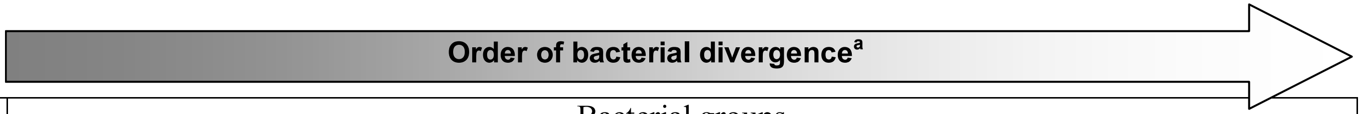


Figure 4.1. Oxidation/reduction schematic of the major oxidative stress regulators of *E. coli*, OxyR (a) and SoxR (b). Placement of disulfide bonds on OxyR after oxidation is based on Choi et al. 2001 [21]. Exposure of [2Fe-2S] clusters on SoxR after oxidation is based on Watanabe et al. 2008 [73]. See text for further details.



Regulation	Bacterial groups					
	Actinobacteria	*Deinococcus-Thermus*	*Bacteroidetes*	Alpha-proteobacteria	Beta-proteobacteria	Gamma-proteobacteria
+		*Deinococcus* [50, 51]	*Bacteroides* [49]	*Agrobacterium* [187] *Brucella* [188] *Caulobacter* [189] *Rhizobium* [190][b] *Rhodobacter* [191] *Sinorhizobium* [40]*	*Burkholderia* [192]* *Neisseria* [193]*	*Erwinia* [194] *Escherichia* [195] *Haemophilus* [31] *Moraxella* [32] *Pseudomonas* [33] *Salmonella* [13] *Vibrio* [196] *Yersinia* [30] *Xanthomonas* [197, 198]
-				*Acetobacter* [199][c] *Sinorhizobium* [40]*	*Burkholderia* [192]* *Neisseria* [37]*	
None	*Streptomyces* [52] *Mycobacterium* [54, 200]			*Bradyrhizobium* [41]		*Legionella* [35][d]

[a] Order of bacterial divergence taken from Gupta & Griffiths 2002 [201].

[b] *katG* of *Rhizobium etli* is induced by H_2O_2, and the *katG* promoter has characteristic OxyR binding motifs.

[c] Catalase regulation by OxyR in *Acetobacter* was not tested under oxidative stress conditions.

[d] *Legionella pneumophila* OxyR_{LP} weakly binds *katG* promoter of *E. coli*.

*Positive regulation of catalase by oxidized OxyR; negative regulation of catalase by reduced OxyR.

Figure 4.2. OxyR regulation of catalase across bacterial species. Only bacterial groups for which OxyR regulation of catalase has been described are included. "+" regulation indicates OxyR positively regulates catalase; "-" regulation indicates OxyR negatively regulates catalase; and "None" regulation indicates OxyR is not known to regulate catalase in these species.

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CHAPTER 5:

Discussion

5.1 General discussion

Modification of regulatory networks, in addition to genetic content, contributes to bacterial diversity. Recently, it was proposed that regulatory networks are highly flexible, possibly providing organisms with the ability to adapt to the environment (Lozada-Chavez *et al*., 2006; Madan *et al*., 2006). The RpoS regulon, as a model of regulon development, supports the theory that regulatory networks are highly flexible and, to extend this theory, that environment is a determining factor in regulatory adaptation. RpoS and RpoS regulon composition have extensive species-specific modifications and are probably adapted with environmental selection. As determined in the present study, RpoS regulon composition is weakly conserved between closely related bacterial species (*E. coli* and *P. aeruginosa*) (Chapter 2), RpoS mutants of *E. coli* are naturally present in the environment, and a prototypical RpoS regulon member, *katE*, is differentially expressed among environmental *E. coli* isolates (Chapter 3). As well, RpoS mutants can be readily selected from environmental *E. coli* isolates under nutrient poor growth conditions (Chapter 3). The conservation of RpoS in many γ-, β-, and δ-proteobacterial species (Chapter 2), as well as the regulon's functional conservation (Chapter 4), suggests that RpoS is advantageous in many bacterial niches. Likewise, the oxidative stress regulons of *E. coli*, OxyR and SoxRS, have considerable functional conservation with flexible regulon composition (Chapter 4). The extent of regulatory flexibility is linked to its selective advantage in a given environment. In this discussion, I will expand on factors underlying the organizational flexibility of the RpoS model system, detailing how environment and evolutionary selection drive expression and composition of RpoS and oxidative stress regulons.

5.2 Evolution of the RpoS regulon: RpoS conservation and regulon flexibility

RpoS is present in γ-, β-, and δ-proteobacteria, as determined by phylogenetic (Figure 2.1) and reciprocal best hits analyses. Considering the divergence of bacteria as determined using indels (Gupta, 2000), a proposed evolutionary development of RpoS is provided in Figure 2.7. Here, RpoS presence in γ-, β-, and δ-proteobacteria is explained by vertical inheritance from a common ancestor. RpoS likely originated from an *rpoD* duplication event before the emergence of β- and δ-proteobacteria and was conserved through speciation to γ-proteobacteria but lost in α-proteobacteria. One possible exception to the proposed vertical inheritance of RpoS is the presence of an annotated RpoS protein in the distantly related spirochaete, *Borrelia* sp. (Elias *et al.*, 2000). However, annotated RpoS of *Borrelia burgdorferi* is only 33.5% identical to RpoS of *E. coli* K-12 and clusters separately from proteobacterial RpoS in a maximum-likelihood phylogeny (Figure 2.1). Therefore, the annotated RpoS sigma factor of *Borrelia* sp. is from an independent evolutionary event. Of course, rare horizontal transfer events may have occurred in other species overlooked in the maximum-likelihood phylogeny. Reciprocal best hits analysis of all sequenced bacterial genomes available from NCBI's RefSeq database (January 11, 2010) confirmed that RpoS is absent in species outside of γ-, β-, and δ-proteobacteria.

The vertical descent of RpoS agrees with the reported slow evolution of global regulatory factors (Price *et al.*, 2008) and the characteristic conservation of sigma factors (Perez-Rueda *et al.*, 2009). A comparison of transcription factor orthologs with the number of genes they regulate in *E. coli* and *Shewanella oneidensis* indicates that transcription factors with more regulatory interactions have higher sequence conservation (Price *et al.*, 2008). A similar correlation is observed for transcriptional regulators of γ-proteobacteria (Rajewsky *et al.*, 2002). Therefore, global regulatory factors evolve more slowly than non-global regulatory factors. As well, sigma factors are 10× less abundant than other transcription factors due to possible constrained evolutionary divergence (Perez-Rueda *et al.*, 2009). Sigma factors have a limited number of regulatory interactions (core RNA polymerase and promoter DNA) and, consequently, limited locations for mutational adaptation (Perez-Rueda *et al.*, 2009). Therefore based on results of this study and those of other studies, duplication and divergence or horizontal transfer of sigma factors, such as RpoS, is likely minimal.

While there is no evidence to support the horizontal transfer of RpoS across species, the genomic region between *rpoS* and the methyl-directed mismatch repair gene, *mutS*, is frequently transferred among strains of natural *E. coli* (Brown *et al.*, 2001). As a result, the *rpoS-mutS* region is highly polymorphic and of dissimilar genomic length, such as a 6.1 kb sequence in *E. coli* K-12 and a 2.9 kb sequence in *E. coli* O157:H7 (LeClerc *et al.*, 1999), and has been termed a "recombinational hot spot" in *E. coli* (Brown *et al.*, 2001). The transfer of *rpoS* with the *rpoS-mutS* intergenic region is not known. While *rpoS* transfer within species or between closely related species cannot be ruled out, this study did not identify any incidences of *rpoS* transfer to species outside of γ-, β-, and δ-proteobacteria. The transfer of RpoS without its cognate regulon may not provide a selective advantage and, therefore, would be lost through deletion. Although unlikely for RpoS, horizontal transfer may have occurred for other bacterial sigma factors. For example, a group one sigma factor in the spirochaete *Leptospira borgpetersenii*, has uncharacteristically low G/C content, possibly as a result of transfer from a low G/C, Gram-positive species (Gruber & Bryant, 1997).

While RpoS is conserved in γ-, β-, and δ-proteobacteria, RpoS regulon composition is highly flexible. Regulon flexibility may be attributed to both genomic divergence and regulatory rewiring. *E. coli* and *P. aeruginosa* are within the gammaproteobacterial class based on 16s rRNA, yet these species have unique bacterial niches and significant genomic differences. In particular, *E. coli* is commonly present in the gastrointestinal tract of humans and many warm-blooded animals, and *P. aeruginosa* is ubiquitous in aquatic and soil habitats. *P. aeruginosa* is found in diverse environments because its genome, while largely conserved among strains, carries regions of plasticity that allow environmental adaptation (Mathee *et al.*, 2008). G/C content of *E. coli* and *P. aeruginosa*, 50.8% (Blattner *et al.*, 1997) and 66.6% (Stover *et al.*, 2000) respectively, also demonstrates genomic divergence. Therefore, it is not surprising that only 50 genes of 186 RpoS-regulated in *E. coli* have orthologs in *P. aeruginosa*. As for regulatory conservation, of the 50 orthologous genes that are regulated by RpoS in *E. coli*, 12 genes are commonly regulated by RpoS in *P. aeruginosa* (Table 2.2). A central role for the conserved genes is elusive because many of these genes are of unknown or poorly defined function. In addition, these genes are induced under different stress conditions, such as phosphate starvation (*psiF*) (Metcalf *et al.*, 1990) or osmotic stress (*ygaU*) (Weber *et al.*, 2006). Flexibility of RpoS regulon composition may be the result of environmental selection in different bacterial habitats.

Flexibility of regulon composition, as seen in the RpoS regulon, is similarly characteristic of other regulons (Erill *et al.*, 2007; Monsieurs *et al.*, 2005), which supports the use of RpoS as a model for regulon development. For example, the PhoPQ regulons in *E. coli* and *Salmonella enterica* serovar Typhimurium, responsible for the expression of virulence genes in Mg^{2+} starvation conditions, have ~200 highly PhoPQ-dependent genes with many lineage-specific modifications (Monsieurs *et al.*, 2005). PhoP in *E. coli* and *S. enterica*, regulates only 13 operons in common (Monsieurs *et al.*, 2005), and among ten species of Enterobacteriaceae, only two regulatory units, *phoPQ* and *slyB*, are conserved under PhoP regulation (Perez *et al.*, 2009). At a regulatory level, genes are recruited or lost to the regulon by mutations in the regulator or the promoter recognition sequence.

Because promoter sequences recognized by RpoS are variable, microarray data for *E. coli* and *S. enterica* was used in the present study to define the RpoS regulon, as opposed to a motif search for RpoS recognition sequences. RpoS regulon expression, however, is dependent on RpoS recognition of promoters and activity of accessory transcription factors. As an example, the RpoS-dependent *yciGFE* operon is present in both *S. enterica* and *E. coli*, but expression of *yciGFE* in *E. coli* is much lower (Beraud *et al.*, 2010). The upstream promoter sequence of *E. coli yciGFE*, unlike in *S. enterica*, allows for repression by the histone-like protein, H-NS (Beraud *et al.*, 2010). Despite some promoter dissimilarity, the promoter consensus sequence for the RpoS regulons of *E. coli* and of *P. aeruginosa* is analogous. *E. coli* RpoS recognizes the –10 motif, "CTATACT" (Typas *et al.*, 2007b), and two defined RpoS-dependent promoters in *P. aeruginosa*, *cheY2* and *mcpA*, have similar –10 sequences, namely "CTTTACT" and "CTAGTTT," respectively (Schuster *et al.*, 2004). As well, region 2 of the RpoS protein, which recognizes the –10 motif, is highly conserved in *E. coli* and in *P. aeruginosa* (Tanaka & Takahashi, 1994). Although not considered in the present study due to promoter variability, co-evolution of RpoS and the regulon necessarily requires adaptation of both RpoS and promoter sequences.

Conservation of RpoS and RpoS regulon function across species suggests that this regulon provides a selective advantage in many bacterial niches. How then do species without RpoS survive in stationary phase and stress? In α-proteobacteria, which do not contain RpoS, SkgA is a regulator of the oxidative stress response. Although not a global regulator of stationary phase, an *skgA* mutant expresses 70% less *katG* and is more susceptible to H_2O_2 toxicity (Rava *et al.*, 1999). As well, in Gram-positive species such as *Bacillus subtilis*, the SigB regulator is functionally similar to RpoS and regulates several homologous genes of the RpoS regulon (Hengge-Aronis, 2000). RpoS and SigB are present in discrete bacterial groups, with the exception of *Mycobacterium* sp. (Mittenhuber, 2002). Following publication of chapter 2 of this thesis, a separate study was published that compared conservation of the RpoS regulons of *E. coli* K-12, *Geobacter sulfurreducens* (δ-proteobacterium), *Borrelia burgdorferi* (spirochaete), and the SigB regulon of *Bacillus subtilis* (firmicute; Gram-positive) (Santos-Zavaleta *et al.*, 2011). Santos-Zavaleta *et al.* (2011) also found that these regulons have considerable species-specific differences. While RpoS is conserved in many proteobacterial species, regulon composition is highly flexible. Environmental adaptation is likely a principal means for diversifying the RpoS regulon and is seen even among strains of the same species.

5.3 Adaptation of environmental *E. coli* due to changes in RpoS regulon expression

RpoS regulon expression is dramatically affected by acquisition of deleterious mutations in the *rpoS* gene. Although RpoS increases cell survival in stationary phase and stress, the loss of RpoS increases expression of genes required for metabolism (Patten *et al.*, 2004), and therefore, *rpoS* mutations provide a selective advantage in certain conditions, such as a low nutrient environment. Among 2,040 environmental *E. coli* isolates, which are subject to diverse environmental pressures, RpoS mutants comprise 0.3% (Table 3.3). RpoS mutants in environmental *E. coli* populations suggest that some natural environments select for *rpoS* mutations. RpoS mutants have also been identified among laboratory (Atlung *et al.*, 2002) and pathogenic (Dong *et al.*, 2009) *E. coli* strains. In a standard reference collection of *E. coli* composed of genetically diverse isolates (ECOR collection) (Ochman & Selander, 1984), 29% of strains carry deleterious *rpoS* mutations (Ferenci *et al.*, 2011). The higher frequency of RpoS mutants in the ECOR collection might be expected because of genetic diversity and accumulation of *rpoS* mutations during long-term storage.

In the laboratory, RpoS mutants are selected from laboratory and pathogenic *E. coli* when grown on poor carbon or in low nutrients. Loss of RpoS allows for the increased expression of genes under other sigma factor regulation, such as RpoD-dependent genes needed for growth (Farewell *et al.*, 1998). Therefore, RpoS mutants of laboratory (Chen *et al.*, 2004) and pathogenic (Dong *et al.*, 2009) *E. coli* have a selective advantage over wild type when grown on the poor carbon source, succinate. As well, in nutrient-limited growth, RpoS mutants take over wild type culture within ten generations (Notley-McRobb *et al.*, 2002). The ability to select for RpoS mutants from environmental *E. coli* isolates grown with succinate as the sole carbon source (Table 3.5) indicates that natural environments can similarly select for RpoS mutants in environmental strains. RpoS mutants of environmental *E. coli*, whether selected in the laboratory or isolated from the environment, also have characteristic growth advantage on poor carbon (Table 3.6) and reduced stress resistance (Figure 3.5) compared to wild type. Natural environments that select for RpoS mutants could not be determined from the present study, since RpoS mutants were identified from different sources (beach sand or sewage). *E. coli* thriving in the environment is, however, exposed to diverse conditions, which may select for adaptation of RpoS and the RpoS regulon.

The natural habitat of *E. coli* is in the gastrointestinal tract and, as a nutrient-rich environment, likely ensures conservation of RpoS function. On the other hand, some environmental conditions outside the host may preferentially select for RpoS mutants. After inoculation into stream water or soil, *E. coli* O157:H7, a pathogenic *E. coli* strain, decreased in viability after three days in water and no significant decrease was detected after 30 days in soil (Duffitt *et al.*, 2011). Long-term persistence in sub-optimal conditions may select for beneficial mutations. Indeed, *E. coli* isolated from soil has 0.8% genetic differences, as determined through multilocus sequencing, compared to *E. coli* isolated from fecal matter (Bergholz *et al.*, 2011), and naturalized soil isolates are not only able to persist but multiply in soil (Ishii *et al.*, 2006). Long-term growth, possibly under nutrient-limitation, may select for RpoS mutants.

Selection of *rpoS* mutations is a rapid and global means of regulatory adaptation. From Chapter 2 of this thesis, regulon composition between species is also highly flexible. Changes to regulon composition, while not of a global impact, allows the cell to fine-tune to the environment. This is perhaps why regulon flexibility is observable even among environmental *E. coli* isolates. Among these isolates, *katE* is differentially dependent on RpoS for expression (Figure 3.4). Isolates CCF06, ECC07, and ECH01 from beach water, ABB10 from untreated combined sewer overflow, and ABH11 from beach sand contain a functional RpoS protein, as indicated by expression of both RpoS and RpoS-dependent AppA, but do not express functional KatE. The mechanism for loss of KatE activity, whether by *katE* mutation, loss of RpoS regulation, or mutation of another transcription factor, is not known. As well, although RpoS and AppA in isolate ECF11 (Canada Goose dropping) are expressed to similar levels as the wild type control, KatE activity is significantly reduced. These results suggest that RpoS regulon composition is driven by *rpoS* mutation, as well as regulatory modulation, as selected by environment.

5.4 Lessons from the RpoS regulon: Do they apply to OxyR and SoxRS oxidative stress regulons?

Like the RpoS regulon, other regulons, such as the LexA (Erill *et al.*, 2007) and PhoPQ regulons (Monsieurs *et al.*, 2005), are flexible across species. The literature review (Chapter 4) on oxidative stress regulons of *E. coli* also supports regulator conservation with regulon flexibility. The review establishes that OxyR is present in divergent species, from proteobacteria to spirochaetes, yet regulon composition is highly modified, as OxyR regulates almost 40 genes in *E. coli* (Table 4.1) and only three genes in the β-proteobacterium *Neisseria gonorrhoeae* (Seib *et al.*, 2007). Despite regulon changes, OxyR is maintained as an oxidative stress response regulator in most species.

The *soxRS* genes are present in only proteobacteria, and the *soxRS* alleles are also highly polymorphic among clinical *E. coli* isolates (Koutsolioutsou *et al.*, 2005). Unlike the RpoS regulon, SoxRS regulon function is weakly conserved. While SoxRS regulates genes that respond to superoxide stress in many species, such as *E. coli* (Greenberg *et al.*, 1990) and the α-proteobacterium *Agrobacterium tumefaciens* (Eiamphungporn *et al.*, 2006), SoxRS regulates efflux pump genes in *P. aeruginosa* (Palma *et al.*, 2005), *S. enterica* (Kehrenberg *et al.*, 2009), and *Klebsiella pneumoniae* (Bialek-Davenet *et al.*, 2011). The SoxRS regulon may be an example of a regulon that has adapted without strong positive selection. Because superoxide is a weak oxidant and not a significant cause of oxidative damage in the cell (Imlay, 2003), conservation of a coordinated regulatory response to superoxide stress may be unnecessary in some species.

5.5 Concluding remarks

This thesis provides strong support for regulon flexibility as a result of mutation and environmental selection. While RpoS is conserved in γ-, β-, and δ-proteobacteria, the RpoS regulon is flexible, as even closely related species have extensive regulon composition differences. Among environmental *E. coli* isolates, environmental selection is a key factor in RpoS and RpoS regulon diversification, and rapid adaptation is possible by selection of RpoS mutants. Regulon flexibility is similarly observed in oxidative stress regulons, OxyR and SoxRS. As well despite composition flexibility, the RpoS and OxyR regulons are functionally conserved, while the SoxRS regulon function has diverged. This thesis indicates that the extent of regulon flexibility and of functional conservation is linked to the strength of environmental selection. Therefore, regulon adaptation must be considered in the context of environment.

Research that may be of future interest includes analysis of regulatory conservation for essential versus nonessential genes. RpoS regulates genes that are required in stationary phase and stress survival but are nonessential for growth phase in optimal laboratory conditions. Therefore, selection for RpoS regulon genes and for RpoS regulation may be weak. As more genomic data and regulatory pathways are compiled, it may be feasible to determine if regulation for essential genes is more highly conserved than regulation for nonessential genes due to the necessity of essential gene expression. Another element in RpoS regulon development that remains to be investigated is the role of promoter mutations. Because RpoS and RpoD promoter recognition sites are similar and because RpoS tolerates deviations from the promoter consensus sequence (Typas *et* *al*., 2007b), it is a challenge to characterize promoter mutations that result in gene recruitment or loss from the RpoS regulon. However, studies on the strength of RpoS recognition for specific promoter sequences, such as Miksch *et al*. (2005), may provide the necessary information to predict the effect of promoter changes on regulon composition. A comparison of RpoS regulon composition between strains of one species, e.g., environmental *E. coli* isolates, may also identify promoter mutations that mediate RpoS regulation.

The RpoS regulon is modulated among environmental *E. coli* isolates by environmental selection. RpoS mutants are present among environmental *E. coli*, but a natural selective condition for RpoS mutants is unknown. Important future research on environmental *E. coli* would be to identify natural conditions that select for, or allow for persistence of, RpoS mutants. For this purpose, it may be preferable to use *E. coli* that is naturalized to a given environment, e.g., sand, in the laboratory, since *E. coli* from the environment may be recent deposits. An extension of this work would be to examine RpoS regulon plasticity among naturalized *E. coli* isolates. Lastly, bacteria exist in the environment predominantly as biofilm (Costerton *et al*., 1995), and RpoS is implicated in biofilm production of laboratory *E. coli* (Ito *et al*., 2008). Therefore, it may be of future interest to determine if RpoS is also necessary for environmental *E. coli* biofilm, possibly the primary form of *E. coli* in nature. Continued research on RpoS function and RpoS regulon modulation will be useful to further understanding of regulon evolution and adaptation of bacteria to environmental conditions.

5.6 References for Introduction and Discussion

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CHAPTER 6: APPENDIX

Appendix A. Reciprocal best hits of *E. coli* RpoS to NCBI's RefSeq database using an *E*-value threshold of 1e-70. Data is organized from the most to the least significant reciprocal best hit.

gi Number	Species	Class
16130648	Escherichia coli str. K-12 substr. MG1655	Gamma-proteobacteria
170082316	Escherichia coli str. K-12 substr. DH10B	Gamma-proteobacteria
238901878	Escherichia coli BW2952	Gamma-proteobacteria
15832849	Escherichia coli O157:H7 str. Sakai	Gamma-proteobacteria
74313307	Shigella sonnei Ss046	Gamma-proteobacteria
110642882	Escherichia coli 536	Gamma-proteobacteria
110806628	Shigella flexneri 5 str. 8401	Gamma-proteobacteria
157157336	Escherichia coli E24377A	Gamma-proteobacteria
157162189	Escherichia coli HS	Gamma-proteobacteria
168749933	Escherichia coli O157:H7 str. EC4113	Gamma-proteobacteria
168755487	Escherichia coli O157:H7 str. EC4401	Gamma-proteobacteria
168778725	Escherichia coli O157:H7 str. EC4076	Gamma-proteobacteria
168787997	Escherichia coli O157:H7 str. EC869	Gamma-proteobacteria
170019013	Escherichia coli ATCC 8739	Gamma-proteobacteria
170684160	Escherichia coli SMS-3-5	Gamma-proteobacteria
191166775	Escherichia coli B7A	Gamma-proteobacteria
191171134	Escherichia coli F11	Gamma-proteobacteria
193065048	Escherichia coli E22	Gamma-proteobacteria
193069671	Escherichia coli E110019	Gamma-proteobacteria
194431745	Shigella dysenteriae 1012	Gamma-proteobacteria
194438995	Escherichia coli 101-1	Gamma-proteobacteria
208812039	Escherichia coli O157:H7 str. EC4045	Gamma-proteobacteria
209395891	Escherichia coli O157:H7 str. EC4115	Gamma-proteobacteria
209920184	Escherichia coli SE11	Gamma-proteobacteria
217326648	Escherichia coli O157:H7 str. TW14588	Gamma-proteobacteria
218559734	Escherichia coli S88	Gamma-proteobacteria
218696339	Escherichia coli 55989	Gamma-proteobacteria
218706235	Escherichia coli UMN026	Gamma-proteobacteria
253772403	Escherichia coli BL21(DE3)	Gamma-proteobacteria
254162672	Escherichia coli B str. REL606	Gamma-proteobacteria
256019474	Shigella sp. D9	Gamma-proteobacteria
256024751	Escherichia sp. 4_1_40B	Gamma-proteobacteria
260845388	Escherichia coli O103:H2 str. 12009	Gamma-proteobacteria
260869420	Escherichia coli O111:H- str. 11128	Gamma-proteobacteria
261226035	Escherichia coli O157:H7 str. FRIK2000	Gamma-proteobacteria
261256708	Escherichia coli O157:H7 str. FRIK966	Gamma-proteobacteria
91212108	Escherichia coli UTI89	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
117624976	Escherichia coli APEC O1	Gamma-proteobacteria
227888284	Escherichia coli 83972	Gamma-proteobacteria
237706630	Escherichia sp. 3_2_53FAA	Gamma-proteobacteria
254037782	Escherichia sp. 1_1_43	Gamma-proteobacteria
283786707	Citrobacter rodentium ICC168	Gamma-proteobacteria
24114036	Shigella flexneri 2a str. 301	Gamma-proteobacteria
218701232	Escherichia coli IAI39	Gamma-proteobacteria
208821597	Escherichia coli O157:H7 str. EC4042	Gamma-proteobacteria
218690868	Escherichia coli ED1a	Gamma-proteobacteria
260856852	Escherichia coli O26:H11 str. 11368	Gamma-proteobacteria
82778108	Shigella dysenteriae Sd197	Gamma-proteobacteria
254794681	Escherichia coli O157:H7 str. TW14359	Gamma-proteobacteria
16761696	Salmonella enterica subsp. enterica serovar Typhi str. CT18	Gamma-proteobacteria
16766230	Salmonella typhimurium LT2	Gamma-proteobacteria
56414872	Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150	Gamma-proteobacteria
62181426	Salmonella enterica subsp. enterica serovar Choleraesuis str. SC-B67	Gamma-proteobacteria
157148284	Citrobacter koseri ATCC BAA-895	Gamma-proteobacteria
167550225	Salmonella enterica subsp. enterica serovar Saintpaul str. SARA29	Gamma-proteobacteria
168230998	Salmonella enterica subsp. enterica serovar Kentucky str. CDC 191	Gamma-proteobacteria
168235962	Salmonella enterica subsp. enterica serovar Schwarzengrund str. SL480	Gamma-proteobacteria
168242664	Salmonella enterica subsp. enterica serovar Heidelberg str. SL486	Gamma-proteobacteria
168261948	Salmonella enterica subsp. enterica serovar Hadar str. RI_05P066	Gamma-proteobacteria
168463936	Salmonella enterica subsp. enterica serovar Newport str. SL317	Gamma-proteobacteria
168820376	Salmonella enterica subsp. enterica serovar Weltevreden str. HI_N05-537	Gamma-proteobacteria
194442500	Salmonella enterica subsp. enterica serovar Newport str. SL254	Gamma-proteobacteria
194451079	Salmonella enterica subsp. enterica serovar Heidelberg str. SL476	Gamma-proteobacteria
194470798	Salmonella enterica subsp. enterica serovar Kentucky str. CVM29188	Gamma-proteobacteria
194738113	Salmonella enterica subsp. enterica serovar Schwarzengrund str. CVM19633	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
197263839	Salmonella enterica subsp. enterica serovar Saintpaul str. SARA23	Gamma-proteobacteria
197363800	Salmonella enterica subsp. enterica serovar Paratyphi A str. AKU_12601	Gamma-proteobacteria
198243199	Salmonella enterica subsp. enterica serovar Dublin str. CT_02021853	Gamma-proteobacteria
200388856	Salmonella enterica subsp. enterica serovar Virchow str. SL491	Gamma-proteobacteria
204928102	Salmonella enterica subsp. enterica serovar Javiana str. GA_MM04042433	Gamma-proteobacteria
207858184	Salmonella enterica subsp. enterica serovar Enteritidis str. P125109	Gamma-proteobacteria
213052392	Salmonella enterica subsp. enterica serovar Typhi str. E00-7866	Gamma-proteobacteria
213648321	Salmonella enterica subsp. enterica serovar Typhi str. J185	Gamma-proteobacteria
224584701	Salmonella enterica subsp. enterica serovar Paratyphi C strain RKS4594	Gamma-proteobacteria
237729723	Citrobacter sp. 30_2	Gamma-proteobacteria
238909692	Salmonella enterica subsp. enterica serovar Tennessee str. CDC07-0191	Gamma-proteobacteria
283835495	Citrobacter youngae ATCC 29220	Gamma-proteobacteria
15803258	Escherichia coli O157:H7 EDL933	Gamma-proteobacteria
82545186	Shigella boydii Sb227	Gamma-proteobacteria
205353865	Salmonella enterica subsp. enterica serovar Gallinarum str. 287/91	Gamma-proteobacteria
161502017	Salmonella enterica subsp. arizonae serovar 62:z4,z23:--	Gamma-proteobacteria
238896221	Klebsiella pneumoniae NTUH-K2044	Gamma-proteobacteria
168768834	Escherichia coli O157:H7 str. EC4486	Gamma-proteobacteria
167993163	Salmonella enterica subsp. enterica serovar 4,[5	Gamma-proteobacteria
152971625	Klebsiella pneumoniae subsp. pneumoniae MGH 78578	Gamma-proteobacteria
206580660	Klebsiella pneumoniae 342	Gamma-proteobacteria
262041489	Klebsiella pneumoniae subsp. rhinoscleromatis ATCC 13884	Gamma-proteobacteria
269915854	Klebsiella variicola At-22	Gamma-proteobacteria
260599105	Cronobacter turicensis	Gamma-proteobacteria
146312853	Enterobacter sp. 638	Gamma-proteobacteria
213975649	Salmonella enterica subsp. enterica serovar Typhi str. AG3	Gamma-proteobacteria
258638949	Pantoea sp. At-9b	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
213859421	Salmonella enterica subsp. enterica serovar Typhi str. M223	Gamma-proteobacteria
188534815	Erwinia tasmaniensis Et1/99	Gamma-proteobacteria
238754524	Yersinia ruckeri ATCC 29473	Gamma-proteobacteria
238797130	Yersinia mollaretii ATCC 43969	Gamma-proteobacteria
157369077	Serratia proteamaculans 568	Gamma-proteobacteria
213029652	Salmonella enterica subsp. enterica serovar Typhi str. 404ty	Gamma-proteobacteria
29143180	Salmonella enterica subsp. enterica serovar Typhi str. Ty2	Gamma-proteobacteria
238791265	Yersinia intermedia ATCC 29909	Gamma-proteobacteria
270264030	Serratia odorifera 4Rx13	Gamma-proteobacteria
238750591	Yersinia rohdei ATCC 43380	Gamma-proteobacteria
123441128	Yersinia enterocolitica subsp. enterocolitica 8081	Gamma-proteobacteria
238761664	Yersinia kristensenii ATCC 33638	Gamma-proteobacteria
238786006	Yersinia bercovieri ATCC 43970	Gamma-proteobacteria
238758207	Yersinia aldovae ATCC 35236	Gamma-proteobacteria
271501815	Dickeya dadantii Ech586	Gamma-proteobacteria
22124745	Yersinia pestis KIM	Gamma-proteobacteria
45440189	Yersinia pestis biovar Microtus str. 91001	Gamma-proteobacteria
108808779	Yersinia pestis Antiqua	Gamma-proteobacteria
108810903	Yersinia pestis Nepal516	Gamma-proteobacteria
145600262	Yersinia pestis Pestoides F	Gamma-proteobacteria
153997601	Yersinia pestis CA88-4125	Gamma-proteobacteria
162420853	Yersinia pestis Angola	Gamma-proteobacteria
165925871	Yersinia pestis biovar Orientalis str. F1991016	Gamma-proteobacteria
165936700	Yersinia pestis biovar Orientalis str. IP275	Gamma-proteobacteria
166010016	Yersinia pestis biovar Antiqua str. E1979001	Gamma-proteobacteria
166212931	Yersinia pestis biovar Antiqua str. B42003004	Gamma-proteobacteria
167398742	Yersinia pestis biovar Antiqua str. UG05-0454	Gamma-proteobacteria
167421704	Yersinia pestis biovar Orientalis str. MG05-1020	Gamma-proteobacteria
167423190	Yersinia pestis biovar Mediaevalis str. K1973002	Gamma-proteobacteria
218930373	Yersinia pestis CO92	Gamma-proteobacteria
229838982	Yersinia pestis biovar Orientalis str. PEXU2	Gamma-proteobacteria
229896462	Yersinia pestis Pestoides A	Gamma-proteobacteria
229899549	Yersinia pestis biovar Orientalis str. India 195	Gamma-proteobacteria
270489294	Yersinia pestis KIM D27	Gamma-proteobacteria
51595126	Yersinia pseudotuberculosis IP 32953	Gamma-proteobacteria
153948385	Yersinia pseudotuberculosis IP 31758	Gamma-proteobacteria
170025640	Yersinia pseudotuberculosis YPIII	Gamma-proteobacteria
186894139	Yersinia pseudotuberculosis PB1/+	Gamma-proteobacteria
251788478	Dickeya zeae Ech1591	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
195939453	Escherichia coli O157:H7 str. EC4024	Gamma-proteobacteria
253689710	Pectobacterium carotovorum subsp. carotovorum PC1	Gamma-proteobacteria
242238323	Dickeya dadantii Ech703	Gamma-proteobacteria
50122451	Pectobacterium atrosepticum SCRI1043	Gamma-proteobacteria
227114060	Pectobacterium carotovorum subsp. brasiliensis PBR1692	Gamma-proteobacteria
227327750	Pectobacterium carotovorum subsp. carotovorum WPP14	Gamma-proteobacteria
261822742	Pectobacterium wasabiae WPP163	Gamma-proteobacteria
213425678	Salmonella enterica subsp. enterica serovar Typhi str. E02-1180	Gamma-proteobacteria
89109528	Escherichia coli str. K-12 substr. W3110	Gamma-proteobacteria
218547743	Escherichia fergusonii ATCC 35469	Gamma-proteobacteria
269140216	Edwardsiella tarda EIB202	Gamma-proteobacteria
238921111	Edwardsiella ictaluri 93-146	Gamma-proteobacteria
213417289	Salmonella enterica subsp. enterica serovar Typhi str. E01-6750	Gamma-proteobacteria
238786819	Yersinia frederiksenii ATCC 33641	Gamma-proteobacteria
253988173	Photorhabdus asymbiotica	Gamma-proteobacteria
37524720	Photorhabdus luminescens subsp. laumondii TTO1	Gamma-proteobacteria
170765845	Escherichia albertii TW07627	Gamma-proteobacteria
259909441	Erwinia pyrifoliae Ep1/96	Gamma-proteobacteria
167469361	Yersinia pestis FV-1	Gamma-proteobacteria
226328647	Proteus penneri ATCC 35198	Gamma-proteobacteria
197286082	Proteus mirabilis HI4320	Gamma-proteobacteria
227356592	Proteus mirabilis ATCC 29906	Gamma-proteobacteria
197247378	Salmonella enterica subsp. enterica serovar Agona str. SL483	Gamma-proteobacteria
212711364	Providencia alcalifaciens DSM 30120	Gamma-proteobacteria
261345855	Providencia rustigianii DSM 4541	Gamma-proteobacteria
183600216	Providencia stuartii ATCC 25827	Gamma-proteobacteria
268591356	Providencia rettgeri DSM 1131	Gamma-proteobacteria
238898782	Candidatus Hamiltonella defensa 5AT (Acyrthosiphon pisum)	Gamma-proteobacteria
90580346	Vibrio angustum S14	Gamma-proteobacteria
89075068	Photobacterium sp. SKA34	Gamma-proteobacteria
88858314	Pseudoalteromonas tunicata D2	Gamma-proteobacteria
149911458	Moritella sp. PE36	Gamma-proteobacteria
90412104	Photobacterium profundum 3TCK	Gamma-proteobacteria
54310169	Photobacterium profundum SS9	Gamma-proteobacteria
260902364	Vibrio parahaemolyticus AQ4037	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	**Species**	**Class**
254509205	Vibrio parahaemolyticus 16	Gamma-proteobacteria
261250229	Vibrio orientalis CIP 102891	Gamma-proteobacteria
260767191	Vibrio furnissii CIP 102972	Gamma-proteobacteria
260775503	Vibrio coralliilyticus ATCC BAA-450	Gamma-proteobacteria
117617733	Aeromonas hydrophila subsp. hydrophila ATCC 7966	Gamma-proteobacteria
269101863	Photobacterium damselae subsp. damselae CIP 102761	Gamma-proteobacteria
254230502	Vibrio sp. Ex25	Gamma-proteobacteria
163802977	Vibrio sp. AND4	Gamma-proteobacteria
91227834	Vibrio alginolyticus 12G01	Gamma-proteobacteria
269965239	Vibrio alginolyticus 40B	Gamma-proteobacteria
145300341	Aeromonas salmonicida subsp. salmonicida A449	Gamma-proteobacteria
153835750	Vibrio harveyi HY01	Gamma-proteobacteria
156975773	Vibrio harveyi ATCC BAA-1116	Gamma-proteobacteria
260878982	Vibrio parahaemolyticus AN-5034	Gamma-proteobacteria
260895455	Vibrio parahaemolyticus Peru-466	Gamma-proteobacteria
149190125	Vibrio shilonii AK1	Gamma-proteobacteria
127512145	Shewanella loihica PV-4	Gamma-proteobacteria
260361829	Vibrio parahaemolyticus K5030	Gamma-proteobacteria
196158524	Alteromonas macleodii 'Deep ecotype'	Gamma-proteobacteria
37680992	Vibrio vulnificus YJ016	Gamma-proteobacteria
27364956	Vibrio vulnificus CMCP6	Gamma-proteobacteria
170727680	Shewanella woodyi ATCC 51908	Gamma-proteobacteria
84390122	Vibrio splendidus 12B01	Gamma-proteobacteria
209695924	Aliivibrio salmonicida LFI1238	Gamma-proteobacteria
86146341	Vibrio sp. MED222	Gamma-proteobacteria
157374437	Shewanella sediminis HAW-EB3	Gamma-proteobacteria
157961021	Shewanella pealeana ATCC 700345	Gamma-proteobacteria
148978207	Vibrionales bacterium SWAT-3	Gamma-proteobacteria
218710550	Vibrio splendidus LGP32	Gamma-proteobacteria
239995277	Alteromonas macleodii ATCC 27126	Gamma-proteobacteria
258623525	Vibrio mimicus VM573	Gamma-proteobacteria
262172378	Vibrio mimicus MB-451	Gamma-proteobacteria
258623793	Vibrio mimicus VM603	Gamma-proteobacteria
167623164	Shewanella halifaxensis HAW-EB4	Gamma-proteobacteria
119468990	Alteromonadales bacterium TW-7	Gamma-proteobacteria
237809473	Tolumonas auensis DSM 9187	Gamma-proteobacteria
262164038	Vibrio mimicus VM223	Gamma-proteobacteria
147674644	Vibrio cholerae O395	Gamma-proteobacteria
262169845	Vibrio cholerae RC27	Gamma-proteobacteria
15640556	Vibrio cholerae O1 biovar El Tor str. N16961	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
121590676	Vibrio cholerae 2740-80	Gamma-proteobacteria
121728563	Vibrio cholerae V52	Gamma-proteobacteria
153223807	Vibrio cholerae MAK 757	Gamma-proteobacteria
153819103	Vibrio cholerae NCTC 8457	Gamma-proteobacteria
153823615	Vibrio cholerae B33	Gamma-proteobacteria
227080717	Vibrio cholerae M66-2	Gamma-proteobacteria
229507145	Vibrio cholerae BX 330286	Gamma-proteobacteria
229519660	Vibrio cholerae RC9	Gamma-proteobacteria
229606176	Vibrio cholerae MJ-1236	Gamma-proteobacteria
254850774	Vibrio cholerae MO10	Gamma-proteobacteria
255744238	Vibrio cholera CIRS 101	Gamma-proteobacteria
262149032	Vibrio cholerae INDRE 91/1	Gamma-proteobacteria
153214922	Vibrio cholerae 1587	Gamma-proteobacteria
153826929	Vibrio cholerae MZO-2	Gamma-proteobacteria
153829928	Vibrio cholerae 623-39	Gamma-proteobacteria
229530348	Vibrio cholerae 12129(1)	Gamma-proteobacteria
254226840	Vibrio cholerae V51	Gamma-proteobacteria
254291219	Vibrio cholerae AM-19226	Gamma-proteobacteria
262190768	Vibrio cholerae CT 5369-93	Gamma-proteobacteria
229512804	Vibrio cholerae TMA 21	Gamma-proteobacteria
254224068	Vibrio cholerae RC385	Gamma-proteobacteria
212634213	Shewanella piezotolerans WP3	Gamma-proteobacteria
153802558	Vibrio cholerae MZO-3	Gamma-proteobacteria
120598086	Shewanella sp. W3-18-1	Gamma-proteobacteria
146293843	Shewanella putrefaciens CN-32	Gamma-proteobacteria
261210105	Vibrio sp. RC341	Gamma-proteobacteria
197335185	Vibrio fischeri MJ11	Gamma-proteobacteria
59712674	Vibrio fischeri ES114	Gamma-proteobacteria
85712119	Idiomarina baltica OS145	Gamma-proteobacteria
153001641	Shewanella baltica OS185	Gamma-proteobacteria
160876377	Shewanella baltica OS195	Gamma-proteobacteria
217972427	Shewanella baltica OS223	Gamma-proteobacteria
229520830	Vibrio cholerae TM 11079-80	Gamma-proteobacteria
163749990	Shewanella benthica KT99	Gamma-proteobacteria
262401766	Vibrio sp. RC586	Gamma-proteobacteria
56459854	Idiomarina loihiensis L2TR	Gamma-proteobacteria
126175320	Shewanella baltica OS155	Gamma-proteobacteria
119774181	Shewanella amazonensis SB2B	Gamma-proteobacteria
91792562	Shewanella denitrificans OS217	Gamma-proteobacteria
77359643	Pseudoalteromonas haloplanktis TAC125	Gamma-proteobacteria
24374942	Shewanella oneidensis MR-1	Gamma-proteobacteria
113969467	Shewanella sp. MR-4	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
114046700	Shewanella sp. MR-7	Gamma-proteobacteria
117919573	Shewanella sp. ANA-3	Gamma-proteobacteria
109900163	Pseudoalteromonas atlantica T6c	Gamma-proteobacteria
153839213	Vibrio parahaemolyticus AQ3810	Gamma-proteobacteria
71278347	Colwellia psychrerythraea 34H	Gamma-proteobacteria
114562238	Shewanella frigidimarina NCIMB 400	Gamma-proteobacteria
88798286	Reinekea sp. MED297	Gamma-proteobacteria
90407885	Psychromonas sp. CNPT3	Gamma-proteobacteria
119944449	Psychromonas ingrahamii 37	Gamma-proteobacteria
254786794	Teredinibacter turnerae T7901	Gamma-proteobacteria
226945910	Azotobacter vinelandii DJ	Gamma-proteobacteria
254481825	marine gamma proteobacterium HTCC2148	Gamma-proteobacteria
90020900	Saccharophagus degradans 2-40	Gamma-proteobacteria
94500747	Oceanobacter sp. RED65	Gamma-proteobacteria
83644704	Hahella chejuensis KCTC 2396	Gamma-proteobacteria
15598818	Pseudomonas aeruginosa PAO1	Gamma-proteobacteria
116051619	Pseudomonas aeruginosa UCBPP-PA14	Gamma-proteobacteria
218890153	Pseudomonas aeruginosa LESB58	Gamma-proteobacteria
254242320	Pseudomonas aeruginosa 2192	Gamma-proteobacteria
152985831	Pseudomonas aeruginosa PA7	Gamma-proteobacteria
104783164	Pseudomonas entomophila L48	Gamma-proteobacteria
229588834	Pseudomonas fluorescens SBW25	Gamma-proteobacteria
237800176	Pseudomonas syringae pv. oryzae str. 1_6	Gamma-proteobacteria
157169065	Pseudomonas fluorescens Pf-5	Gamma-proteobacteria
71736784	Pseudomonas syringae pv. phaseolicola 1448A	Gamma-proteobacteria
146308041	Pseudomonas mendocina ymp	Gamma-proteobacteria
26988355	Pseudomonas putida KT2440	Gamma-proteobacteria
148549360	Pseudomonas putida F1	Gamma-proteobacteria
167032190	Pseudomonas putida GB-1	Gamma-proteobacteria
87118598	Marinomonas sp. MED121	Gamma-proteobacteria
77457359	Pseudomonas fluorescens Pf0-1	Gamma-proteobacteria
120553862	Marinobacter aquaeolei VT8	Gamma-proteobacteria
170723213	Pseudomonas putida W619	Gamma-proteobacteria
28868771	Pseudomonas syringae pv. tomato str. DC3000	Gamma-proteobacteria
66044622	Pseudomonas syringae pv. syringae B728a	Gamma-proteobacteria
257487049	Pseudomonas syringae pv. tabaci ATCC 11528	Gamma-proteobacteria
149378178	Marinobacter algicola DG893	Gamma-proteobacteria
146281946	Pseudomonas stutzeri A1501	Gamma-proteobacteria
192358973	Cellvibrio japonicus Ueda107	Gamma-proteobacteria
213969149	Pseudomonas syringae pv. tomato T1	Gamma-proteobacteria
28899327	Vibrio parahaemolyticus RIMD 2210633	Gamma-proteobacteria
126667564	Marinobacter sp. ELB17	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
114771615	Rhodobacterales bacterium HTCC2255	Alpha-proteobacteria
221134005	Glaciecola sp. HTCC2999	Gamma-proteobacteria
89092114	Oceanospirillum sp. MED92	Gamma-proteobacteria
92114749	Chromohalobacter salexigens DSM 3043	Gamma-proteobacteria
152995337	Marinomonas sp. MWYL1	Gamma-proteobacteria
269960386	Vibrio harveyi 1DA3	Gamma-proteobacteria
220934609	Thioalkalivibrio sp. HL-EbGR7	Gamma-proteobacteria
213621937	Salmonella enterica subsp. enterica serovar Typhi str. E98-2068	Gamma-proteobacteria
52841515	Legionella pneumophila subsp. pneumophila str. Philadelphia 1	Gamma-proteobacteria
114320976	Alkalilimnicola ehrlichii MLHE-1	Gamma-proteobacteria
148358826	Legionella pneumophila str. Corby	Gamma-proteobacteria
54294183	Legionella pneumophila str. Lens	Gamma-proteobacteria
54297202	Legionella pneumophila str. Paris	Gamma-proteobacteria
270157097	Legionella longbeachae D-4968	Gamma-proteobacteria
254496518	Legionella drancourtii LLAP12	Gamma-proteobacteria
241776538	Allochromatium vinosum DSM 180	Gamma-proteobacteria
88705821	Congregibacter litoralis KT71	Gamma-proteobacteria
260773542	Vibrio metschnikovii CIP 69.14	Gamma-proteobacteria
88812406	Nitrococcus mobilis Nb-231	Gamma-proteobacteria
254514875	gamma proteobacterium NOR5-3	Gamma-proteobacteria
90416198	marine gamma proteobacterium HTCC2207	Gamma-proteobacteria
160872453	Rickettsiella grylli	Gamma-proteobacteria
121998209	Halorhodospira halophila SL1	Gamma-proteobacteria
254449272	gamma proteobacterium HTCC5015	Gamma-proteobacteria
107103136	Pseudomonas aeruginosa PACS2	Gamma-proteobacteria
77163721	Nitrosococcus oceani ATCC 19707	Gamma-proteobacteria
254436220	Nitrosococcus oceani AFC27	Gamma-proteobacteria
262273681	Grimontia hollisae CIP 101886	Gamma-proteobacteria
26249145	Escherichia coli CFT073	Gamma-proteobacteria
261342188	Enterobacter cancerogenus ATCC 35316	Gamma-proteobacteria
153875311	Beggiatoa sp. PS	Gamma-proteobacteria
154706690	Coxiella burnetii Dugway 5J108-111	Gamma-proteobacteria
224818509	Thioalkalivibrio sp. K90mix	Gamma-proteobacteria
153207564	Coxiella burnetii 'MSU Goat Q177'	Gamma-proteobacteria
161829847	Coxiella burnetii RSA 331	Gamma-proteobacteria
165918356	Coxiella burnetii RSA 334	Gamma-proteobacteria
212211994	Coxiella burnetii CbuG_Q212	Gamma-proteobacteria
212218001	Coxiella burnetii CbuK_Q154	Gamma-proteobacteria
29654959	Coxiella burnetii RSA 493	Gamma-proteobacteria
261856530	Halothiobacillus neapolitanus c2	Gamma-proteobacteria

Appendix A (*continued*).

gi Number	Species	Class
224824776	Lutiella nitroferrum 2002	Beta-proteobacteria
34499137	Chromobacterium violaceum ATCC 12472	Beta-proteobacteria
255020521	Acidithiobacillus caldus ATCC 51756	Gamma-proteobacteria
198282770	Acidithiobacillus ferrooxidans ATCC 53993	Gamma-proteobacteria
218666687	Acidithiobacillus ferrooxidans ATCC 23270	Gamma-proteobacteria
167950928	Endoriftia persephone 'Hot96_1+Hot96_2'	Gamma-proteobacteria
74316856	Thiobacillus denitrificans ATCC 25259	Beta-proteobacteria
257092978	Candidatus Accumulibacter phosphatis clade IIA str. UW-1	Beta-proteobacteria
71908137	Dechloromonas aromatica RCB	Beta-proteobacteria
119476594	marine gamma proteobacterium HTCC2143	Gamma-proteobacteria
82701632	Nitrosospira multiformis ATCC 25196	Beta-proteobacteria
152980760	Janthinobacterium sp. Marseille	Beta-proteobacteria
134094484	Herminiimonas arsenicoxydans	Beta-proteobacteria
83747939	Ralstonia solanacearum UW551	Beta-proteobacteria
17545926	Ralstonia solanacearum GMI1000	Beta-proteobacteria
241662747	Ralstonia pickettii 12D	Beta-proteobacteria
187928144	Ralstonia pickettii 12J	Beta-proteobacteria
113868347	Ralstonia eutropha H16	Beta-proteobacteria
194290015	Cupriavidus taiwanensis	Beta-proteobacteria
94311053	Ralstonia metallidurans CH34	Beta-proteobacteria
73541783	Ralstonia eutropha JMP134	Beta-proteobacteria

Appendix B. Reciprocal best hits of *E. coli* RpoS to NCBI's RefSeq database using an *E*-value threshold of 1e-04. Data is organized from the most to the least significant reciprocal best hit.

gi Number	Species	Class
16130648	Escherichia coli str. K-12 substr. MG1655	Gamma-proteobacteria
170082316	Escherichia coli str. K-12 substr. DH10B	Gamma-proteobacteria
238901878	Escherichia coli BW2952	Gamma-proteobacteria
15832849	Escherichia coli O157:H7 str. Sakai	Gamma-proteobacteria
74313307	Shigella sonnei Ss046	Gamma-proteobacteria
110642882	Escherichia coli 536	Gamma-proteobacteria
110806628	Shigella flexneri 5 str. 8401	Gamma-proteobacteria
157157336	Escherichia coli E24377A	Gamma-proteobacteria
157162189	Escherichia coli HS	Gamma-proteobacteria
168749933	Escherichia coli O157:H7 str. EC4113	Gamma-proteobacteria
168755487	Escherichia coli O157:H7 str. EC4401	Gamma-proteobacteria
168778725	Escherichia coli O157:H7 str. EC4076	Gamma-proteobacteria
168787997	Escherichia coli O157:H7 str. EC869	Gamma-proteobacteria
170019013	Escherichia coli ATCC 8739	Gamma-proteobacteria
170684160	Escherichia coli SMS-3-5	Gamma-proteobacteria
191166775	Escherichia coli B7A	Gamma-proteobacteria
191171134	Escherichia coli F11	Gamma-proteobacteria
193065048	Escherichia coli E22	Gamma-proteobacteria
193069671	Escherichia coli E110019	Gamma-proteobacteria
194431745	Shigella dysenteriae 1012	Gamma-proteobacteria
194438995	Escherichia coli 101-1	Gamma-proteobacteria
208812039	Escherichia coli O157:H7 str. EC4045	Gamma-proteobacteria
209395891	Escherichia coli O157:H7 str. EC4115	Gamma-proteobacteria
209920184	Escherichia coli SE11	Gamma-proteobacteria
217326648	Escherichia coli O157:H7 str. TW14588	Gamma-proteobacteria
218559734	Escherichia coli S88	Gamma-proteobacteria
218696339	Escherichia coli 55989	Gamma-proteobacteria
218706235	Escherichia coli UMN026	Gamma-proteobacteria
253772403	Escherichia coli BL21(DE3)	Gamma-proteobacteria
254162672	Escherichia coli B str. REL606	Gamma-proteobacteria
256019474	Shigella sp. D9	Gamma-proteobacteria
256024751	Escherichia sp. 4_1_40B	Gamma-proteobacteria
260845388	Escherichia coli O103:H2 str. 12009	Gamma-proteobacteria
260869420	Escherichia coli O111:H- str. 11128	Gamma-proteobacteria
261226035	Escherichia coli O157:H7 str. FRIK2000	Gamma-proteobacteria
261256708	Escherichia coli O157:H7 str. FRIK966	Gamma-proteobacteria
91212108	Escherichia coli UTI89	Gamma-proteobacteria
117624976	Escherichia coli APEC O1	Gamma-proteobacteria
227888284	Escherichia coli 83972	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
237706630	Escherichia sp. 3_2_53FAA	Gamma-proteobacteria
254037782	Escherichia sp. 1_1_43	Gamma-proteobacteria
283786707	Citrobacter rodentium ICC168	Gamma-proteobacteria
24114036	Shigella flexneri 2a str. 301	Gamma-proteobacteria
218701232	Escherichia coli IAI39	Gamma-proteobacteria
208821597	Escherichia coli O157:H7 str. EC4042	Gamma-proteobacteria
218690868	Escherichia coli ED1a	Gamma-proteobacteria
260856852	Escherichia coli O26:H11 str. 11368	Gamma-proteobacteria
82778108	Shigella dysenteriae Sd197	Gamma-proteobacteria
254794681	Escherichia coli O157:H7 str. TW14359	Gamma-proteobacteria
16761696	Salmonella enterica subsp. enterica serovar Typhi str. CT18	Gamma-proteobacteria
16766230	Salmonella typhimurium LT2	Gamma-proteobacteria
56414872	Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150	Gamma-proteobacteria
62181426	Salmonella enterica subsp. enterica serovar Choleraesuis str. SC-B67	Gamma-proteobacteria
157148284	Citrobacter koseri ATCC BAA-895	Gamma-proteobacteria
167550225	Salmonella enterica subsp. enterica serovar Saintpaul str. SARA29	Gamma-proteobacteria
168230998	Salmonella enterica subsp. enterica serovar Kentucky str. CDC 191	Gamma-proteobacteria
168235962	Salmonella enterica subsp. enterica serovar Schwarzengrund str. SL480	Gamma-proteobacteria
168242664	Salmonella enterica subsp. enterica serovar Heidelberg str. SL486	Gamma-proteobacteria
168261948	Salmonella enterica subsp. enterica serovar Hadar str. RI_05P066	Gamma-proteobacteria
168463936	Salmonella enterica subsp. enterica serovar Newport str. SL317	Gamma-proteobacteria
168820376	Salmonella enterica subsp. enterica serovar Weltevreden str. HI_N05-537	Gamma-proteobacteria
194442500	Salmonella enterica subsp. enterica serovar Newport str. SL254	Gamma-proteobacteria
194451079	Salmonella enterica subsp. enterica serovar Heidelberg str. SL476	Gamma-proteobacteria
194470798	Salmonella enterica subsp. enterica serovar Kentucky str. CVM29188	Gamma-proteobacteria
194738113	Salmonella enterica subsp. enterica serovar Schwarzengrund str. CVM19633	Gamma-proteobacteria
197263839	Salmonella enterica subsp. enterica serovar Saintpaul str. SARA23	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
197363800	Salmonella enterica subsp. enterica serovar Paratyphi A str. AKU_12601	Gamma-proteobacteria
198243199	Salmonella enterica subsp. enterica serovar Dublin str. CT_02021853	Gamma-proteobacteria
200388856	Salmonella enterica subsp. enterica serovar Virchow str. SL491	Gamma-proteobacteria
204928102	Salmonella enterica subsp. enterica serovar Javiana str. GA_MM04042433	Gamma-proteobacteria
207858184	Salmonella enterica subsp. enterica serovar Enteritidis str. P125109	Gamma-proteobacteria
213052392	Salmonella enterica subsp. enterica serovar Typhi str. E00-7866	Gamma-proteobacteria
213648321	Salmonella enterica subsp. enterica serovar Typhi str. J185	Gamma-proteobacteria
224584701	Salmonella enterica subsp. enterica serovar Paratyphi C strain RKS4594	Gamma-proteobacteria
237729723	Citrobacter sp. 30_2	Gamma-proteobacteria
238909692	Salmonella enterica subsp. enterica serovar Tennessee str. CDC07-0191	Gamma-proteobacteria
283835495	Citrobacter youngae ATCC 29220	Gamma-proteobacteria
15803258	Escherichia coli O157:H7 EDL933	Gamma-proteobacteria
82545186	Shigella boydii Sb227	Gamma-proteobacteria
205353865	Salmonella enterica subsp. enterica serovar Gallinarum str. 287/91	Gamma-proteobacteria
161502017	Salmonella enterica subsp. arizonae serovar 62:z4,z23:--	Gamma-proteobacteria
238896221	Klebsiella pneumoniae NTUH-K2044	Gamma-proteobacteria
168768834	Escherichia coli O157:H7 str. EC4486	Gamma-proteobacteria
167993163	Salmonella enterica subsp. enterica serovar 4,[5	Gamma-proteobacteria
152971625	Klebsiella pneumoniae subsp. pneumoniae MGH 78578	Gamma-proteobacteria
206580660	Klebsiella pneumoniae 342	Gamma-proteobacteria
262041489	Klebsiella pneumoniae subsp. rhinoscleromatis ATCC 13884	Gamma-proteobacteria
269915854	Klebsiella variicola At-22	Gamma-proteobacteria
260599105	Cronobacter turicensis	Gamma-proteobacteria
146312853	Enterobacter sp. 638	Gamma-proteobacteria
213975649	Salmonella enterica subsp. enterica serovar Typhi str. AG3	Gamma-proteobacteria
258638949	Pantoea sp. At-9b	Gamma-proteobacteria
213859421	Salmonella enterica subsp. enterica serovar Typhi str. M223	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
188534815	Erwinia tasmaniensis Et1/99	Gamma-proteobacteria
238754524	Yersinia ruckeri ATCC 29473	Gamma-proteobacteria
238797130	Yersinia mollaretii ATCC 43969	Gamma-proteobacteria
157369077	Serratia proteamaculans 568	Gamma-proteobacteria
213029652	Salmonella enterica subsp. enterica serovar Typhi str. 404ty	Gamma-proteobacteria
29143180	Salmonella enterica subsp. enterica serovar Typhi str. Ty2	Gamma-proteobacteria
238791265	Yersinia intermedia ATCC 29909	Gamma-proteobacteria
270264030	Serratia odorifera 4Rx13	Gamma-proteobacteria
238750591	Yersinia rohdei ATCC 43380	Gamma-proteobacteria
123441128	Yersinia enterocolitica subsp. enterocolitica 8081	Gamma-proteobacteria
238761664	Yersinia kristensenii ATCC 33638	Gamma-proteobacteria
238786006	Yersinia bercovieri ATCC 43970	Gamma-proteobacteria
238758207	Yersinia aldovae ATCC 35236	Gamma-proteobacteria
271501815	Dickeya dadantii Ech586	Gamma-proteobacteria
22124745	Yersinia pestis KIM	Gamma-proteobacteria
45440189	Yersinia pestis biovar Microtus str. 91001	Gamma-proteobacteria
108808779	Yersinia pestis Antiqua	Gamma-proteobacteria
108810903	Yersinia pestis Nepal516	Gamma-proteobacteria
145600262	Yersinia pestis Pestoides F	Gamma-proteobacteria
153997601	Yersinia pestis CA88-4125	Gamma-proteobacteria
162420853	Yersinia pestis Angola	Gamma-proteobacteria
165925871	Yersinia pestis biovar Orientalis str. F1991016	Gamma-proteobacteria
165936700	Yersinia pestis biovar Orientalis str. IP275	Gamma-proteobacteria
166010016	Yersinia pestis biovar Antiqua str. E1979001	Gamma-proteobacteria
166212931	Yersinia pestis biovar Antiqua str. B42003004	Gamma-proteobacteria
167398742	Yersinia pestis biovar Antiqua str. UG05-0454	Gamma-proteobacteria
167421704	Yersinia pestis biovar Orientalis str. MG05-1020	Gamma-proteobacteria
167423190	Yersinia pestis biovar Mediaevalis str. K1973002	Gamma-proteobacteria
218930373	Yersinia pestis CO92	Gamma-proteobacteria
229838982	Yersinia pestis biovar Orientalis str. PEXU2	Gamma-proteobacteria
229896462	Yersinia pestis Pestoides A	Gamma-proteobacteria
229899549	Yersinia pestis biovar Orientalis str. India 195	Gamma-proteobacteria
270489294	Yersinia pestis KIM D27	Gamma-proteobacteria
51595126	Yersinia pseudotuberculosis IP 32953	Gamma-proteobacteria
153948385	Yersinia pseudotuberculosis IP 31758	Gamma-proteobacteria
170025640	Yersinia pseudotuberculosis YPIII	Gamma-proteobacteria
186894139	Yersinia pseudotuberculosis PB1/+	Gamma-proteobacteria
251788478	Dickeya zeae Ech1591	Gamma-proteobacteria
195939453	Escherichia coli O157:H7 str. EC4024	Gamma-proteobacteria
253689710	Pectobacterium carotovorum subsp. carotovorum	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
	PC1	
242238323	Dickeya dadantii Ech703	Gamma-proteobacteria
50122451	Pectobacterium atrosepticum SCRI1043	Gamma-proteobacteria
227114060	Pectobacterium carotovorum subsp. brasiliensis PBR1692	Gamma-proteobacteria
227327750	Pectobacterium carotovorum subsp. carotovorum WPP14	Gamma-proteobacteria
261822742	Pectobacterium wasabiae WPP163	Gamma-proteobacteria
213425678	Salmonella enterica subsp. enterica serovar Typhi str. E02-1180	Gamma-proteobacteria
89109528	Escherichia coli str. K-12 substr. W3110	Gamma-proteobacteria
218547743	Escherichia fergusonii ATCC 35469	Gamma-proteobacteria
269140216	Edwardsiella tarda EIB202	Gamma-proteobacteria
238921111	Edwardsiella ictaluri 93-146	Gamma-proteobacteria
213417289	Salmonella enterica subsp. enterica serovar Typhi str. E01-6750	Gamma-proteobacteria
238786819	Yersinia frederiksenii ATCC 33641	Gamma-proteobacteria
253988173	Photorhabdus asymbiotica	Gamma-proteobacteria
37524720	Photorhabdus luminescens subsp. laumondii TTO1	Gamma-proteobacteria
170765845	Escherichia albertii TW07627	Gamma-proteobacteria
259909441	Erwinia pyrifoliae Ep1/96	Gamma-proteobacteria
167469361	Yersinia pestis FV-1	Gamma-proteobacteria
226328647	Proteus penneri ATCC 35198	Gamma-proteobacteria
197286082	Proteus mirabilis HI4320	Gamma-proteobacteria
227356592	Proteus mirabilis ATCC 29906	Gamma-proteobacteria
197247378	Salmonella enterica subsp. enterica serovar Agona str. SL483	Gamma-proteobacteria
212711364	Providencia alcalifaciens DSM 30120	Gamma-proteobacteria
261345855	Providencia rustigianii DSM 4541	Gamma-proteobacteria
183600216	Providencia stuartii ATCC 25827	Gamma-proteobacteria
268591356	Providencia rettgeri DSM 1131	Gamma-proteobacteria
238898782	Candidatus Hamiltonella defensa 5AT (Acyrthosiphon pisum)	Gamma-proteobacteria
90580346	Vibrio angustum S14	Gamma-proteobacteria
89075068	Photobacterium sp. SKA34	Gamma-proteobacteria
88858314	Pseudoalteromonas tunicata D2	Gamma-proteobacteria
149911458	Moritella sp. PE36	Gamma-proteobacteria
90412104	Photobacterium profundum 3TCK	Gamma-proteobacteria
54310169	Photobacterium profundum SS9	Gamma-proteobacteria
260902364	Vibrio parahaemolyticus AQ4037	Gamma-proteobacteria
254509205	Vibrio parahaemolyticus 16	Gamma-proteobacteria
261250229	Vibrio orientalis CIP 102891	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
260767191	Vibrio furnissii CIP 102972	Gamma-proteobacteria
260775503	Vibrio coralliilyticus ATCC BAA-450	Gamma-proteobacteria
117617733	Aeromonas hydrophila subsp. hydrophila ATCC 7966	Gamma-proteobacteria
269101863	Photobacterium damselae subsp. damselae CIP 102761	Gamma-proteobacteria
254230502	Vibrio sp. Ex25	Gamma-proteobacteria
163802977	Vibrio sp. AND4	Gamma-proteobacteria
91227834	Vibrio alginolyticus 12G01	Gamma-proteobacteria
269965239	Vibrio alginolyticus 40B	Gamma-proteobacteria
145300341	Aeromonas salmonicida subsp. salmonicida A449	Gamma-proteobacteria
153835750	Vibrio harveyi HY01	Gamma-proteobacteria
156975773	Vibrio harveyi ATCC BAA-1116	Gamma-proteobacteria
260878982	Vibrio parahaemolyticus AN-5034	Gamma-proteobacteria
260895455	Vibrio parahaemolyticus Peru-466	Gamma-proteobacteria
149190125	Vibrio shilonii AK1	Gamma-proteobacteria
127512145	Shewanella loihica PV-4	Gamma-proteobacteria
260361829	Vibrio parahaemolyticus K5030	Gamma-proteobacteria
196158524	Alteromonas macleodii 'Deep ecotype'	Gamma-proteobacteria
37680992	Vibrio vulnificus YJ016	Gamma-proteobacteria
27364956	Vibrio vulnificus CMCP6	Gamma-proteobacteria
170727680	Shewanella woodyi ATCC 51908	Gamma-proteobacteria
84390122	Vibrio splendidus 12B01	Gamma-proteobacteria
209695924	Aliivibrio salmonicida LFI1238	Gamma-proteobacteria
86146341	Vibrio sp. MED222	Gamma-proteobacteria
157374437	Shewanella sediminis HAW-EB3	Gamma-proteobacteria
157961021	Shewanella pealeana ATCC 700345	Gamma-proteobacteria
148978207	Vibrionales bacterium SWAT-3	Gamma-proteobacteria
218710550	Vibrio splendidus LGP32	Gamma-proteobacteria
239995277	Alteromonas macleodii ATCC 27126	Gamma-proteobacteria
258623525	Vibrio mimicus VM573	Gamma-proteobacteria
262172378	Vibrio mimicus MB-451	Gamma-proteobacteria
258623793	Vibrio mimicus VM603	Gamma-proteobacteria
167623164	Shewanella halifaxensis HAW-EB4	Gamma-proteobacteria
119468990	Alteromonadales bacterium TW-7	Gamma-proteobacteria
237809473	Tolumonas auensis DSM 9187	Gamma-proteobacteria
262164038	Vibrio mimicus VM223	Gamma-proteobacteria
147674644	Vibrio cholerae O395	Gamma-proteobacteria
262169845	Vibrio cholerae RC27	Gamma-proteobacteria
15640556	Vibrio cholerae O1 biovar El Tor str. N16961	Gamma-proteobacteria
121590676	Vibrio cholerae 2740-80	Gamma-proteobacteria
121728563	Vibrio cholerae V52	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
153223807	Vibrio cholerae MAK 757	Gamma-proteobacteria
153819103	Vibrio cholerae NCTC 8457	Gamma-proteobacteria
153823615	Vibrio cholerae B33	Gamma-proteobacteria
227080717	Vibrio cholerae M66-2	Gamma-proteobacteria
229507145	Vibrio cholerae BX 330286	Gamma-proteobacteria
229519660	Vibrio cholerae RC9	Gamma-proteobacteria
229606176	Vibrio cholerae MJ-1236	Gamma-proteobacteria
254850774	Vibrio cholerae MO10	Gamma-proteobacteria
255744238	Vibrio cholera CIRS 101	Gamma-proteobacteria
262149032	Vibrio cholerae INDRE 91/1	Gamma-proteobacteria
153214922	Vibrio cholerae 1587	Gamma-proteobacteria
153826929	Vibrio cholerae MZO-2	Gamma-proteobacteria
153829928	Vibrio cholerae 623-39	Gamma-proteobacteria
229530348	Vibrio cholerae 12129(1)	Gamma-proteobacteria
254226840	Vibrio cholerae V51	Gamma-proteobacteria
254291219	Vibrio cholerae AM-19226	Gamma-proteobacteria
262190768	Vibrio cholerae CT 5369-93	Gamma-proteobacteria
229512804	Vibrio cholerae TMA 21	Gamma-proteobacteria
254224068	Vibrio cholerae RC385	Gamma-proteobacteria
212634213	Shewanella piezotolerans WP3	Gamma-proteobacteria
153802558	Vibrio cholerae MZO-3	Gamma-proteobacteria
120598086	Shewanella sp. W3-18-1	Gamma-proteobacteria
146293843	Shewanella putrefaciens CN-32	Gamma-proteobacteria
261210105	Vibrio sp. RC341	Gamma-proteobacteria
197335185	Vibrio fischeri MJ11	Gamma-proteobacteria
59712674	Vibrio fischeri ES114	Gamma-proteobacteria
85712119	Idiomarina baltica OS145	Gamma-proteobacteria
153001641	Shewanella baltica OS185	Gamma-proteobacteria
160876377	Shewanella baltica OS195	Gamma-proteobacteria
217972427	Shewanella baltica OS223	Gamma-proteobacteria
229520830	Vibrio cholerae TM 11079-80	Gamma-proteobacteria
163749990	Shewanella benthica KT99	Gamma-proteobacteria
262401766	Vibrio sp. RC586	Gamma-proteobacteria
56459854	Idiomarina loihiensis L2TR	Gamma-proteobacteria
126175320	Shewanella baltica OS155	Gamma-proteobacteria
119774181	Shewanella amazonensis SB2B	Gamma-proteobacteria
91792562	Shewanella denitrificans OS217	Gamma-proteobacteria
77359643	Pseudoalteromonas haloplanktis TAC125	Gamma-proteobacteria
24374942	Shewanella oneidensis MR-1	Gamma-proteobacteria
113969467	Shewanella sp. MR-4	Gamma-proteobacteria
114046700	Shewanella sp. MR-7	Gamma-proteobacteria
117919573	Shewanella sp. ANA-3	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
109900163	Pseudoalteromonas atlantica T6c	Gamma-proteobacteria
153839213	Vibrio parahaemolyticus AQ3810	Gamma-proteobacteria
71278347	Colwellia psychrerythraea 34H	Gamma-proteobacteria
114562238	Shewanella frigidimarina NCIMB 400	Gamma-proteobacteria
88798286	Reinekea sp. MED297	Gamma-proteobacteria
90407885	Psychromonas sp. CNPT3	Gamma-proteobacteria
119944449	Psychromonas ingrahamii 37	Gamma-proteobacteria
254786794	Teredinibacter turnerae T7901	Gamma-proteobacteria
226945910	Azotobacter vinelandii DJ	Gamma-proteobacteria
254481825	marine gamma proteobacterium HTCC2148	Gamma-proteobacteria
90020900	Saccharophagus degradans 2-40	Gamma-proteobacteria
94500747	Oceanobacter sp. RED65	Gamma-proteobacteria
83644704	Hahella chejuensis KCTC 2396	Gamma-proteobacteria
15598818	Pseudomonas aeruginosa PAO1	Gamma-proteobacteria
116051619	Pseudomonas aeruginosa UCBPP-PA14	Gamma-proteobacteria
218890153	Pseudomonas aeruginosa LESB58	Gamma-proteobacteria
254242320	Pseudomonas aeruginosa 2192	Gamma-proteobacteria
152985831	Pseudomonas aeruginosa PA7	Gamma-proteobacteria
104783164	Pseudomonas entomophila L48	Gamma-proteobacteria
229588834	Pseudomonas fluorescens SBW25	Gamma-proteobacteria
237800176	Pseudomonas syringae pv. oryzae str. 1_6	Gamma-proteobacteria
157169065	Pseudomonas fluorescens Pf-5	Gamma-proteobacteria
71736784	Pseudomonas syringae pv. phaseolicola 1448A	Gamma-proteobacteria
146308041	Pseudomonas mendocina ymp	Gamma-proteobacteria
26988355	Pseudomonas putida KT2440	Gamma-proteobacteria
148549360	Pseudomonas putida F1	Gamma-proteobacteria
167032190	Pseudomonas putida GB-1	Gamma-proteobacteria
87118598	Marinomonas sp. MED121	Gamma-proteobacteria
77457359	Pseudomonas fluorescens Pf0-1	Gamma-proteobacteria
120553862	Marinobacter aquaeolei VT8	Gamma-proteobacteria
170723213	Pseudomonas putida W619	Gamma-proteobacteria
28868771	Pseudomonas syringae pv. tomato str. DC3000	Gamma-proteobacteria
66044622	Pseudomonas syringae pv. syringae B728a	Gamma-proteobacteria
257487049	Pseudomonas syringae pv. tabaci ATCC 11528	Gamma-proteobacteria
149378178	Marinobacter algicola DG893	Gamma-proteobacteria
146281946	Pseudomonas stutzeri A1501	Gamma-proteobacteria
192358973	Cellvibrio japonicus Ueda107	Gamma-proteobacteria
213969149	Pseudomonas syringae pv. tomato T1	Gamma-proteobacteria
28899327	Vibrio parahaemolyticus RIMD 2210633	Gamma-proteobacteria
126667564	Marinobacter sp. ELB17	Gamma-proteobacteria
114771615	Rhodobacterales bacterium HTCC2255	Alpha-proteobacteria
221134005	Glaciecola sp. HTCC2999	Gamma-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
89092114	Oceanospirillum sp. MED92	Gamma-proteobacteria
92114749	Chromohalobacter salexigens DSM 3043	Gamma-proteobacteria
152995337	Marinomonas sp. MWYL1	Gamma-proteobacteria
269960386	Vibrio harveyi 1DA3	Gamma-proteobacteria
220934609	Thioalkalivibrio sp. HL-EbGR7	Gamma-proteobacteria
213621937	Salmonella enterica subsp. enterica serovar Typhi str. E98-2068	Gamma-proteobacteria
52841515	Legionella pneumophila subsp. pneumophila str. Philadelphia 1	Gamma-proteobacteria
114320976	Alkalilimnicola ehrlichii MLHE-1	Gamma-proteobacteria
148358826	Legionella pneumophila str. Corby	Gamma-proteobacteria
54294183	Legionella pneumophila str. Lens	Gamma-proteobacteria
54297202	Legionella pneumophila str. Paris	Gamma-proteobacteria
270157097	Legionella longbeachae D-4968	Gamma-proteobacteria
254496518	Legionella drancourtii LLAP12	Gamma-proteobacteria
241776538	Allochromatium vinosum DSM 180	Gamma-proteobacteria
88705821	Congregibacter litoralis KT71	Gamma-proteobacteria
260773542	Vibrio metschnikovii CIP 69.14	Gamma-proteobacteria
88812406	Nitrococcus mobilis Nb-231	Gamma-proteobacteria
254514875	gamma proteobacterium NOR5-3	Gamma-proteobacteria
90416198	marine gamma proteobacterium HTCC2207	Gamma-proteobacteria
160872453	Rickettsiella grylli	Gamma-proteobacteria
121998209	Halorhodospira halophila SL1	Gamma-proteobacteria
254449272	gamma proteobacterium HTCC5015	Gamma-proteobacteria
107103136	Pseudomonas aeruginosa PACS2	Gamma-proteobacteria
77163721	Nitrosococcus oceani ATCC 19707	Gamma-proteobacteria
254436220	Nitrosococcus oceani AFC27	Gamma-proteobacteria
262273681	Grimontia hollisae CIP 101886	Gamma-proteobacteria
26249145	Escherichia coli CFT073	Gamma-proteobacteria
261342188	Enterobacter cancerogenus ATCC 35316	Gamma-proteobacteria
153875311	Beggiatoa sp. PS	Gamma-proteobacteria
154706690	Coxiella burnetii Dugway 5J108-111	Gamma-proteobacteria
224818509	Thioalkalivibrio sp. K90mix	Gamma-proteobacteria
153207564	Coxiella burnetii 'MSU Goat Q177'	Gamma-proteobacteria
161829847	Coxiella burnetii RSA 331	Gamma-proteobacteria
165918356	Coxiella burnetii RSA 334	Gamma-proteobacteria
212211994	Coxiella burnetii CbuG_Q212	Gamma-proteobacteria
212218001	Coxiella burnetii CbuK_Q154	Gamma-proteobacteria
29654959	Coxiella burnetii RSA 493	Gamma-proteobacteria
261856530	Halothiobacillus neapolitanus c2	Gamma-proteobacteria
224824776	Lutiella nitroferrum 2002	Beta-proteobacteria
34499137	Chromobacterium violaceum ATCC 12472	Beta-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
255020521	Acidithiobacillus caldus ATCC 51756	Gamma-proteobacteria
198282770	Acidithiobacillus ferrooxidans ATCC 53993	Gamma-proteobacteria
218666687	Acidithiobacillus ferrooxidans ATCC 23270	Gamma-proteobacteria
167950928	Endoriftia persephone 'Hot96_1+Hot96_2'	Gamma-proteobacteria
74316856	Thiobacillus denitrificans ATCC 25259	Beta-proteobacteria
257092978	Candidatus Accumulibacter phosphatis clade IIA str. UW-1	Gamma-proteobacteria
71908137	Dechloromonas aromatica RCB	Beta-proteobacteria
119476594	marine gamma proteobacterium HTCC2143	Gamma-proteobacteria
82701632	Nitrosospira multiformis ATCC 25196	Beta-proteobacteria
152980760	Janthinobacterium sp. Marseille	Beta-proteobacteria
134094484	Herminiimonas arsenicoxydans	Beta-proteobacteria
119897381	Azoarcus sp. BH72	Beta-proteobacteria
83747939	Ralstonia solanacearum UW551	Beta-proteobacteria
17545926	Ralstonia solanacearum GMI1000	Beta-proteobacteria
241662747	Ralstonia pickettii 12D	Beta-proteobacteria
187928144	Ralstonia pickettii 12J	Beta-proteobacteria
113868347	Ralstonia eutropha H16	Beta-proteobacteria
194290015	Cupriavidus taiwanensis	Beta-proteobacteria
94311053	Ralstonia metallidurans CH34	Beta-proteobacteria
73541783	Ralstonia eutropha JMP134	Beta-proteobacteria
56475838	Aromatoleum aromaticum EbN1	Beta-proteobacteria
107029001	Burkholderia cenocepacia AU 1054	Beta-proteobacteria
77919031	Pelobacter carbinolicus DSM 2380	Delta-proteoabacteria
115351809	Burkholderia ambifaria AMMD	Beta-proteobacteria
172060780	Burkholderia ambifaria MC40-6	Beta-proteobacteria
171321354	Burkholderia ambifaria MEX-5	Beta-proteobacteria
116689841	Burkholderia cenocepacia HI2424	Beta-proteobacteria
170733180	Burkholderia cenocepacia MC0-3	Beta-proteobacteria
167587040	Burkholderia ubonensis Bu	Beta-proteobacteria
254252249	Burkholderia dolosa AUO158	Beta-proteobacteria
161524626	Burkholderia multivorans ATCC 17616	Beta-proteobacteria
221212761	Burkholderia multivorans CGD1	Beta-proteobacteria
53719139	Burkholderia pseudomallei K96243	Beta-proteobacteria
53723558	Burkholderia mallei ATCC 23344	Beta-proteobacteria
67639892	Burkholderia mallei GB8 horse 4	Beta-proteobacteria
76810144	Burkholderia pseudomallei 1710b	Beta-proteobacteria
121600326	Burkholderia mallei SAVP1	Beta-proteobacteria
124385284	Burkholderia mallei NCTC 10229	Beta-proteobacteria
126440772	Burkholderia pseudomallei 668	Beta-proteobacteria
126450398	Burkholderia mallei NCTC 10247	Beta-proteobacteria
126452489	Burkholderia pseudomallei 1106a	Beta-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
134277449	Burkholderia pseudomallei 305	Beta-proteobacteria
167002252	Burkholderia mallei PRL-20	Beta-proteobacteria
167719257	Burkholderia pseudomallei DM98	Beta-proteobacteria
167738254	Burkholderia pseudomallei 14	Beta-proteobacteria
167815444	Burkholderia pseudomallei 91	Beta-proteobacteria
167823855	Burkholderia pseudomallei 9	Beta-proteobacteria
167845396	Burkholderia pseudomallei B7210	Beta-proteobacteria
167893937	Burkholderia pseudomallei 7894	Beta-proteobacteria
167902387	Burkholderia pseudomallei NCTC 13177	Beta-proteobacteria
167910629	Burkholderia pseudomallei 112	Beta-proteobacteria
167918658	Burkholderia pseudomallei BCC215	Beta-proteobacteria
217421435	Burkholderia pseudomallei 576	Beta-proteobacteria
226197238	Burkholderia pseudomallei Pakistan 9	Beta-proteobacteria
237812557	Burkholderia pseudomallei MSHR346	Beta-proteobacteria
242315690	Burkholderia pseudomallei 1106b	Beta-proteobacteria
254178572	Burkholderia mallei ATCC 10399	Beta-proteobacteria
254179542	Burkholderia pseudomallei 1655	Beta-proteobacteria
254189067	Burkholderia pseudomallei Pasteur 52237	Beta-proteobacteria
254197294	Burkholderia pseudomallei S13	Beta-proteobacteria
254199960	Burkholderia mallei FMH	Beta-proteobacteria
254206293	Burkholderia mallei JHU	Beta-proteobacteria
254261861	Burkholderia pseudomallei 1710a	Beta-proteobacteria
254297419	Burkholderia pseudomallei 406e	Beta-proteobacteria
254358292	Burkholderia mallei 2002721280	Beta-proteobacteria
206560255	Burkholderia cenocepacia J2315	Beta-proteobacteria
170703618	Burkholderia ambifaria IOP40-10	Beta-proteobacteria
78066590	Burkholderia sp. 383	Beta-proteobacteria
83721572	Burkholderia thailandensis E264	Beta-proteobacteria
167581696	Burkholderia thailandensis TXDOH	Beta-proteobacteria
167619812	Burkholderia thailandensis Bt4	Beta-proteobacteria
221198089	Burkholderia multivorans CGD2M	Beta-proteobacteria
221204352	Burkholderia multivorans CGD2	Beta-proteobacteria
134295851	Burkholderia vietnamiensis G4	Beta-proteobacteria
167562539	Burkholderia oklahomensis EO147	Beta-proteobacteria
167569723	Burkholderia oklahomensis C6786	Beta-proteobacteria
238027063	Burkholderia glumae BGR1	Beta-proteobacteria
167836384	Burkholderia thailandensis MSMB43	Beta-proteobacteria
237653675	Thauera sp. MZ1T	Beta-proteobacteria
95930284	Desulfuromonas acetoxidans DSM 684	Delta-proteoabacteria
118581147	Pelobacter propionicus DSM 2379	Delta-proteoabacteria
124546593	Shewanella putrefaciens 200	Gamma-proteobacteria
222056415	Geobacter sp. FRC-32	Delta-proteoabacteria

Appendix B (*continued*).

gi Number	Species	Class
187923827	Burkholderia phytofirmans PsJN	Beta-proteobacteria
282887685	Burkholderia sp. CCGE1001	Beta-proteobacteria
170692344	Burkholderia graminis C4D1M	Beta-proteobacteria
209522220	Burkholderia sp. H160	Beta-proteobacteria
91783466	Burkholderia xenovorans LB400	Beta-proteobacteria
197119058	Geobacter bemidjiensis Bem	Delta-proteoabacteria
186475727	Burkholderia phymatum STM815	Beta-proteobacteria
272529353	Burkholderia sp. CCGE1002	Beta-proteobacteria
148264661	Geobacter uraniireducens Rf4	Delta-proteoabacteria
255057776	Geobacter sp. M18	Delta-proteoabacteria
78222633	Geobacter metallireducens GS-15	Delta-proteoabacteria
39996625	Geobacter sulfurreducens PCA	Delta-proteoabacteria
124266448	Methylibium petroleiphilum PM1	Beta-proteobacteria
253700182	Geobacter sp. M21	Delta-proteobacteria
227422546	Denitrovibrio acetiphilus DSM 12809	Deferribacteres
189424151	Geobacter lovleyi SZ	Delta-proteobacteria
256758263	Thiomonas intermedia K12	Beta-proteobacteria
114776318	Mariprofundus ferrooxydans PV-1	Zeta-proteobacteria
189218244	Methylacidiphilum infernorum V4	unclassified Verrucomicrobia
225159269	Opitutaceae bacterium TAV2	Opitutae
254432880	Cyanobium sp. PCC 7001	Cyanobacteria
81300366	Synechococcus elongatus PCC 7942	Cyanobacteria
56750024	Synechococcus elongatus PCC 6301	Cyanobacteria
171909626	Verrucomicrobium spinosum DSM 4136	Verrucomicrobiae
196232822	Chthoniobacter flavus Ellin428	Verrucomicrobiae
239814806	Variovorax paradoxus S110	Beta-proteobacteria
87301096	Synechococcus sp. WH 5701	Cyanobacteria
113954655	Synechococcus sp. CC9311	Cyanobacteria
87125501	Synechococcus sp. RS9917	Cyanobacteria
223936758	bacterium Ellin514	Verrucomicrobiae
78185087	Synechococcus sp. CC9902	Cyanobacteria
116072338	Synechococcus sp. BL107	Cyanobacteria
124006216	Microscilla marina ATCC 23134	Sphingobacteria
126645437	Algoriphagus sp. PR1	Sphingobacteria
255034849	Dyadobacter fermentans DSM 18053	Sphingobacteria
116073370	Synechococcus sp. RS9916	Cyanobacteria
254476970	Ruegeria sp. R11	Alpha-proteobacteria
284036901	Spirosoma linguale DSM 74	Sphingobacteria
148242803	Synechococcus sp. RCC307	Cyanobacteria
218528655	Methylobacterium chloromethanicum CM4	Alpha-proteobacteria
240137186	Methylobacterium extorquens AM1	Alpha-proteobacteria

Appendix B (*continued*).

gi Number	Species	Class
158340427	Acaryochloris marina MBIC11017	Cyanobacteria
227499774	Anaerococcus tetradius ATCC 35098	Clostridia
89901551	Rhodoferax ferrireducens T118	Beta-proteobacteria
149177995	Planctomyces maris DSM 8797	Planctomycetacia
188994502	Porphyromonas gingivalis ATCC 33277	Bacteroidia
149278388	Pedobacter sp. BAL39	Sphingobacteria
255532892	Pedobacter heparinus DSM 2366	Sphingobacteria
172037252	Cyanothece sp. ATCC 51142	Cyanobacteria
153952229	Campylobacter jejuni subsp. doylei 269.97	Epsilon-proteobacteria
227536516	Sphingobacterium spiritivorum ATCC 33300	Sphingobacteria
241893907	Sphingobacterium spiritivorum ATCC 33861	Sphingobacteria
258648419	Prevotella tannerae ATCC 51259	Bacteroidia
207743452	Ralstonia solanacearum IPO1609	Beta-proteobacteria
207723156	Ralstonia solanacearum MolK2	Beta-proteobacteria
167932999	candidate division TM7 single-cell isolate TM7b	Not specified
229539716	Planctomyces limnophilus DSM 3776	Planctomycetacia
281357500	Victivallis vadensis ATCC BAA-548	Lentisphaerae
149200267	Lentisphaera araneosa HTCC2155	Lentisphaerae
261416342	Fibrobacter succinogenes subsp. succinogenes S85	Fibrobacteres
255017998	Listeria monocytogenes FSL F2-515	Bacilli
208742286	Bacillus cereus	Bacilli
229076790	Bacillus cereus Rock4-18	Bacilli
177654897	Bacillus anthracis str. A0174	Bacilli
190566146	Bacillus anthracis Tsiankovskii-I	Bacilli
196038626	Bacillus cereus NVH0597-99	Bacilli
206976716	Bacillus cereus H3081.97	Bacilli
254729575	Cyanophage PSS2	Virus
194014382	Bacillus pumilus ATCC 7061	Bacilli
148747769	Geobacillus virus E2	Bacilli
77020162	Bacillus phage Gamma	Bacilli
85701416	Bacillus phage WBeta	Bacilli
89152515	Bacillus phage Fah	Bacilli

Appendix C. Collection of 2,040 environmental *E. coli* isolates used in this study.

Ability to grow on succinate (Suc^{++}) and catalase expression are indicated.

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0031939416	04AB	A01	ABA01	Bayfront Park beach	Canada Goose (Branta canadensis)	BP6/7/2004CG7-3	07-Jun-04	-	+
0031941467	04AB	A02	ABA02	Dundas sewage treatment plant	Sewage plant final effluent	DS6/2/2004FE1-2	02-Jun-04	-	+
0031941778	04AB	A03	ABA03	Hamilton sewage treatment plant	Sewage plant final effluent	HS6/3/2004FE2-1	03-Jun-04	-	+
0036632478	04AB	A04	ABA04	Hamilton bypass	Sewage plant final effluent	BYP6/2/2004FE2-5	02-Jun-04	-	+
0036632483	04AB	A05	ABA05	Waterdown sewage treatment plant	Sewage plant final effluent	WS6/2/2004FE15-2	02-Jun-04	-	+
0036632481	04AB	A06	ABA06	Hamilton bypass	Sewage plant final effluent	BYP6/1/2004FE1-1	01-Jun-04	-	+
0036635090	04AB	A07	ABA07	Bayfront Park beach	Gull (Larus delawarensis)	BP6/14/2004G2-2	14-Jun-04	-	+
0029880839	04AB	A08	ABA08	Bayfront Park beach	Canada Goose (Branta canadensis)	BP6/21/2004CG7-3	21-Jun-04	-	+
0026737990	04AB	A09	ABA09	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS7/30/2004DG10-5	30-Jul-04	-	+
0031941106	04AB	A10	ABA10	Bayfront Park beach	Beach sand	BP5/10/2004SD1-2	10-May-04	-	+
0026738473	04AB	A11	ABA11	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS7/30/2004DG14-3	30-Jul-04	-	+
0031939747	04AB	A12	ABA12	Bayfront Park beach	Beach sand	bp7/19/2004SD1-2	19-Jul-04	-	+
0036635093	04AB	B01	ABB01	Bayfront Park beach	Gull (Larus delawarensis)	BP6/14/2004G3-3	14-Jun-04	-	+
0034436092	04AB	B02	ABB02	Bayfront Park beach	Beach sand	BP7/19/2004SD2-3	19-Jul-04	-	+
0034437418	04AB	B03	ABB03	Bayfront Park beach	Beach sand	BP7/19/2004SD2-5	19-Jul-04	-	+
0034436097	04AB	B04	ABB04	Bayfront Park beach	Beach sand	BP7/19/2004SD2-6	19-Jul-04	-	+
0034437415	04AB	B05	ABB05	Bayfront Park beach	Beach sand	BP7/19/2004SD2-8	19-Jul-04	-	+
0026736807	04AB	B06	ABB06	Bayfront Park beach	Beach sand	BP7/26/2004SD1-1	26-Jul-04	-	+
0026736808	04AB	B07	ABB07	Bayfront Park beach	Beach sand	BP7/26/2004SD1-2	26-Jul-04	-	+
0026736809	04AB	B08	ABB08	Bayfront Park beach	Beach sand	BP7/26/2004SD1-3	26-Jul-04	-	+
0026738776	04AB	B09	ABB09	Hamilton CSO	Untreated CSO sewage	IN7/28/2004CSO2-3	28-Jul-04	-	+
0026738749	04AB	B10	ABB10	Eastwood	Untreated CSO sewage	EW7/31/2004CSO1-1	31-Jul-04	-	-
0026738751	04AB	B11	ABB11	Eastwood	Untreated CSO sewage	EW7/31/2004CSO1-3	31-Jul-04	-	+
0026738739	04AB	B12	ABB12	Eastwood	Untreated CSO sewage	EW7/31/2004CSO2-1	31-Jul-04	-	+
0029864711	04AB	C01	ABC01	Bayfront Park beach	Beach sand	BP8/9/2004SD1-3	09-Aug-04	-	+
0029864708	04AB	C02	ABC02	Bayfront Park beach	Beach sand	BP8/9/2004SD1-6	09-Aug-04	-	+
0029864696	04AB	C03	ABC03	Bayfront Park beach	Beach sand	BP8/9/2004SD1-9	09-Aug-04	+	+
0029864705	04AB	C04	ABC04	Bayfront Park beach	Beach sand	BP8/9/2004SD2-6	09-Aug-04	-	+
0036630616	04AB	C05	ABC05	Hamilton sewage treatment plant	Sewage plant final effluent	HS7/8/2004FE1-1	08-Jul-04	-	+
0031939252	04AB	C06	ABC06	Hamilton bypass	Sewage plant final effluent	BYP7/14/2004FE1-6	14-Jul-04	-	+
0034437116	04AB	C07	ABC07	Bayfront Park beach	Canada Goose (Branta canadensis)	BP7/19/2004CG6-3	19-Jul-04	-	+
0034437076	04AB	C08	ABC08	Bayfront Park beach	Gull (Larus delawarensis)	BP7/19/2004G5-3	19-Jul-04	-	+
0029864504	04AB	C09	ABC09	Hamilton sewage treatment plant	Sewage plant final effluent	HS8/9/2004FE2-6	09-Aug-04	-	+
0026738424	04AB	C10	ABC10	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS7/30/2004C14-3	30-Jul-04	-	+
0026738422	04AB	C11	ABC11	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS7/30/2004C14-5	30-Jul-04	-	+
0029879123	04AB	C12	ABC12	Hamilton sewage treatment plant	Sewage plant final effluent	HS8/31/2004FE1-2	31-Aug-04	-	+
0026738597	04AB	D01	ABD01	Bayfront Park beach	Beach sand	BP7/26/2004SD2-4	26-Jul-04	-	+
0026736796	04AB	D02	ABD02	Bayfront Park beach	Beach sand	BP7/26/2004SD1-7	26-Jul-04	-	+
0026736789	04AB	D03	ABD03	Bayfront Park beach	Beach sand	BP7/26/2004SD2-2	26-Jul-04	-	+
0029880869	04AB	D04	ABD04	Bayfront Park beach	Gull (Larus delawarensis)	BP6/21/2004G5-3	21-Jun-04	-	+
0029861624	04AB	D05	ABD05	Bayfront Park beach	Beach sand	BP8/16/2004SD1-6	16-Aug-04	-	+
0029861621	04AB	D06	ABD06	Bayfront Park beach	Beach sand	BP8/16/2004SD2-6	16-Aug-04	-	+
0029861619	04AB	D07	ABD07	Bayfront Park beach	Beach sand	BP8/16/2004SD2-8	16-Aug-04	-	+
0029879127	04AB	D08	ABD08	Dundas sewage treatment plant	Sewage plant final effluent	DS8/31/2004FE1-6	31-Aug-04	-	+
0026738753	04AB	D09	ABD09	Eastwood	Untreated CSO sewage	EW7/31/2004CSO1-5	31-Jul-04	-	+
0026739575	04AB	D10	ABD10	Bayfront Park beach	Dog (Canis lupus familiaris)	BP8/3/2004DG1-4	03-Aug-04	-	+
0026740010	04AB	D11	ABD11	Bayfront Park beach	Canada Goose (Branta canadensis)	BP8/3/2004CG7-3	03-Aug-04	-	+
0026737928	04AB	D12	ABD12	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS7/30/2004DG4-2	30-Jul-04	-	+
0036632690	04AB	E01	ABE01	Bayfront Park beach	Gull (Larus delawarensis)	BP4/5/2004G1-2	05-Apr-04	-	+
0036632694	04AB	E02	ABE02	Bayfront Park beach	Gull (Larus delawarensis)	BP4/5/2004G3-1	05-Apr-04	-	+
0026732453	04AB	E03	ABE03	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS7/30/2004C15-3	30-Jul-04	-	+
0034437183	04AB	E04	ABE04	Hamilton sewage treatment plant	Sewage plant final effluent	HS4/27/2004FE2-5	27-Apr-04	-	+
0034435231	04AB	E05	ABE05	Bayfront Park beach	Canada Goose (Branta canadensis)	BP5/3/2004CG2-2	03-May-04	-	+
0029864419	04AB	E06	ABE06	Bayfront Park beach	Gull (Larus delawarensis)	BP8/9/2004G1-3	09-Aug-04	-	+
0029862039	04AB	E07	ABE07	Hamilton bypass	Sewage plant final effluent	BYP7/29/2004FE1-5	29-Jul-04	-	+
0029862003	04AB	E08	ABE08	Hamilton bypass	Sewage plant final effluent	BYP7/31/2004FE2-3	31-Jul-04	-	+
0036633762	04AB	E09	ABE09	Bayfront Park beach	Beach sand	BP7/12/2004SD1-3	12-Jul-04	-	+
0036633761	04AB	E10	ABE10	Bayfront Park beach	Beach sand	BP7/12/2004SD1-4	12-Jul-04	-	+
0036634580	04AB	E11	ABE11	Bayfront Park beach	Beach sand	BP7/12/2004SD1-7	12-Jul-04	-	+
0036634586	04AB	E12	ABE12	Bayfront Park beach	Beach sand	BP7/12/2004SD2-1	12-Jul-04	-	+
0036633748	04AB	F01	ABF01	Bayfront Park beach	Beach sand	BP7/12/2004SD2-5	12-Jul-04	-	+
0029879368	04AB	F02	ABF02	Bayfront Park beach	Beach sand	BP8/23/2004SD1-7	23-Aug-04	-	+
0029879355	04AB	F03	ABF03	Bayfront Park beach	Beach sand	BP8/23/2004SD2-3	23-Aug-04	-	+
0029879352	04AB	F04	ABF04	Bayfront Park beach	Beach sand	BP8/23/2004SD2-6	23-Aug-04	-	+
0029898965	04AB	F05	ABF05	Bayfront Park beach	Beach sand	BP8/30/2004SD1-5	30-Aug-04	-	+
0029898964	04AB	F06	ABF06	Bayfront Park beach	Beach sand	BP8/30/2004SD1-6	30-Aug-04	-	+
0029898961	04AB	F07	ABF07	Bayfront Park beach	Beach sand	BP8/30/2004SD1-9	30-Aug-04	-	+
0029898943	04AB	F08	ABF08	Bayfront Park beach	Beach sand	BP8/30/2004SD2-4	30-Aug-04	-	+
0029898947	04AB	F09	ABF09	Bayfront Park beach	Beach sand	BP8/30/2004SD2-8	30-Aug-04	-	+
0029898948	04AB	F10	ABF10	Bayfront Park beach	Beach sand	BP8/30/2004SD2-9	30-Aug-04	-	+
0029879599	04AB	F11	ABF11	Bayfront Park beach	Beach sand	BP5/10/2004SD1-3	10-May-04	-	+
0029879600	04AB	F12	ABF12	Bayfront Park beach	Beach sand	BP5/10/2004SD1-4	10-May-04	-	+
0029881153	04AB	G01	ABG01	Bayfront Park beach	Beach sand	BP5/10/2004SD1-8	10-May-04	-	+
0029881138	04AB	G02	ABG02	Bayfront Park beach	Beach sand	BP5/10/2004SD2-6	10-May-04	-	+
0029898817	04AB	G03	ABG03	Bayfront Park beach	Canada Goose (Branta canadensis)	BP8/30/2004CG5-3	30-Aug-04	-	+
0029879572	04AB	G04	ABG04	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS8/27/2004C9-4	27-Aug-04	-	+
0029879568	04AB	G05	ABG05	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS8/27/2004C11-5	27-Aug-04	-	+
0029878037	04AB	G06	ABG06	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO3-3	14-Sep-04	+	+
0031921885	04AB	G07	ABG07	Main and King	Untreated CSO sewage	MK5/4/2004CSO1-1	04-May-04	-	+
0031921873	04AB	G08	ABG08	Eastwood	Untreated CSO sewage	EW5/4/2004CSO1-2	04-May-04	-	+
0029880844	04AB	G09	ABG09	Bayfront Park beach	Canada Goose (Branta canadensis)	BP6/21/2004CG5-3	21-Jun-04	-	+
0029878509	04AB	G10	ABG10	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS9/7/2004DG7-5	07-Sep-04	-	+
0031942474	04AB	G11	ABG11	Bayfront Park beach	Beach sand	BP5/17/2004SD1-9	17-May-04	-	+
0029880225	04AB	G12	ABG12	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS8/27/2004C23-3	27-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0029880227	04AB	H01	ABH01	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS8/27/2004C23-5	27-Aug-04	-	+
0029880213	04AB	H02	ABH02	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS8/27/2004DG3-4	27-Aug-04	-	+
0029877761	04AB	H03	ABH03	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS9/7/2004C13-3	07-Sep-04	-	+
0029878543	04AB	H04	ABH04	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS9/7/2004C13-4	07-Sep-04	-	+
0029878544	04AB	H05	ABH05	Hamilton SPCA Animal Shelter	Cat (Felis catus)	AS9/7/2004C13-5	07-Sep-04	-	+
0029878521	04AB	H06	ABH06	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS9/7/2004DG4-1	07-Sep-04	-	+
0029879177	04AB	H07	ABH07	Dundas sewage treatment plant	Sewage plant final effluent	DS9/8/2004FE33-2	08-Sep-04	-	+
0029864516	04AB	H08	ABH08	Hamilton sewage treatment plant	Sewage plant final effluent	HS8/9/2004FE2-3	09-Aug-04	-	+
0031939287	04AB	H09	ABH09	Bayfront Park beach	Beach sand	bp7/19/2004SD1-1	19-Jul-04	-	+
0034435236	04AB	H10	ABH10	Bayfront Park beach	Dog (Canis lupus familiaris)	BP5/3/2004DG1-1	03-May-04	-	+
0029861638	04AB	H11	ABH11	Bayfront Park beach	Beach sand	BP8/16/2004SD1-1	16-Aug-04	-	-
0026739984	04AB	H12	ABH12	Bayfront Park beach	Gull (Larus delawarensis)	BP8/3/2004G1-2	03-Aug-04	-	+
0031940098	04AH	A01	AHA01	Toronto	Beach sand	KW26/14/2004SD2-6	14-Jun-04	-	+
0031940097	04AH	A02	AHA02	Toronto	Beach sand	KW26/14/2004SD2-7	14-Jun-04	-	+
0029860247	04AH	A03	AHA03	Toronto	Beach sand	KW36/7/2004SD2-7	07-Jun-04	-	+
0031940096	04AH	A04	AHA04	Toronto	Beach sand	KW26/14/2004SD2-8	14-Jun-04	-	+
0031940095	04AH	A05	AHA05	Toronto	Beach sand	KW26/14/2004SD2-9	14-Jun-04	+	+
0031940112	04AH	A06	AHA06	Toronto	Beach sand	KW36/14/2004SD1-4	14-Jun-04	-	+
0031940115	04AH	A07	AHA07	Toronto	Beach sand	KW36/14/2004SD1-8	14-Jun-04	-	+
0031940128	04AH	A08	AHA08	Toronto	Beach sand	KW36/14/2004SD2-3	14-Jun-04	-	+
0031940127	04AH	A09	AHA09	Toronto	Beach sand	KW36/14/2004SD2-4	14-Jun-04	-	+
0031940124	04AH	A10	AHA10	Toronto	Beach sand	KW36/14/2004SD2-7	14-Jun-04	-	+
0031940121	04AH	A11	AHA11	Toronto	Beach sand	KW36/14/2004SD2-10	14-Jun-04	-	+
0031940135	04AH	A12	AHA12	Toronto	Beach sand	CI16/14/2004SD3-4	14-Jun-04	-	+
0031940134	04AH	B01	AHB01	Toronto	Beach sand	CI16/14/2004SD3-3	14-Jun-04	-	+
0031940137	04AH	B02	AHB02	Toronto	Beach sand	CI16/14/2004SD3-6	14-Jun-04	+	+
0031940139	04AH	B03	AHB03	Toronto	Beach sand	CI16/14/2004SD3-8	14-Jun-04	-	+
0031940141	04AH	B04	AHB04	Toronto	Beach sand	CI16/14/2004SD3-10	14-Jun-04	-	+
0031940142	04AH	B05	AHB05	Toronto	Beach sand	CI16/14/2004SD3-11	14-Jun-04	-	+
0031940152	04AH	B06	AHB06	Toronto	Beach sand	CI26/14/2004SD3-2	14-Jun-04	-	+
0031939717	04AH	B07	AHB07	Toronto	Beach sand	CI26/14/2004SD3-3	14-Jun-04	-	+
0031940149	04AH	B08	AHB08	Toronto	Beach sand	CI26/14/2004SD3-5	14-Jun-04	-	+
0031940146	04AH	B09	AHB09	Toronto	Beach sand	CI26/14/2004SD3-10	14-Jun-04	+	+
0031938659	04AH	B10	AHB10	Toronto	Beach sand	KW17/19/2004SD1-2	19-Jul-04	-	+
0031938658	04AH	B11	AHB11	Toronto	Beach sand	KW17/19/2004SD1-3	19-Jul-04	-	+
0031938657	04AH	B12	AHB12	Toronto	Beach sand	KW17/19/2004SD1-4	19-Jul-04	-	+
0026733017	04AH	C01	AHC01	Toronto	Beach sand	CI28/3/2004SD1-7	03-Aug-04	-	+
0026733018	04AH	C02	AHC02	Toronto	Beach sand	CI28/3/2004SD1-8	03-Aug-04	-	+
0026733001	04AH	C03	AHC03	Toronto	Beach sand	CI28/3/2004SD2-3	03-Aug-04	-	+
0026733000	04AH	C04	AHC04	Toronto	Beach sand	CI28/3/2004SD2-4	03-Aug-04	-	+
0026732999	04AH	C05	AHC05	Toronto	Beach sand	CI28/3/2004SD2-5	03-Aug-04	-	+
0026732998	04AH	C06	AHC06	Toronto	Beach sand	CI28/3/2004SD2-6	03-Aug-04	-	+
0026732997	04AH	C07	AHC07	Toronto	Beach sand	CI28/3/2004SD2-7	03-Aug-04	-	+
0026738109	04AH	C08	AHC08	Toronto	Beach water	KW3E8/3/2004WC1-5	03-Aug-04	-	+
0031938673	04AH	C09	AHC09	Toronto	Beach sand	KW17/19/2004SD2-2	19-Jul-04	-	+
0029898574	04AH	C10	AHC10	Toronto	Beach water	KW18/17/2004WK1-7	17-Aug-04	-	+
0029898571	04AH	C11	AHC11	Toronto	Beach water	KW18/17/2004WK1-10	17-Aug-04	-	-
0029898561	04AH	C12	AHC12	Toronto	Beach water	KW18/17/2004WK2-1	17-Aug-04	-	+
0029898564	04AH	D01	AHD01	Toronto	Beach water	KW18/17/2004WK2-4	17-Aug-04	-	+
0029898565	04AH	D02	AHD02	Toronto	Beach water	KW18/17/2004WK2-5	17-Aug-04	-	+
0029898566	04AH	D03	AHD03	Toronto	Beach water	KW18/17/2004WK2-6	17-Aug-04	-	+
0029898570	04AH	D04	AHD04	Toronto	Beach water	KW18/17/2004WK2-10	17-Aug-04	-	+
0029898556	04AH	D05	AHD05	Toronto	Beach water	KW28/17/2004WA1-1	17-Aug-04	-	+
0029898555	04AH	D06	AHD06	Toronto	Beach water	KW28/17/2004WA1-2	17-Aug-04	-	+
0029898554	04AH	D07	AHD07	Toronto	Beach water	KW28/17/2004WA1-3	17-Aug-04	-	+
0029898553	04AH	D08	AHD08	Toronto	Beach water	KW28/17/2004WA1-4	17-Aug-04	-	+
0029898551	04AH	D09	AHD09	Toronto	Beach water	KW28/17/2004WA1-6	17-Aug-04	-	+
0029895774	04AH	D10	AHD10	Toronto	Beach water	KW28/17/2004WA1-9	17-Aug-04	-	+
0029895754	04AH	D11	AHD11	Toronto	Beach water	KW28/17/2004WA1-10	17-Aug-04	-	+
0029895755	04AH	D12	AHD12	Toronto	Beach water	KW28/17/2004WA1-11	17-Aug-04	-	+
0029895740	04AH	E01	AHE01	Toronto	Beach water	KW28/17/2004WA2-7	17-Aug-04	-	+
0029895729	04AH	E02	AHE02	Toronto	Beach water	KW28/17/2004WA2-11	17-Aug-04	-	+
0029895730	04AH	E03	AHE03	Toronto	Beach water	KW28/17/2004WA2-12	17-Aug-04	-	+
0029895732	04AH	E04	AHE04	Toronto	Beach water	KW28/17/2004WK1-2	17-Aug-04	-	+
0029895736	04AH	E05	AHE05	Toronto	Beach water	KW28/17/2004WK1-6	17-Aug-04	-	+
0029895737	04AH	E06	AHE06	Toronto	Beach water	KW28/17/2004WK1-7	17-Aug-04	-	+
0029895722	04AH	E07	AHE07	Toronto	Beach water	KW28/17/2004WK2-1	17-Aug-04	-	+
0029895720	04AH	E08	AHE08	Toronto	Beach water	KW28/17/2004WK2-3	17-Aug-04	-	+
0026733453	04AH	E09	AHE09	Toronto	Sewage plant final effluent	AF28/9/2004FE2-10	09-Aug-04	-	+
0026737982	04AH	E10	AHE10	Toronto	Sewage plant final effluent	AF18/3/2004FE1-7	03-Aug-04	-	+
0026737981	04AH	E11	AHE11	Toronto	Sewage plant final effluent	AF18/3/2004FE1-8	03-Aug-04	-	+
0026738689	04AH	E12	AHE12	Toronto	Sewage plant final effluent	AF18/3/2004FE1-12	03-Aug-04	-	+
0026737976	04AH	F01	AHF01	Toronto	Sewage plant final effluent	AF18/3/2004FE2-6	03-Aug-04	-	+
0026738671	04AH	F02	AHF02	Toronto	Sewage plant final effluent	AF18/3/2004FE2-7	03-Aug-04	-	+
0026738672	04AH	F03	AHF03	Toronto	Sewage plant final effluent	AF18/3/2004FE2-8	03-Aug-04	-	+
0026738680	04AH	F04	AHF04	Toronto	Sewage plant final effluent	AF28/3/2004FE1-4	03-Aug-04	-	+
0026738682	04AH	F05	AHF05	Toronto	Sewage plant final effluent	AF28/3/2004FE1-6	03-Aug-04	-	+
0026738666	04AH	F06	AHF06	Toronto	Sewage plant final effluent	AF28/3/2004FE1-11	03-Aug-04	-	+
0026738665	04AH	F07	AHF07	Toronto	Sewage plant final effluent	AF28/3/2004FE1-12	03-Aug-04	-	+
0026738647	04AH	F08	AHF08	Toronto	Sewage plant final effluent	AF28/3/2004FE2-8	03-Aug-04	-	+
0026738655	04AH	F09	AHF09	Toronto	Sewage plant final effluent	AF8/3/2004FE1-4	03-Aug-04	-	+
0026738656	04AH	F10	AHF10	Toronto	Sewage plant final effluent	AF8/3/2004FE2-1	03-Aug-04	-	+
0026738657	04AH	F11	AHF11	Toronto	Sewage plant final effluent	AF8/3/2004FE2-4	03-Aug-04	-	-
0026738658	04AH	F12	AHF12	Toronto	Sewage plant final effluent	AF8/3/2004FE2-5	03-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0029879059	04AH	G01	AHG01	Toronto	Sewage plant final effluent	AF19/7/2004FE2-14	07-Sep-04	-	+
0029877768	04AH	G02	AHG02	Toronto	Sewage plant final effluent	AF19/7/2004FE1-3	07-Sep-04	-	-
0029879104	04AH	G03	AHG03	Toronto	Sewage plant final effluent	AF19/7/2004FE1-4	07-Sep-04	-	+
0029879093	04AH	G04	AHG04	Toronto	Sewage plant final effluent	AF19/7/2004FE1-9	07-Sep-04	-	+
0029879090	04AH	G05	AHG05	Toronto	Sewage plant final effluent	AF19/7/2004FE1-12	07-Sep-04	-	+
0029879086	04AH	G06	AHG06	Toronto	Sewage plant final effluent	AF19/7/2004FE1-16	07-Sep-04	-	+
0029879083	04AH	G07	AHG07	Toronto	Sewage plant final effluent	AF19/7/2004FE1-19	07-Sep-04	-	+
0029879076	04AH	G08	AHG08	Toronto	Sewage plant final effluent	AF19/7/2004FE2-6	07-Sep-04	-	-
0029879077	04AH	G09	AHG09	Toronto	Sewage plant final effluent	AF19/7/2004FE2-7	07-Sep-04	-	+
0029879082	04AH	G10	AHG10	Toronto	Sewage plant final effluent	AF19/7/2004FE2-12	07-Sep-04	-	+
0029879061	04AH	G11	AHG11	Toronto	Sewage plant final effluent	AF29/7/2004FE1-3	07-Sep-04	-	+
0029879069	04AH	G12	AHG12	Toronto	Sewage plant final effluent	AF19/7/2004FE2-15	07-Sep-04	-	+
0029861026	04AL	A01	ALA01	Hamilton	Beach water	HH16/14/2004W2-3	14-Jun-04	-	+
0029861882	04AL	A02	ALA02	Hamilton	Beach water	HH16/14/2004W2-4	14-Jun-04	-	+
0029861881	04AL	A03	ALA03	Hamilton	Beach water	HH16/14/2004W2-5	14-Jun-04	-	+
0031939465	04AL	A04	ALA04	Hamilton	Beach water	BP6/7/2004WA1-1	07-Jun-04	+	+
0031939466	04AL	A05	ALA05	Hamilton	Beach water	BP6/7/2004WA1-2	07-Jun-04	-	+
0031939467	04AL	A06	ALA06	Hamilton	Beach water	BP6/7/2004WA1-3	07-Jun-04	-	+
0031939468	04AL	A07	ALA07	Hamilton	Beach water	BP6/7/2004WA1-4	07-Jun-04	-	+
0031939469	04AL	A08	ALA08	Hamilton	Beach water	BP6/7/2004WA1-5	07-Jun-04	-	+
0031939470	04AL	A09	ALA09	Hamilton	Beach water	BP6/7/2004WA1-6	07-Jun-04	-	+
0031939482	04AL	A10	ALA10	Hamilton	Beach water	BP6/7/2004WA1-7	07-Jun-04	-	+
0031939477	04AL	A11	ALA11	Hamilton	Beach water	BP6/7/2004WA2-1	07-Jun-04	-	+
0031938273	04AL	A12	ALA12	Hamilton	Beach water	BP6/7/2004WA2-3	07-Jun-04	-	+
0031938272	04AL	B01	ALB01	Hamilton	Beach water	BP6/7/2004WA2-5	07-Jun-04	-	+
0031939474	04AL	B02	ALB02	Hamilton	Beach water	BP6/7/2004WA2-6	07-Jun-04	+	+
0031939473	04AL	B03	ALB03	Hamilton	Beach water	BP6/7/2004WA2-4	07-Jun-04	-	+
0031940274	04AL	B04	ALB04	Hamilton	Beach sand	BP6/21/2004SD1-4	21-Jun-04	-	+
0031940273	04AL	B05	ALB05	Hamilton	Beach sand	BP6/21/2004SD1-5	21-Jun-04	-	+
0031940272	04AL	B06	ALB06	Hamilton	Beach sand	BP6/21/2004SD1-6	21-Jun-04	-	+
0031940271	04AL	B07	ALB07	Hamilton	Beach sand	BP6/21/2004SD1-7	21-Jun-04	-	+
0031940270	04AL	B08	ALB08	Hamilton	Beach sand	BP6/21/2004SD1-8	21-Jun-04	-	+
0031940269	04AL	B09	ALB09	Hamilton	Beach sand	BP6/21/2004SD1-9	21-Jun-04	-	+
0031940265	04AL	B10	ALB10	Hamilton	Beach sand	BP6/21/2004SD2-1	21-Jun-04	-	+
0031940264	04AL	B11	ALB11	Hamilton	Beach sand	BP6/21/2004SD2-2	21-Jun-04	-	+
0031940263	04AL	B12	ALB12	Hamilton	Beach sand	BP6/21/2004SD2-3	21-Jun-04	-	+
0031940275	04AL	C01	ALC01	Hamilton	Beach sand	BP6/21/2004SD2-4	21-Jun-04	-	+
0031940276	04AL	C02	ALC02	Hamilton	Beach sand	BP6/21/2004SD2-5	21-Jun-04	+	+
0031940277	04AL	C03	ALC03	Hamilton	Beach sand	BP6/21/2004SD2-6	21-Jun-04	-	+
0031939748	04AL	C04	ALC04	Hamilton	Beach sand	BP6/21/2004SD2-7	21-Jun-04	-	+
0031940279	04AL	C05	ALC05	Hamilton	Beach sand	BP6/21/2004SD2-8	21-Jun-04	-	+
0031940280	04AL	C06	ALC06	Hamilton	Beach sand	BP6/21/2004SD2-9	21-Jun-04	-	+
0029881989	04AL	C07	ALC07	Hamilton	Beach water	BP6/21/2004WA1-1	21-Jun-04	-	+
0029881988	04AL	C08	ALC08	Hamilton	Beach water	BP6/21/2004WA1-2	21-Jun-04	-	+
0029881987	04AL	C09	ALC09	Hamilton	Beach water	BP6/21/2004WA1-3	21-Jun-04	-	+
0029881975	04AL	C10	ALC10	Hamilton	Beach water	BP6/21/2004WA1-4	21-Jun-04	-	+
0029881976	04AL	C11	ALC11	Hamilton	Beach water	BP6/21/2004WA1-5	21-Jun-04	-	+
0029881977	04AL	C12	ALC12	Hamilton	Beach water	BP6/21/2004WA1-6	21-Jun-04	+	+
0029881983	04AL	D01	ALD01	Hamilton	Beach water	BP6/21/2004WA2-1	21-Jun-04	-	+
0029881984	04AL	D02	ALD02	Hamilton	Beach water	BP6/21/2004WA2-2	21-Jun-04	-	+
0029881985	04AL	D03	ALD03	Hamilton	Beach water	BP6/21/2004WA2-3	21-Jun-04	-	+
0029881986	04AL	D04	ALD04	Hamilton	Beach water	BP6/21/2004WA2-4	21-Jun-04	-	+
0029881974	04AL	D05	ALD05	Hamilton	Beach water	BP6/21/2004WA2-5	21-Jun-04	-	+
0029881973	04AL	D06	ALD06	Hamilton	Beach water	BP6/21/2004WA2-6	21-Jun-04	-	+
0029881972	04AL	D07	ALD07	Hamilton	Beach water	BP6/21/2004WA2-7	21-Jun-04	-	+
0029881966	04AL	D08	ALD08	Hamilton	Beach water	BP6/21/2004WK1-1	21-Jun-04	-	+
0029881965	04AL	D09	ALD09	Hamilton	Beach water	BP6/21/2004WK1-2	21-Jun-04	-	+
0029881964	04AL	D10	ALD10	Hamilton	Beach water	BP6/21/2004WK1-3	21-Jun-04	-	+
0029881963	04AL	D11	ALD11	Hamilton	Beach water	BP6/21/2004WK1-4	21-Jun-04	+	+
0029881951	04AL	D12	ALD12	Hamilton	Beach water	BP6/21/2004WK1-5	21-Jun-04	+	+
0029881952	04AL	E01	ALE01	Hamilton	Beach water	BP6/21/2004WK1-6	21-Jun-04	-	+
0029881953	04AL	E02	ALE02	Hamilton	Beach water	BP6/21/2004WK1-7	21-Jun-04	+	+
0029881954	04AL	E03	ALE03	Hamilton	Beach water	BP6/21/2004WK1-8	21-Jun-04	+	+
0029881958	04AL	E04	ALE04	Hamilton	Beach water	BP6/21/2004WK2-1	21-Jun-04	+	+
0029881959	04AL	E05	ALE05	Hamilton	Beach water	BP6/21/2004WK2-2	21-Jun-04	+	+
0029881960	04AL	E06	ALE06	Hamilton	Beach water	BP6/21/2004WK2-3	21-Jun-04	-	+
0029881961	04AL	E07	ALE07	Hamilton	Beach water	BP6/21/2004WK2-4	21-Jun-04	+	+
0029881962	04AL	E08	ALE08	Hamilton	Beach water	BP6/21/2004WK2-5	21-Jun-04	-	+
0029881950	04AL	E09	ALE09	Hamilton	Beach water	BP6/21/2004WK2-6	21-Jun-04	-	+
0029881949	04AL	E10	ALE10	Hamilton	Beach water	BP6/21/2004WK2-7	21-Jun-04	-	+
0029881948	04AL	E11	ALE11	Hamilton	Beach water	BP6/21/2004WK2-8	21-Jun-04	-	+
0029881943	04AL	E12	ALE12	Hamilton	Beach sand	BP6/21/2004SD1-1	21-Jun-04	-	+
0029881942	04AL	F01	ALF01	Hamilton	Beach sand	BP6/21/2004SD1-2	21-Jun-04	+	+
0029881941	04AL	F02	ALF02	Hamilton	Beach sand	BP6/21/2004SD1-3	21-Jun-04	-	+
0031941002	04AL	F03	ALF03	Hamilton	Beach sand	BP6/7/2004SD2-7	07-Jun-04	-	+
0031940285	04AL	F04	ALF04	Hamilton	Beach water	BP6/21/2004WC1-2	21-Jun-04	-	+
0031940286	04AL	F05	ALF05	Hamilton	Beach water	BP6/21/2004WC1-3	21-Jun-04	-	+
0031941003	04AL	F06	ALF06	Hamilton	Beach sand	BP6/7/2004SD2-8	07-Jun-04	-	+
0031940297	04AL	F07	ALF07	Hamilton	Beach water	BP6/21/2004WC1-5	21-Jun-04	-	+
0031940296	04AL	F08	ALF08	Hamilton	Beach water	BP6/21/2004WC1-6	21-Jun-04	+	+
0031939745	04AL	F09	ALF09	Hamilton	Beach water	BP6/21/2004WC1-7	21-Jun-04	-	+
0031940294	04AL	F10	ALF10	Hamilton	Beach water	BP6/21/2004WC1-8	21-Jun-04	-	+
0031940293	04AL	F11	ALF11	Hamilton	Beach water	BP6/21/2004WC1-9	21-Jun-04	-	+
0031940292	04AL	F12	ALF12	Hamilton	Beach water	BP6/21/2004WC1-10	21-Jun-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0031940291	04AL	G01	ALG01	Hamilton	Beach water	BP6/21/2004WC1-11	21-Jun-04	-	+
0031940290	04AL	G02	ALG02	Hamilton	Beach water	BP6/21/2004WC1-12	21-Jun-04	-	+
0031940289	04AL	G03	ALG03	Hamilton	Beach water	BP6/21/2004WC2-1	21-Jun-04	-	+
0031940288	04AL	G04	ALG04	Hamilton	Beach water	BP6/21/2004WC2-2	21-Jun-04	-	+
0031940287	04AL	G05	ALG05	Hamilton	Beach water	BP6/21/2004WC2-3	21-Jun-04	-	+
0031940299	04AL	G06	ALG06	Hamilton	Beach water	BP6/21/2004WC2-4	21-Jun-04	-	+
0031941004	04AL	G07	ALG07	Hamilton	Beach sand	BP6/7/2004SD2-9	07-Jun-04	-	+
0031940301	04AL	G08	ALG08	Hamilton	Beach water	BP6/21/2004WC2-6	21-Jun-04	-	+
0031940302	04AL	G09	ALG09	Hamilton	Beach water	BP6/21/2004WC2-7	21-Jun-04	-	+
0031940303	04AL	G10	ALG10	Hamilton	Beach water	BP6/21/2004WC2-8	21-Jun-04	-	+
0031940304	04AL	G11	ALG11	Hamilton	Beach water	BP6/21/2004WC2-9	21-Jun-04	-	+
0031940305	04AL	G12	ALG12	Hamilton	Beach water	BP6/21/2004WC2-10	21-Jun-04	-	+
0036635094	04AS	A01	ASA01	Hamilton	Gull (Larus delawarensis)	BP6/14/2004G4-1	14-Jun-04	-	+
0036635106	04AS	A02	ASA02	Hamilton	Gull (Larus delawarensis)	BP6/14/2004G4-2	14-Jun-04	+	+
0036635105	04AS	A03	ASA03	Hamilton	Gull (Larus delawarensis)	BP6/14/2004G4-3	14-Jun-04	-	+
0036635104	04AS	A04	ASA04	Hamilton	Gull (Larus delawarensis)	BP6/14/2004G5-1	14-Jun-04	-	+
0036635103	04AS	A05	ASA05	Hamilton	Gull (Larus delawarensis)	BP6/14/2004G5-2	14-Jun-04	-	+
0036635102	04AS	A06	ASA06	Hamilton	Gull (Larus delawarensis)	BP6/14/2004G5-3	14-Jun-04	-	+
0041014464	04AS	A07	ASA07	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-1	22-Jun-04	-	+
0041014463	04AS	A08	ASA08	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-2	22-Jun-04	-	+
0041014462	04AS	A09	ASA09	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-3	22-Jun-04	-	+
0041014461	04AS	A10	ASA10	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-4	22-Jun-04	-	+
0041014460	04AS	A11	ASA11	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-5	22-Jun-04	-	+
0041014459	04AS	A12	ASA12	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-6	22-Jun-04	+	+
0041014447	04AS	B01	ASB01	Hamilton	Sewage plant final effluent	HS6/22/2004FE1-7	22-Jun-04	-	+
0041014448	04AS	B02	ASB02	Hamilton	Sewage plant final effluent	HS6/22/2004FE2-1	22-Jun-04	-	+
0041014449	04AS	B03	ASB03	Hamilton	Sewage plant final effluent	HS6/22/2004FE2-2	22-Jun-04	-	-
0041014450	04AS	B04	ASB04	Hamilton	Sewage plant final effluent	HS6/22/2004FE2-3	22-Jun-04	-	+
0041014451	04AS	B05	ASB05	Hamilton	Sewage plant final effluent	HS6/22/2004FE2-4	22-Jun-04	-	+
0041014452	04AS	B06	ASB06	Hamilton	Sewage plant final effluent	HS6/22/2004FE2-5	22-Jun-04	-	+
0041014453	04AS	B07	ASB07	Hamilton	Sewage plant final effluent	HS6/22/2004FE2-6	22-Jun-04	-	+
0031940328	04AS	B08	ASB08	Hamilton	Sewage plant final effluent	DS6/16/2004FE1-1	16-Jun-04	-	+
0031940329	04AS	B09	ASB09	Hamilton	Sewage plant final effluent	DS6/16/2004FE1-3	16-Jun-04	-	+
0031940330	04AS	B10	ASB10	Hamilton	Sewage plant final effluent	WS6/16/2004FE1-2	16-Jun-04	+	+
0031940331	04AS	B11	ASB11	Hamilton	Sewage plant final effluent	WS6/16/2004FE1-3	16-Jun-04	-	+
0031940332	04AS	B12	ASB12	Hamilton	Sewage plant final effluent	WS6/16/2004FE1-4	16-Jun-04	-	+
0031940333	04AS	C01	ASC01	Hamilton	Sewage plant final effluent	WS6/16/2004FE1-5	16-Jun-04	-	+
0031940334	04AS	C02	ASC02	Hamilton	Sewage plant final effluent	WS6/16/2004FE1-6	16-Jun-04	+	-
0031940344	04AS	C03	ASC03	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G2-1	21-Jun-04	-	+
0031940343	04AS	C04	ASC04	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G2-2	21-Jun-04	-	+
0031940342	04AS	C05	ASC05	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G2-3	21-Jun-04	-	+
0031940341	04AS	C06	ASC06	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G3-1	21-Jun-04	-	+
0031940340	04AS	C07	ASC07	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G3-2	21-Jun-04	-	+
0031940339	04AS	C08	ASC08	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G4-1	21-Jun-04	-	+
0031940338	04AS	C09	ASC09	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G4-2	21-Jun-04	-	+
0031940337	04AS	C10	ASC10	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G5-1	21-Jun-04	-	+
0029880870	04AS	C11	ASC11	Hamilton	Gull (Larus delawarensis)	BP6/21/2004G5-2	21-Jun-04	-	+
0029898168	04AS	C12	ASC12	Hamilton	Cat (Felis catus)	AS9/7/2004C10-3	07-Sep-04	-	+
0029880847	04AS	D01	ASD01	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG1-1	21-Jun-04	-	+
0029880848	04AS	D02	ASD02	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG1-2	21-Jun-04	-	+
0029880849	04AS	D03	ASD03	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG1-3	21-Jun-04	-	+
0029880850	04AS	D04	ASD04	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG2-1	21-Jun-04	-	+
0029880851	04AS	D05	ASD05	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG2-2	21-Jun-04	-	+
0029880852	04AS	D06	ASD06	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG2-3	21-Jun-04	-	+
0029880853	04AS	D07	ASD07	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG3-1	21-Jun-04	-	+
0029880854	04AS	D08	ASD08	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG3-2	21-Jun-04	-	+
0029880855	04AS	D09	ASD09	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG3-3	21-Jun-04	-	+
0029880856	04AS	D10	ASD10	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG4-1	21-Jun-04	-	+
0029880857	04AS	D11	ASD11	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG4-2	21-Jun-04	-	+
0029880858	04AS	D12	ASD12	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG4-3	21-Jun-04	-	+
0029880846	04AS	E01	ASE01	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG5-1	21-Jun-04	-	+
0029880845	04AS	E02	ASE02	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG5-2	21-Jun-04	-	+
0031942605	04AS	E03	ASE03	Hamilton	Beach sand	BP5/25/2004SD2-1	25-May-04	-	+
0029880843	04AS	E04	ASE04	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG6-1	21-Jun-04	-	+
0029880842	04AS	E05	ASE05	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG6-2	21-Jun-04	-	+
0029880841	04AS	E06	ASE06	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG6-3	21-Jun-04	-	+
0029880840	04AS	E07	ASE07	Hamilton	Canada Goose (Branta canadensis)	BP6/21/2004CG7-1	21-Jun-04	-	+
0036632198	04AS	E08	ASE08	Hamilton	Beach water	BP4/5/2004WA1-6	05-Apr-04	+	+
0026737993	04AS	E09	ASE09	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG8-4	30-Jul-04	-	+
0026738501	04AS	E10	ASE10	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG8-5	30-Jul-04	-	+
0026737994	04AS	E11	ASE11	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG9-1	30-Jul-04	-	+
0026738499	04AS	E12	ASE12	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG9-2	30-Jul-04	-	+
0026738498	04AS	F01	ASF01	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG9-3	30-Jul-04	-	+
0026738497	04AS	F02	ASF02	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG9-4	30-Jul-04	-	+
0026738496	04AS	F03	ASF03	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG9-5	30-Jul-04	-	+
0026738495	04AS	F04	ASF04	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG10-1	30-Jul-04	-	+
0026738494	04AS	F05	ASF05	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG10-2	30-Jul-04	-	+
0026737991	04AS	F06	ASF06	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG10-3	30-Jul-04	-	+
0026738492	04AS	F07	ASF07	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG10-4	30-Jul-04	-	+
0036632195	04AS	F08	ASF08	Hamilton	Beach water	BP4/5/2004WA1-7	05-Apr-04	-	+
0026738479	04AS	F09	ASF09	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG11-1	30-Jul-04	-	+
0026738477	04AS	F10	ASF10	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG13-4	30-Jul-04	-	+
0041014445	04AS	F11	ASF11	Hamilton	Sewage plant final effluent	HS6/23/2004FE1-1	23-Jun-04	-	+
0041014444	04AS	F12	ASF12	Hamilton	Sewage plant final effluent	HS6/23/2004FE1-4	23-Jun-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0041014443	04AS	G01	ASG01	Hamilton	Sewage plant final effluent	HS6/23/2004FE1-5	23-Jun-04	-	+
0041014442	04AS	G02	ASG02	Hamilton	Sewage plant final effluent	HS6/23/2004FE2-1	23-Jun-04	-	+
0041014441	04AS	G03	ASG03	Hamilton	Sewage plant final effluent	HS6/23/2004FE2-2	23-Jun-04	-	+
0041014440	04AS	G04	ASG04	Hamilton	Sewage plant final effluent	HS6/23/2004FE2-3	23-Jun-04	-	+
0041014439	04AS	G05	ASG05	Hamilton	Sewage plant final effluent	HS6/23/2004FE2-4	23-Jun-04	-	+
0041014438	04AS	G06	ASG06	Hamilton	Sewage plant final effluent	HS6/23/2004FE2-5	23-Jun-04	-	+
0041014437	04AS	G07	ASG07	Hamilton	Sewage plant final effluent	HS6/23/2004FE2-6	23-Jun-04	-	+
0041014426	04AS	G08	ASG08	Hamilton	Sewage plant final effluent	HS6/24/2004FE1-1	24-Jun-04	-	+
0041014427	04AS	G09	ASG09	Hamilton	Sewage plant final effluent	HS6/24/2004FE1-2	24-Jun-04	-	+
0041014428	04AS	G10	ASG10	Hamilton	Sewage plant final effluent	HS6/24/2004FE1-4	24-Jun-04	+	+
0041014429	04AS	G11	ASG11	Hamilton	Sewage plant final effluent	HS6/24/2004FE1-5	24-Jun-04	-	+
0041014430	04AS	G12	ASG12	Hamilton	Sewage plant final effluent	HS6/24/2004FE1-6	24-Jun-04	-	+
0031938691	04AW	A01	AWA01	Toronto	Beach sand	KW27/19/2004SD1-8	19-Jul-04	-	+
0031938692	04AW	A02	AWA02	Toronto	Beach sand	KW27/19/2004SD1-9	19-Jul-04	-	+
0031938693	04AW	A03	AWA03	Toronto	Beach sand	KW27/19/2004SD1-10	19-Jul-04	-	+
0031938694	04AW	A04	AWA04	Toronto	Beach sand	KW27/19/2004SD1-11	19-Jul-04	-	+
0031938695	04AW	A05	AWA05	Toronto	Beach sand	KW27/19/2004SD1-12	19-Jul-04	-	+
0031938697	04AW	A06	AWA06	Toronto	Beach sand	KW27/19/2004SD2-2	19-Jul-04	-	+
0031938698	04AW	A07	AWA07	Toronto	Beach sand	KW27/19/2004SD2-3	19-Jul-04	-	+
0029880908	04AW	A08	AWA08	Toronto	Beach sand	KW17/5/2004SD1-2	05-Jul-04	-	+
0029880907	04AW	A09	AWA09	Toronto	Beach sand	KW17/5/2004SD1-3	05-Jul-04	-	+
0029880895	04AW	A10	AWA10	Toronto	Beach sand	KW17/5/2004SD1-4	05-Jul-04	-	+
0029880896	04AW	A11	AWA11	Toronto	Beach sand	KW17/5/2004SD1-5	05-Jul-04	-	+
0029880897	04AW	A12	AWA12	Toronto	Beach sand	KW17/5/2004SD1-6	05-Jul-04	-	+
0029880898	04AW	B01	AWB01	Toronto	Beach sand	KW17/5/2004SD1-7	05-Jul-04	-	+
0029880899	04AW	B02	AWB02	Toronto	Beach sand	KW17/5/2004SD1-8	05-Jul-04	-	+
0029880900	04AW	B03	AWB03	Toronto	Beach sand	KW17/5/2004SD1-9	05-Jul-04	-	+
0029880901	04AW	B04	AWB04	Toronto	Beach sand	KW17/5/2004SD1-11	05-Jul-04	-	+
0029880902	04AW	B05	AWB05	Toronto	Beach sand	KW17/5/2004SD1-12	05-Jul-04	-	+
0031937677	04AW	B06	AWB06	Toronto	Beach sand	CI27/5/2004SD1-7	05-Jul-04	-	+
0031937676	04AW	B07	AWB07	Toronto	Beach sand	CI27/5/2004SD1-8	05-Jul-04	-	+
0031937675	04AW	B08	AWB08	Toronto	Beach sand	CI27/5/2004SD1-9	05-Jul-04	-	+
0031937674	04AW	B09	AWB09	Toronto	Beach sand	CI27/5/2004SD1-10	05-Jul-04	-	+
0031937673	04AW	B10	AWB10	Toronto	Beach sand	CI27/5/2004SD1-11	05-Jul-04	+	+
0031937672	04AW	B11	AWB11	Toronto	Beach sand	CI27/5/2004SD1-12	05-Jul-04	-	+
0031937671	04AW	B12	AWB12	Toronto	Beach sand	CI27/5/2004SD1-13	05-Jul-04	-	+
0031937683	04AW	C01	AWC01	Toronto	Beach sand	CI27/5/2004SD1-14	05-Jul-04	-	+
0031937684	04AW	C02	AWC02	Toronto	Beach sand	CI27/5/2004SD1-15	05-Jul-04	-	+
0031937685	04AW	C03	AWC03	Toronto	Beach sand	CI27/5/2004SD1-16	05-Jul-04	-	+
0031937686	04AW	C04	AWC04	Toronto	Beach sand	CI27/5/2004SD1-17	05-Jul-04	-	+
0031937687	04AW	C05	AWC05	Toronto	Beach sand	CI27/5/2004SD1-18	05-Jul-04	-	+
0031937688	04AW	C06	AWC06	Toronto	Beach sand	CI27/5/2004SD1-19	05-Jul-04	-	+
0031937689	04AW	C07	AWC07	Toronto	Beach sand	CI27/5/2004SD1-20	05-Jul-04	-	+
0031937706	04AW	C08	AWC08	Toronto	Beach sand	CI27/5/2004SD2-6	05-Jul-04	-	+
0031937705	04AW	C09	AWC09	Toronto	Beach sand	CI27/5/2004SD2-7	05-Jul-04	-	+
0031937704	04AW	C10	AWC10	Toronto	Beach sand	CI27/5/2004SD2-9	05-Jul-04	+	+
0031937703	04AW	C11	AWC11	Toronto	Beach sand	CI27/5/2004SD2-10	05-Jul-04	-	+
0031937702	04AW	C12	AWC12	Toronto	Beach sand	CI27/5/2004SD2-11	05-Jul-04	+	+
0031937701	04AW	D01	AWD01	Toronto	Beach sand	CI27/5/2004SD2-12	05-Jul-04	-	+
0031937700	04AW	D02	AWD02	Toronto	Beach sand	CI27/5/2004SD2-13	05-Jul-04	+	+
0031937699	04AW	D03	AWD03	Toronto	Beach sand	CI27/5/2004SD2-14	05-Jul-04	-	+
0031937698	04AW	D04	AWD04	Toronto	Beach sand	CI27/5/2004SD2-15	05-Jul-04	-	+
0031937697	04AW	D05	AWD05	Toronto	Beach sand	CI27/5/2004SD2-16	05-Jul-04	-	+
0031937696	04AW	D06	AWD06	Toronto	Beach sand	CI27/5/2004SD2-17	05-Jul-04	-	+
0031937009	04AW	D07	AWD07	Toronto	Beach sand	CI27/5/2004SD2-18	05-Jul-04	-	+
0031937707	04AW	D08	AWD08	Toronto	Beach sand	CI27/5/2004SD2-19	05-Jul-04	-	+
0031937708	04AW	D09	AWD09	Toronto	Beach sand	CI27/5/2004SD2-20	05-Jul-04	-	+
0031938714	04AW	D10	AWD10	Toronto	Beach sand	KW27/19/2004SD2-8	19-Jul-04	-	+
0031938713	04AW	D11	AWD11	Toronto	Beach sand	KW27/19/2004SD2-9	19-Jul-04	-	+
0031938712	04AW	D12	AWD12	Toronto	Beach sand	KW27/19/2004SD2-10	19-Jul-04	-	+
0031924729	04AW	E01	AWE01	Toronto	Beach sand	CI27/12/2004SD1-18	12-Jul-04	-	+
0031924728	04AW	E02	AWE02	Toronto	Beach sand	CI27/12/2004SD1-19	12-Jul-04	+	+
0031938711	04AW	E03	AWE03	Toronto	Beach sand	KW27/19/2004SD2-11	19-Jul-04	-	+
0031924727	04AW	E04	AWE04	Toronto	Beach sand	CI27/12/2004SD1-20	12-Jul-04	-	+
0031938710	04AW	E05	AWE05	Toronto	Beach sand	KW27/19/2004SD2-12	19-Jul-04	-	+
0031938709	04AW	E06	AWE06	Toronto	Beach sand	KW37/19/2004SD1-2	19-Jul-04	-	+
0031938708	04AW	E07	AWE07	Toronto	Beach sand	KW37/19/2004SD1-3	19-Jul-04	-	+
0031938707	04AW	E08	AWE08	Toronto	Beach sand	KW37/19/2004SD1-4	19-Jul-04	-	+
0026733025	04AW	E09	AWE09	Toronto	Beach sand	CI18/3/2004SD2-2	03-Aug-04	-	+
0026733024	04AW	E10	AWE10	Toronto	Beach sand	CI18/3/2004SD2-3	03-Aug-04	-	+
0026733023	04AW	E11	AWE11	Toronto	Beach sand	CI18/3/2004SD2-4	03-Aug-04	-	+
0026733022	04AW	E12	AWE12	Toronto	Beach sand	CI18/3/2004SD2-5	03-Aug-04	-	+
0026733021	04AW	F01	AWF01	Toronto	Beach sand	CI18/3/2004SD2-6	03-Aug-04	-	+
0026733012	04AW	F02	AWF02	Toronto	Beach sand	CI28/3/2004SD1-2	03-Aug-04	-	+
0031938706	04AW	F03	AWF03	Toronto	Beach sand	KW37/19/2004SD1-5	19-Jul-04	-	+
0031938705	04AW	F04	AWF04	Toronto	Beach sand	KW37/19/2004SD1-6	19-Jul-04	-	+
0026733013	04AW	F05	AWF05	Toronto	Beach sand	CI28/3/2004SD1-3	03-Aug-04	-	+
0036633793	04AW	F06	AWF06	Toronto	Beach sand	CI27/19/2004SD2-9	19-Jul-04	-	+
0036633809	04AW	F07	AWF07	Toronto	Beach sand	CI27/19/2004SD2-10	19-Jul-04	+	+
0036633795	04AW	F08	AWF08	Toronto	Beach sand	CI27/19/2004SD2-11	19-Jul-04	-	+
0036633807	04AW	F09	AWF09	Toronto	Beach sand	CI27/19/2004SD2-12	19-Jul-04	-	+
0036633806	04AW	F10	AWF10	Toronto	Beach sand	CI27/19/2004SD2-13	19-Jul-04	-	+
0036633805	04AW	F11	AWF11	Toronto	Beach sand	CI27/19/2004SD2-14	19-Jul-04	-	+
0036633804	04AW	F12	AWF12	Toronto	Beach sand	CI27/19/2004SD2-15	19-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0036633803	04AW	G01	AWG01	Toronto	Beach sand	CI27/19/2004SD2-16	19-Jul-04	-	+
0036633802	04AW	G02	AWG02	Toronto	Beach sand	CI27/19/2004SD2-17	19-Jul-04	-	+
0036633798	04AW	G03	AWG03	Toronto	Beach sand	CI27/19/2004SD2-18	19-Jul-04	-	+
0036633797	04AW	G04	AWG04	Toronto	Beach sand	CI27/19/2004SD2-19	19-Jul-04	-	+
0031924804	04AW	G05	AWG05	Toronto	Beach sand	KW17/12/2004SD1-2	12-Jul-04	-	+
0031924803	04AW	G06	AWG06	Toronto	Beach sand	KW17/12/2004SD1-3	12-Jul-04	-	+
0031924802	04AW	G07	AWG07	Toronto	Beach sand	KW17/12/2004SD1-4	12-Jul-04	-	+
0031924801	04AW	G08	AWG08	Toronto	Beach sand	KW17/12/2004SD1-5	12-Jul-04	-	+
0031924800	04AW	G09	AWG09	Toronto	Beach sand	KW17/12/2004SD1-6	12-Jul-04	-	+
0031924799	04AW	G10	AWG10	Toronto	Beach sand	KW17/12/2004SD1-7	12-Jul-04	-	+
0031924798	04AW	G11	AWG11	Toronto	Beach sand	KW17/12/2004SD1-8	12-Jul-04	-	+
0031924797	04AW	G12	AWG12	Toronto	Beach sand	KW17/12/2004SD1-9	12-Jul-04	-	+
0031941010	04AZ	A01	AZA01	Hamilton	Beach water	HH16/7/2004W1-9	07-Jun-04	-	+
0031941009	04AZ	A02	AZA02	Hamilton	Beach water	HH16/7/2004W1-10	07-Jun-04	-	+
0036632187	04AZ	A03	AZA03	Hamilton	Beach water	BP4/5/2004WA2-1	05-Apr-04	-	+
0031941425	04AZ	A04	AZA04	Hamilton	Beach water	HH16/7/2004W2-2	07-Jun-04	-	+
0031941424	04AZ	A05	AZA05	Hamilton	Beach water	HH16/7/2004W2-3	07-Jun-04	-	+
0031941423	04AZ	A06	AZA06	Hamilton	Beach water	HH16/7/2004W2-4	07-Jun-04	-	+
0031941422	04AZ	A07	AZA07	Hamilton	Beach water	HH16/7/2004W2-5	07-Jun-04	-	+
0031941421	04AZ	A08	AZA08	Hamilton	Beach water	HH16/7/2004W2-7	07-Jun-04	-	+
0031941760	04AZ	A09	AZA09	Hamilton	Beach water	HH16/7/2004W2-8	07-Jun-04	-	+
0031941419	04AZ	A10	AZA10	Hamilton	Beach water	HH16/7/2004W2-10	07-Jun-04	-	+
0031941761	04AZ	A11	AZA11	Hamilton	Beach water	HH16/7/2004W2-11	07-Jun-04	-	-
0031941417	04AZ	A12	AZA12	Hamilton	Beach water	HH16/7/2004W2-12	07-Jun-04	-	+
0031941441	04AZ	B01	AZB01	Hamilton	Beach water	BP6/7/2004WK1-1	07-Jun-04	+	+
0031941757	04AZ	B02	AZB02	Hamilton	Beach water	BP6/7/2004WK1-2	07-Jun-04	-	+
0026739557	04AZ	B03	AZB03	Hamilton	Beach sand	BP8/3/2004SD1-4	03-Aug-04	-	+
0036635715	04AZ	B04	AZB04	Hamilton	Beach sand	BP4/5/2004SD2-3	05-Apr-04	-	+
0036635537	04AZ	B05	AZB05	Hamilton	Beach sand	BP4/5/2004SD2-4	05-Apr-04	-	+
0036635536	04AZ	B06	AZB06	Hamilton	Beach sand	BP4/5/2004SD2-5	05-Apr-04	-	+
0036635535	04AZ	B07	AZB07	Hamilton	Beach sand	BP4/5/2004SD2-6	05-Apr-04	+	-
0036635534	04AZ	B08	AZB08	Hamilton	Beach sand	BP4/5/2004SD2-7	05-Apr-04	-	+
0036635533	04AZ	B09	AZB09	Hamilton	Beach sand	BP4/5/2004SD2-8	05-Apr-04	-	+
0036635532	04AZ	B10	AZB10	Hamilton	Beach sand	BP4/5/2004SD2-9	05-Apr-04	-	-
0036633715	04AZ	B11	AZB11	Hamilton	Beach water	BP4/13/2004WA1-1	13-Apr-04	-	+
0036633714	04AZ	B12	AZB12	Hamilton	Beach water	BP4/13/2004WA1-2	13-Apr-04	-	+
0036635539	04AZ	C01	AZC01	Hamilton	Beach water	BP4/13/2004WA1-3	13-Apr-04	-	+
0036635540	04AZ	C02	AZC02	Hamilton	Beach water	BP4/13/2004WA1-4	13-Apr-04	-	+
0036635541	04AZ	C03	AZC03	Hamilton	Beach water	BP4/13/2004WA1-5	13-Apr-04	-	+
0036635542	04AZ	C04	AZC04	Hamilton	Beach water	BP4/13/2004WA1-6	13-Apr-04	-	+
0036635543	04AZ	C05	AZC05	Hamilton	Beach water	BP4/13/2004WA1-7	13-Apr-04	-	+
0036635548	04AZ	C06	AZC06	Hamilton	Beach water	BP4/13/2004WA2-1	13-Apr-04	-	+
0036635549	04AZ	C07	AZC07	Hamilton	Beach water	BP4/13/2004WA2-2	13-Apr-04	-	+
0036635714	04AZ	C08	AZC08	Hamilton	Beach water	BP4/13/2004WA2-3	13-Apr-04	-	+
0036635562	04AZ	C09	AZC09	Hamilton	Beach water	BP4/13/2004WA2-4	13-Apr-04	-	+
0036635561	04AZ	C10	AZC10	Hamilton	Beach water	BP4/13/2004WA2-6	13-Apr-04	-	+
0036635560	04AZ	C11	AZC11	Hamilton	Beach water	BP4/13/2004WA2-7	13-Apr-04	-	+
0036632201	04AZ	C12	AZC12	Hamilton	Beach water	BP4/5/2004WA1-1	05-Apr-04	-	+
0036635584	04AZ	D01	AZD01	Hamilton	Beach water	HH14/26/2004W1-1	26-Apr-04	-	+
0036635583	04AZ	D02	AZD02	Hamilton	Beach water	HH14/26/2004W1-2	26-Apr-04	-	+
0036635582	04AZ	D03	AZD03	Hamilton	Beach water	HH14/26/2004W1-5	26-Apr-04	-	+
0029878501	04AZ	D04	AZD04	Hamilton	Beach water	BP9/7/2004WC1-9	07-Sep-04	-	+
0036635580	04AZ	D05	AZD05	Hamilton	Beach water	HH14/26/2004W2-1	26-Apr-04	-	+
0036635579	04AZ	D06	AZD06	Hamilton	Beach water	HH14/26/2004W2-3	26-Apr-04	-	+
0036635578	04AZ	D07	AZD07	Hamilton	Beach water	HH14/26/2004W2-4	26-Apr-04	-	+
0036635577	04AZ	D08	AZD08	Hamilton	Beach water	HH14/26/2004W2-5	26-Apr-04	-	+
0036635576	04AZ	D09	AZD09	Hamilton	Beach water	BP4/26/2004WC1-2	26-Apr-04	-	+
0036635575	04AZ	D10	AZD10	Hamilton	Beach water	BP4/26/2004WC1-3	26-Apr-04	-	+
0036635587	04AZ	D11	AZD11	Hamilton	Beach water	BP4/26/2004WC1-4	26-Apr-04	-	+
0036635588	04AZ	D12	AZD12	Hamilton	Beach water	BP4/26/2004WC2-1	26-Apr-04	-	+
0036635589	04AZ	E01	AZE01	Hamilton	Beach water	BP4/26/2004WC2-2	26-Apr-04	-	+
0036635590	04AZ	E02	AZE02	Hamilton	Beach water	BP4/26/2004WC2-3	26-Apr-04	-	+
0036635591	04AZ	E03	AZE03	Hamilton	Beach water	BP4/26/2004WC2-5	26-Apr-04	-	+
0036635592	04AZ	E04	AZE04	Hamilton	Beach water	BP4/27/2004WA1-1	27-Apr-04	-	+
0036635593	04AZ	E05	AZE05	Hamilton	Beach water	BP4/27/2004WA1-3	27-Apr-04	-	+
0036635594	04AZ	E06	AZE06	Hamilton	Beach water	BP4/27/2004WA1-4	27-Apr-04	-	-
0036635595	04AZ	E07	AZE07	Hamilton	Beach water	BP4/27/2004WA1-5	27-Apr-04	-	+
0036635596	04AZ	E08	AZE08	Hamilton	Beach water	BP4/27/2004WA1-6	27-Apr-04	-	+
0036635597	04AZ	E09	AZE09	Hamilton	Beach water	BP4/27/2004WA1-7	27-Apr-04	-	+
0036635609	04AZ	E10	AZE10	Hamilton	Beach water	BP4/27/2004WA2-3	27-Apr-04	-	+
0036635608	04AZ	E11	AZE11	Hamilton	Beach water	BP4/27/2004WA2-4	27-Apr-04	-	+
0036635607	04AZ	E12	AZE12	Hamilton	Beach water	BP4/27/2004WA2-5	27-Apr-04	-	+
0036635606	04AZ	F01	AZF01	Hamilton	Beach water	BP4/27/2004WA2-7	27-Apr-04	-	+
0036632197	04AZ	F02	AZF02	Hamilton	Beach water	BP4/5/2004WA1-2	05-Apr-04	-	+
0034437195	04AZ	F03	AZF03	Hamilton	Beach sand	BP4/27/2004SD2-8	27-Apr-04	-	+
0034437194	04AZ	F04	AZF04	Hamilton	Beach sand	BP4/27/2004SD2-9	27-Apr-04	-	+
0034435238	04AZ	F05	AZF05	Hamilton	Beach water	BP5/3/2004WK1-1	03-May-04	-	+
0034435239	04AZ	F06	AZF06	Hamilton	Beach water	BP5/3/2004WK1-2	03-May-04	-	+
0034435240	04AZ	F07	AZF07	Hamilton	Beach water	BP5/3/2004WK1-3	03-May-04	-	+
0034435241	04AZ	F08	AZF08	Hamilton	Beach water	BP5/3/2004WK1-4	03-May-04	-	+
0034434751	04AZ	F09	AZF09	Hamilton	Beach water	BP5/3/2004WK1-5	03-May-04	-	+
0034434752	04AZ	F10	AZF10	Hamilton	Beach water	BP5/3/2004WK1-6	03-May-04	-	+
0034435229	04AZ	F11	AZF11	Hamilton	Beach water	BP5/3/2004WK1-7	03-May-04	-	+
0034435228	04AZ	F12	AZF12	Hamilton	Beach water	BP5/3/2004WK1-8	03-May-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0034435225	04AZ	G01	AZG01	Hamilton	Beach water	BP5/3/2004WK2-1	03-May-04	-	+
0034435224	04AZ	G02	AZG02	Hamilton	Beach water	BP5/3/2004WK2-2	03-May-04	-	+
0034435223	04AZ	G03	AZG03	Hamilton	Beach water	BP5/3/2004WK2-3	03-May-04	-	+
0034435222	04AZ	G04	AZG04	Hamilton	Beach water	BP5/3/2004WK2-4	03-May-04	-	+
0034435221	04AZ	G05	AZG05	Hamilton	Beach water	BP5/3/2004WK2-5	03-May-04	-	+
0031941059	04AZ	G06	AZG06	Hamilton	Beach water	BP5/10/2004WA1-1	10-May-04	-	+
0031941058	04AZ	G07	AZG07	Hamilton	Beach water	BP5/10/2004WA1-2	10-May-04	-	+
0031941057	04AZ	G08	AZG08	Hamilton	Beach water	BP5/10/2004WA1-3	10-May-04	-	+
0031941056	04AZ	G09	AZG09	Hamilton	Beach water	BP5/10/2004WA1-4	10-May-04	-	+
0031941055	04AZ	G10	AZG10	Hamilton	Beach water	BP5/10/2004WA1-5	10-May-04	-	+
0034435220	04AZ	G11	AZG11	Hamilton	Beach water	BP5/3/2004WK2-6	03-May-04	-	+
0031941067	04AZ	G12	AZG12	Hamilton	Beach water	BP5/10/2004WA1-6	10-May-04	-	+
0031941068	04B	A01	BA01	Hamilton	Beach water	BP5/10/2004WA1-7	10-May-04	-	+
0031941074	04B	A02	BA02	Hamilton	Beach water	BP5/10/2004WA2-1	10-May-04	-	+
0031941075	04B	A03	BA03	Hamilton	Beach water	BP5/10/2004WA2-2	10-May-04	-	+
0031941076	04B	A04	BA04	Hamilton	Beach water	BP5/10/2004WA2-3	10-May-04	-	+
0031941077	04B	A05	BA05	Hamilton	Beach water	BP5/10/2004WA2-4	10-May-04	-	+
0031942726	04B	A06	BA06	Hamilton	Beach water	BP5/10/2004WA2-5	10-May-04	-	+
0031941090	04B	A07	BA07	Hamilton	Beach water	BP5/10/2004WA2-6	10-May-04	-	+
0031941089	04B	A08	BA08	Hamilton	Beach water	BP5/10/2004WA2-7	10-May-04	-	+
0031941083	04B	A09	BA09	Hamilton	Beach water	BP5/10/2004WK1-1	10-May-04	-	+
0031941082	04B	A10	BA10	Hamilton	Beach water	BP5/10/2004WK1-2	10-May-04	-	+
0031941081	04B	A11	BA11	Hamilton	Beach water	BP5/10/2004WK1-3	10-May-04	-	+
0031941080	04B	A12	BA12	Hamilton	Beach water	BP5/10/2004WK1-4	10-May-04	-	+
0031941079	04B	B01	BB01	Hamilton	Beach water	BP5/10/2004WK1-5	10-May-04	-	+
0031941091	04B	B02	BB02	Hamilton	Beach water	BP5/10/2004WK1-6	10-May-04	-	+
0031941092	04B	B03	BB03	Hamilton	Beach water	BP5/10/2004WK1-7	10-May-04	-	+
0031941093	04B	B04	BB04	Hamilton	Beach water	BP5/10/2004WK1-8	10-May-04	-	+
0031941098	04B	B05	BB05	Hamilton	Beach water	BP5/10/2004WK2-1	10-May-04	-	+
0031941099	04B	B06	BB06	Hamilton	Beach water	BP5/10/2004WK2-2	10-May-04	-	+
0031941100	04B	B07	BB07	Hamilton	Beach water	BP5/10/2004WK2-3	10-May-04	-	+
0031941101	04B	B08	BB08	Hamilton	Beach water	BP5/10/2004WK2-4	10-May-04	-	+
0031941102	04B	B09	BB09	Hamilton	Beach water	BP5/10/2004WK2-5	10-May-04	-	+
0031941114	04B	B10	BB10	Hamilton	Beach water	BP5/10/2004WK2-6	10-May-04	+	+
0031941113	04B	B11	BB11	Hamilton	Beach water	BP5/10/2004WK2-7	10-May-04	-	+
0031941112	04B	B12	BB12	Hamilton	Beach water	BP5/10/2004WK2-8	10-May-04	-	+
0031941107	04B	C01	BC01	Hamilton	Beach sand	BP5/10/2004SD1-1	10-May-04	-	+
0036632170	04B	C02	BC02	Hamilton	Beach water	BP4/5/2004WA2-2	05-Apr-04	-	+
0029861397	04B	C03	BC03	Hamilton	Beach water	BP6/14/2004WA1-1	14-Jun-04	-	+
0029861069	04B	C04	BC04	Hamilton	Beach water	BP6/14/2004WA1-2	14-Jun-04	-	+
0029861048	04B	C05	BC05	Hamilton	Beach water	BP6/14/2004WA1-3	14-Jun-04	-	+
0029861071	04B	C06	BC06	Hamilton	Beach water	BP6/14/2004WA1-4	14-Jun-04	-	+
0029861070	04B	C07	BC07	Hamilton	Beach water	BP6/14/2004WA1-5	14-Jun-04	-	+
0029861954	04B	C08	BC08	Hamilton	Beach water	BP6/14/2004WA1-6	14-Jun-04	-	+
0029861953	04B	C09	BC09	Hamilton	Beach water	BP6/14/2004WA1-7	14-Jun-04	-	+
0029861066	04B	C10	BC10	Hamilton	Beach water	BP6/14/2004WA2-1	14-Jun-04	-	+
0029861935	04B	C11	BC11	Hamilton	Beach water	BP6/14/2004WA2-2	14-Jun-04	-	+
0029861936	04B	C12	BC12	Hamilton	Beach water	BP6/14/2004WA2-3	14-Jun-04	-	+
0029861937	04B	D01	BD01	Hamilton	Beach water	BP6/14/2004WA2-4	14-Jun-04	-	+
0029861938	04B	D02	BD02	Hamilton	Beach water	BP6/14/2004WA2-5	14-Jun-04	-	+
0029861939	04B	D03	BD03	Hamilton	Beach water	BP6/14/2004WA2-6	14-Jun-04	-	+
0029861940	04B	D04	BD04	Hamilton	Beach water	BP6/14/2004WA2-7	14-Jun-04	-	+
0029861946	04B	D05	BD05	Hamilton	Beach water	BP6/14/2004WK1-1	14-Jun-04	-	+
0029861934	04B	D06	BD06	Hamilton	Beach water	BP6/14/2004WK1-2	14-Jun-04	-	+
0029861933	04B	D07	BD07	Hamilton	Beach water	BP6/14/2004WK1-3	14-Jun-04	-	+
0029861932	04B	D08	BD08	Hamilton	Beach water	BP6/14/2004WK1-4	14-Jun-04	-	+
0029861931	04B	D09	BD09	Hamilton	Beach water	BP6/14/2004WK1-5	14-Jun-04	-	+
0029861930	04B	D10	BD10	Hamilton	Beach water	BP6/14/2004WK1-6	14-Jun-04	-	+
0029861929	04B	D11	BD11	Hamilton	Beach water	BP6/14/2004WK1-7	14-Jun-04	-	+
0029861928	04B	D12	BD12	Hamilton	Beach water	BP6/14/2004WK1-8	14-Jun-04	-	+
0029861923	04B	E01	BE01	Hamilton	Beach water	BP6/14/2004WK2-1	14-Jun-04	-	+
0029861911	04B	E02	BE02	Hamilton	Beach water	BP6/14/2004WK2-2	14-Jun-04	-	+
0029861912	04B	E03	BE03	Hamilton	Beach water	BP6/14/2004WK2-3	14-Jun-04	-	+
0029861913	04B	E04	BE04	Hamilton	Beach water	BP6/14/2004WK2-4	14-Jun-04	-	+
0029861914	04B	E05	BE05	Hamilton	Beach water	BP6/14/2004WK2-5	14-Jun-04	-	+
0029861915	04B	E06	BE06	Hamilton	Beach water	BP6/14/2004WK2-6	14-Jun-04	-	+
0029861916	04B	E07	BE07	Hamilton	Beach water	BP6/14/2004WK2-7	14-Jun-04	-	+
0029861917	04B	E08	BE08	Hamilton	Beach water	BP6/14/2004WK2-8	14-Jun-04	-	+
0029861922	04B	E09	BE09	Hamilton	Beach sand	BP6/14/2004SD1-1	14-Jun-04	-	+
0029861910	04B	E10	BE10	Hamilton	Beach sand	BP6/14/2004SD2-1	14-Jun-04	-	+
0029861909	04B	E11	BE11	Hamilton	Beach sand	BP6/14/2004SD2-2	14-Jun-04	-	+
0036632659	04B	E12	BE12	Hamilton	Beach water	BP4/5/2004WA2-3	05-Apr-04	+	+
0029861907	04B	F01	BF01	Hamilton	Beach water	BP6/14/2004WC1-2	14-Jun-04	-	+
0029861906	04B	F02	BF02	Hamilton	Beach water	BP6/14/2004WC1-3	14-Jun-04	-	+
0029861905	04B	F03	BF03	Hamilton	Beach water	BP6/14/2004WC1-4	14-Jun-04	-	+
0029861904	04B	F04	BF04	Hamilton	Beach water	BP6/14/2004WC1-5	14-Jun-04	-	+
0036632660	04B	F05	BF05	Hamilton	Beach water	BP4/5/2004WA2-4	05-Apr-04	-	+
0029861902	04B	F06	BF06	Hamilton	Beach water	BP6/14/2004WC2-2	14-Jun-04	-	+
0029861901	04B	F07	BF07	Hamilton	Beach water	BP6/14/2004WC2-3	14-Jun-04	-	+
0029861900	04B	F08	BF08	Hamilton	Beach water	BP6/14/2004WC2-4	14-Jun-04	-	+
0036632661	04B	F09	BF09	Hamilton	Beach water	BP4/5/2004WA2-5	05-Apr-04	-	+
0029861025	04B	F10	BF10	Hamilton	Beach water	BP6/14/2004WC2-6	14-Jun-04	-	+
0029861888	04B	F11	BF11	Hamilton	Beach water	BP6/14/2004WC2-7	14-Jun-04	-	+
0029861889	04B	F12	BF12	Hamilton	Beach water	BP6/14/2004WC2-8	14-Jun-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0029861890	04B	G01	BG01	Hamilton	Beach water	BP6/14/2004WC2-9	14-Jun-04	-	+
0029861891	04B	G02	BG02	Hamilton	Beach water	BP6/14/2004WC2-10	14-Jun-04	-	+
0029861892	04B	G03	BG03	Hamilton	Beach water	BP6/14/2004WC2-11	14-Jun-04	-	+
0029861893	04B	G04	BG04	Hamilton	Beach water	BP6/14/2004WC2-12	14-Jun-04	-	+
0031942623	04B	G05	BG05	Hamilton	Beach water	BP5/25/2004WA1-3	25-May-04	-	+
0029861895	04B	G06	BG06	Hamilton	Beach water	HH16/14/2004W1-2	14-Jun-04	-	-
0029861896	04B	G07	BG07	Hamilton	Beach water	HH16/14/2004W1-3	14-Jun-04	-	+
0029861897	04B	G08	BG08	Hamilton	Beach water	HH16/14/2004W1-4	14-Jun-04	-	+
0029861051	04B	G09	BG09	Hamilton	Beach water	HH16/14/2004W1-5	14-Jun-04	-	+
0029861886	04B	G10	BG10	Hamilton	Beach water	HH16/14/2004W1-6	14-Jun-04	-	+
0036632662	04B	G11	BG11	Hamilton	Beach water	BP4/5/2004WA2-7	05-Apr-04	-	+
0029861884	04B	G12	BG12	Hamilton	Beach water	HH16/14/2004W2-2	14-Jun-04	-	+
0026738474	04BE	A01	BEA01	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG14-2	30-Jul-04	-	+
0026727916	04BE	A02	BEA02	Hamilton	Cat (Felis catus)	AS7/30/2004C4-1	30-Jul-04	-	+
0026738465	04BE	A03	BEA03	Hamilton	Cat (Felis catus)	AS7/30/2004C6-2	30-Jul-04	-	+
0026738443	04BE	A04	BEA04	Hamilton	Cat (Felis catus)	AS7/30/2004C8-5	30-Jul-04	-	+
0026738431	04BE	A05	BEA05	Hamilton	Cat (Felis catus)	AS7/30/2004C9-1	30-Jul-04	-	+
0026737992	04BE	A06	BEA06	Hamilton	Cat (Felis catus)	AS7/30/2004C13-1	30-Jul-04	-	+
0026727897	04BE	A07	BEA07	Hamilton	Dog (Canis lupus familiaris)	AS7/30/2004DG13-3	30-Jul-04	-	+
0026731631	04BE	A08	BEA08	Toronto	Dog (Canis lupus familiaris)	SPCA8/3/2004DG1-3	03-Aug-04	-	+
0026731629	04BE	A09	BEA09	Toronto	Dog (Canis lupus familiaris)	SPCA8/3/2004DG2-2	03-Aug-04	-	+
0026731628	04BE	A10	BEA10	Toronto	Dog (Canis lupus familiaris)	SPCA8/3/2004DG2-3	03-Aug-04	-	+
0026731615	04BE	A11	BEA11	Toronto	Cat (Felis catus)	SPCA8/3/2004C1-2	03-Aug-04	+	+
0026731618	04BE	A12	BEA12	Toronto	Cat (Felis catus)	SPCA8/3/2004C3-2	03-Aug-04	-	+
0026731672	04BE	B01	BEB01	Toronto	Gull (Larus delawarensis)	CI18/3/2004G6-2	03-Aug-04	-	+
0026731662	04BE	B02	BEB02	Toronto	Gull (Larus delawarensis)	CI18/3/2004G7-2	03-Aug-04	-	+
0026731661	04BE	B03	BEB03	Toronto	Gull (Larus delawarensis)	CI18/3/2004G7-3	03-Aug-04	-	+
0026731653	04BE	B04	BEB04	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG2-2	03-Aug-04	-	+
0026731652	04BE	B05	BEB05	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG2-3	03-Aug-04	-	+
0026731685	04BE	B06	BEB06	Toronto	Canada Goose (Branta canadensis)	CI18/3/2004CG5-1	03-Aug-04	-	+
0026730928	04BE	B07	BEB07	Toronto	Canada Goose (Branta canadensis)	CI18/3/2004CG6-1	03-Aug-04	-	+
0026731657	04BE	B08	BEB08	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG1-1	03-Aug-04	-	+
0026731654	04BE	B09	BEB09	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG2-1	03-Aug-04	-	+
0026731641	04BE	B10	BEB10	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG4-1	03-Aug-04	-	+
0026731644	04BE	B11	BEB11	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG5-1	03-Aug-04	-	+
0026731647	04BE	B12	BEB12	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG6-1	03-Aug-04	-	+
0026731650	04BE	C01	BEC01	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG7-1	03-Aug-04	-	-
0026731636	04BE	C02	BEC02	Toronto	Canada Goose (Branta canadensis)	CI28/3/2004CG8-1	03-Aug-04	-	+
0026732669	04BE	C03	BEC03	Toronto	Cat (Felis catus)	SPCA8/9/2004C11-5	09-Aug-04	-	+
0026732661	04BE	C04	BEC04	Toronto	Cat (Felis catus)	SPCA8/9/2004C16-1	09-Aug-04	-	+
0026731614	04BE	C05	BEC05	Toronto	Cat (Felis catus)	SPCA8/3/2004C5-1	03-Aug-04	-	+
0026731633	04BE	C06	BEC06	Toronto	Dog (Canis lupus familiaris)	SPCA8/3/2004DG1-1	03-Aug-04	-	+
0026731630	04BE	C07	BEC07	Toronto	Dog (Canis lupus familiaris)	SPCA8/3/2004DG2-1	03-Aug-04	-	+
0026731621	04BE	C08	BEC08	Toronto	Cat (Felis catus)	SPCA8/3/2004C3-5	03-Aug-04	-	+
0026731622	04BE	C09	BEC09	Toronto	Cat (Felis catus)	SPCA8/3/2004C4-1	03-Aug-04	-	+
0026731626	04BE	C10	BEC10	Toronto	Cat (Felis catus)	SPCA8/3/2004C4-5	03-Aug-04	-	+
0029894566	04BE	C11	BEC11	Toronto	Cat (Felis catus)	SPCA8/23/2004C4-2	23-Aug-04	-	+
0026732448	04BE	C12	BEC12	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG1-3	03-Aug-04	-	+
0026732447	04BE	D01	BED01	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG2-1	03-Aug-04	-	+
0026732443	04BE	D02	BED02	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG3-2	03-Aug-04	-	+
0026732431	04BE	D03	BED03	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG3-3	03-Aug-04	-	+
0026732438	04BE	D04	BED04	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG6-2	03-Aug-04	-	+
0026732441	04BE	D05	BED05	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG7-2	03-Aug-04	-	+
0026732407	04BE	D06	BED06	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG15-2	03-Aug-04	-	+
0026732425	04BE	D07	BED07	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG12-3	03-Aug-04	-	+
0026732423	04BE	D08	BED08	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG13-3	03-Aug-04	-	+
0026732422	04BE	D09	BED09	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG14-1	03-Aug-04	-	+
0026732420	04BE	D10	BED10	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG14-3	03-Aug-04	-	+
0026732419	04BE	D11	BED11	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG15-1	03-Aug-04	-	+
0026732408	04BE	D12	BED12	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG15-3	03-Aug-04	-	+
0026732409	04BE	E01	BEE01	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG16-1	03-Aug-04	-	+
0026732411	04BE	E02	BEE02	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG16-3	03-Aug-04	-	+
0026732412	04BE	E03	BEE03	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG17-1	03-Aug-04	-	+
0026732398	04BE	E04	BEE04	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG22-1	03-Aug-04	-	+
0026732418	04BE	E05	BEE05	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG19-1	03-Aug-04	-	+
0026732405	04BE	E06	BEE06	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG19-3	03-Aug-04	-	+
0026732403	04BE	E07	BEE07	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG20-2	03-Aug-04	-	+
0026732402	04BE	E08	BEE08	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG20-3	03-Aug-04	-	+
0026732401	04BE	E09	BEE09	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG21-1	03-Aug-04	-	+
0026732400	04BE	E10	BEE10	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG21-2	03-Aug-04	-	+
0026732399	04BE	E11	BEE11	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG21-3	03-Aug-04	-	+
0026732397	04BE	E12	BEE12	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG22-2	03-Aug-04	-	+
0026732396	04BE	F01	BEF01	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG22-3	03-Aug-04	-	+
0026732383	04BE	F02	BEF02	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG23-2	03-Aug-04	-	+
0026732384	04BE	F03	BEF03	Toronto	Canada Goose (Branta canadensis)	AH8/3/2004CG23-3	03-Aug-04	-	+
0029880363	04BE	F04	BEF04	Toronto	Mallard duck (Anas platyrhynchos)	AF9/13/2004D2-3	13-Sep-04	-	+
0029880319	04BE	F05	BEF05	Toronto	Mallard duck (Anas platyrhynchos)	AF9/13/2004D9-1	13-Sep-04	-	+
0029880324	04BE	F06	BEF06	Toronto	Mallard duck (Anas platyrhynchos)	AF9/13/2004D10-1	13-Sep-04	+	+
0029860177	04BE	F07	BEF07	Toronto	Dog (Canis lupus familiaris)	KW5/31/2004DG2-2	31-May-04	-	+
0029860171	04BE	F08	BEF08	Toronto	Canada Goose (Branta canadensis)	AH5/31/2004CG4-3	31-May-04	-	+
0029860159	04BE	F09	BEF09	Toronto	Canada Goose (Branta canadensis)	AH5/31/2004CG8-1	31-May-04	-	+
0029860161	04BE	F10	BEF10	Toronto	Canada Goose (Branta canadensis)	AH5/31/2004CG8-3	31-May-04	-	+
0029859709	04BE	F11	BEF11	Toronto	Gull (Larus delawarensis)	LS6/7/2004G20-3	07-Jun-04	-	+
0029859653	04BE	F12	BEF12	Toronto	Gull (Larus delawarensis)	LS6/7/2004G21-1	07-Jun-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0029859620	04BE	G01	BEG01	Toronto	Gull (Larus delawarensis)	CI6/7/2004G1-3	07-Jun-04	-	+
0029859608	04BE	G02	BEG02	Toronto	Gull (Larus delawarensis)	CI6/7/2004G2-3	07-Jun-04	-	+
0029859609	04BE	G03	BEG03	Toronto	Gull (Larus delawarensis)	CI6/7/2004G3-1	07-Jun-04	-	+
0029859605	04BE	G04	BEG04	Toronto	Gull (Larus delawarensis)	CI6/7/2004G7-1	07-Jun-04	-	+
0029859604	04BE	G05	BEG05	Toronto	Gull (Larus delawarensis)	CI6/7/2004G7-2	07-Jun-04	-	+
0029862725	04BE	G06	BEG06	Toronto	Dog (Canis lupus familiaris)	SPCA8/9/2004Dg1-2	09-Aug-04	-	+
0026732698	04BE	G07	BEG07	Toronto	Cat (Felis catus)	SPCA8/9/2004C1-1	09-Aug-04	-	+
0026732694	04BE	G08	BEG08	Toronto	Cat (Felis catus)	SPCA8/9/2004C3-5	09-Aug-04	-	+
0026732692	04BE	G09	BEG09	Toronto	Cat (Felis catus)	SPCA8/9/2004C5-2	09-Aug-04	-	+
0026732686	04BE	G10	BEG10	Toronto	Cat (Felis catus)	SPCA8/9/2004C6-3	09-Aug-04	-	+
0026731720	04BE	G11	BEG11	Toronto	Cat (Felis catus)	SPCA8/9/2004C9-4	09-Aug-04	-	+
0026732677	04BE	G12	BEG12	Toronto	Cat (Felis catus)	SPCA8/9/2004C10-3	09-Aug-04	-	+
0036633851	04BK	A01	BKA01	Hamilton	Beach water	BP7/19/2004WA1-4	19-Jul-04	-	+
0036633850	04BK	A02	BKA02	Hamilton	Beach water	BP7/19/2004WA1-5	19-Jul-04	-	+
0036633849	04BK	A03	BKA03	Hamilton	Beach water	BP7/19/2004WA1-6	19-Jul-04	-	+
0036633848	04BK	A04	BKA04	Hamilton	Beach water	BP7/19/2004WA1-7	19-Jul-04	-	+
0036633862	04BK	A05	BKA05	Hamilton	Beach water	BP7/19/2004WA2-1	19-Jul-04	-	+
0036633863	04BK	A06	BKA06	Hamilton	Beach water	BP7/19/2004WA2-2	19-Jul-04	-	+
0036633864	04BK	A07	BKA07	Hamilton	Beach water	BP7/19/2004WA2-3	19-Jul-04	-	+
0036633865	04BK	A08	BKA08	Hamilton	Beach water	BP7/19/2004WA2-4	19-Jul-04	-	+
0036633866	04BK	A09	BKA09	Hamilton	Beach water	BP7/19/2004WA2-5	19-Jul-04	-	+
0036633867	04BK	A10	BKA10	Hamilton	Beach water	BP7/19/2004WA2-6	19-Jul-04	-	+
0036633868	04BK	A11	BKA11	Hamilton	Beach water	BP7/19/2004WA2-7	19-Jul-04	-	+
0036633879	04BK	A12	BKA12	Hamilton	Beach water	BP7/19/2004WK1-1	19-Jul-04	-	+
0036633878	04BK	B01	BKB01	Hamilton	Beach water	BP7/19/2004WK1-2	19-Jul-04	-	+
0036633877	04BK	B02	BKB02	Hamilton	Beach water	BP7/19/2004WK1-3	19-Jul-04	-	+
0036633876	04BK	B03	BKB03	Hamilton	Beach water	BP7/19/2004WK1-4	19-Jul-04	-	+
0036633875	04BK	B04	BKB04	Hamilton	Beach water	BP7/19/2004WK1-5	19-Jul-04	-	+
0036633874	04BK	B05	BKB05	Hamilton	Beach water	BP7/19/2004WK1-7	19-Jul-04	-	+
0036633873	04BK	B06	BKB06	Hamilton	Beach water	BP7/19/2004WK1-8	19-Jul-04	-	+
0031915546	04BK	B07	BKB07	Hamilton	Beach water	HH16/1/2004W2-10	01-Jun-04	-	+
0031915547	04BK	B08	BKB08	Hamilton	Beach water	HH16/1/2004W2-11	01-Jun-04	-	+
0031915548	04BK	B09	BKB09	Hamilton	Beach water	HH16/1/2004W2-12	01-Jun-04	-	+
0026739466	04BK	B10	BKB10	Hamilton	Beach sand	BP8/3/2004SD1-5	03-Aug-04	+	+
0026739464	04BK	B11	BKB11	Hamilton	Beach sand	BP8/3/2004SD2-1	03-Aug-04	-	+
0026739471	04BK	B12	BKB12	Hamilton	Beach sand	BP8/3/2004SD2-2	03-Aug-04	-	+
0026739535	04BK	C01	BKC01	Hamilton	Beach sand	BP8/3/2004SD2-3	03-Aug-04	-	+
0029861636	04BK	C02	BKC02	Hamilton	Beach sand	BP8/16/2004SD1-3	16-Aug-04	-	+
0034437196	04BK	C03	BKC03	Hamilton	Beach sand	BP4/27/2004SD2-7	27-Apr-04	-	+
0036632728	04BK	C04	BKC04	Hamilton	Beach sand	BP4/5/2004SD1-3	05-Apr-04	-	+
0031939284	04BK	C05	BKC05	Hamilton	Beach sand	BP7/19/2004SD1-4	19-Jul-04	-	+
0031939283	04BK	C06	BKC06	Hamilton	Beach sand	BP7/19/2004SD1-5	19-Jul-04	-	+
0031939282	04BK	C07	BKC07	Hamilton	Beach sand	BP7/19/2004SD1-6	19-Jul-04	-	+
0031939281	04BK	C08	BKC08	Hamilton	Beach sand	BP7/19/2004SD1-7	19-Jul-04	-	+
0034437426	04BK	C09	BKC09	Hamilton	Beach sand	BP7/19/2004SD1-8	19-Jul-04	-	+
0034436085	04BK	C10	BKC10	Hamilton	Beach sand	BP7/19/2004SD1-9	19-Jul-04	-	+
0034436090	04BK	C11	BKC11	Hamilton	Beach sand	BP7/19/2004SD2-1	19-Jul-04	-	+
0034436091	04BK	C12	BKC12	Hamilton	Beach sand	BP7/19/2004SD2-2	19-Jul-04	+	+
0036632727	04BK	D01	BKD01	Hamilton	Beach sand	BP4/5/2004SD1-4	05-Apr-04	-	+
0036632726	04BK	D02	BKD02	Hamilton	Beach sand	BP4/5/2004SD1-5	05-Apr-04	+	+
0036632725	04BK	D03	BKD03	Hamilton	Beach sand	BP4/5/2004SD1-8	05-Apr-04	-	+
0036632724	04BK	D04	BKD04	Hamilton	Beach sand	BP4/5/2004SD1-9	05-Apr-04	-	+
0034437416	04BK	D05	BKD05	Hamilton	Beach sand	BP7/19/2004SD2-7	19-Jul-04	-	+
0036632721	04BK	D06	BKD06	Hamilton	Beach sand	BP4/5/2004SD2-1	05-Apr-04	-	+
0034436086	04BK	D07	BKD07	Hamilton	Beach sand	BP7/19/2004SD2-9	19-Jul-04	-	+
0029879141	04BK	D08	BKD08	Hamilton	Beach water	HH19/7/2004W1-3	07-Sep-04	-	+
0029877774	04BK	D09	BKD09	Hamilton	Beach water	HH19/7/2004W1-2	07-Sep-04	-	+
0029879138	04BK	D10	BKD10	Hamilton	Beach water	HH19/7/2004W1-6	07-Sep-04	-	+
0029879139	04BK	D11	BKD11	Hamilton	Beach water	HH19/7/2004W1-5	07-Sep-04	-	+
0029879134	04BK	D12	BKD12	Hamilton	Beach water	HH19/7/2004W2-2	07-Sep-04	-	+
0029877785	04BK	E01	BKE01	Hamilton	Beach water	HH19/7/2004W2-3	07-Sep-04	-	+
0029879132	04BK	E02	BKE02	Hamilton	Beach water	HH19/7/2004W2-4	07-Sep-04	-	+
0029877786	04BK	E03	BKE03	Hamilton	Beach water	HH19/7/2004W2-5	07-Sep-04	-	-
0029879120	04BK	E04	BKE04	Hamilton	Beach water	HH19/7/2004W2-6	07-Sep-04	-	+
0026736855	04BK	E05	BKE05	Hamilton	Beach water	BP7/26/2004WA1-1	26-Jul-04	-	+
0026736856	04BK	E06	BKE06	Hamilton	Beach water	BP7/26/2004WA1-2	26-Jul-04	-	+
0026736857	04BK	E07	BKE07	Hamilton	Beach water	BP7/26/2004WA1-3	26-Jul-04	-	+
0026736858	04BK	E08	BKE08	Hamilton	Beach water	BP7/26/2004WA1-4	26-Jul-04	-	+
0026736846	04BK	E09	BKE09	Hamilton	Beach water	BP7/26/2004WA1-5	26-Jul-04	-	+
0026736845	04BK	E10	BKE10	Hamilton	Beach water	BP7/26/2004WA1-6	26-Jul-04	-	+
0026736844	04BK	E11	BKE11	Hamilton	Beach water	BP7/26/2004WA1-7	26-Jul-04	-	+
0026736838	04BK	E12	BKE12	Hamilton	Beach water	BP7/26/2004WA2-1	26-Jul-04	-	+
0026736837	04BK	F01	BKF01	Hamilton	Beach water	BP7/26/2004WA2-2	26-Jul-04	-	+
0026736836	04BK	F02	BKF02	Hamilton	Beach water	BP7/26/2004WA2-3	26-Jul-04	-	+
0026736823	04BK	F03	BKF03	Hamilton	Beach water	BP7/26/2004WA2-5	26-Jul-04	-	+
0026736824	04BK	F04	BKF04	Hamilton	Beach water	BP7/26/2004WA2-6	26-Jul-04	-	+
0026736825	04BK	F05	BKF05	Hamilton	Beach water	BP7/26/2004WA2-7	26-Jul-04	-	+
0026736831	04BK	F06	BKF06	Hamilton	Beach water	BP7/26/2004WK1-1	26-Jul-04	-	+
0026736832	04BK	F07	BKF07	Hamilton	Beach water	BP7/26/2004WK1-2	26-Jul-04	-	+
0026736833	04BK	F08	BKF08	Hamilton	Beach water	BP7/26/2004WK1-3	26-Jul-04	-	+
0026736834	04BK	F09	BKF09	Hamilton	Beach water	BP7/26/2004WK1-4	26-Jul-04	-	+
0026736822	04BK	F10	BKF10	Hamilton	Beach water	BP7/26/2004WK1-5	26-Jul-04	-	+
0026736821	04BK	F11	BKF11	Hamilton	Beach water	BP7/26/2004WK1-6	26-Jul-04	-	+
0026736820	04BK	F12	BKF12	Hamilton	Beach water	BP7/26/2004WK1-7	26-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026736819	04BK	G01	BKG01	Hamilton	Beach water	BP7/26/2004WK1-8	26-Jul-04	-	+
0026736814	04BK	G02	BKG02	Hamilton	Beach water	BP7/26/2004WK2-1	26-Jul-04	-	+
0026736813	04BK	G03	BKG03	Hamilton	Beach water	BP7/26/2004WK2-2	26-Jul-04	-	+
0026736812	04BK	G04	BKG04	Hamilton	Beach water	BP7/26/2004WK2-3	26-Jul-04	-	+
0026736811	04BK	G05	BKG05	Hamilton	Beach water	BP7/26/2004WK2-4	26-Jul-04	-	+
0026736799	04BK	G06	BKG06	Hamilton	Beach water	BP7/26/2004WK2-5	26-Jul-04	-	+
0026736800	04BK	G07	BKG07	Hamilton	Beach water	BP7/26/2004WK2-6	26-Jul-04	-	+
0026736801	04BK	G08	BKG08	Hamilton	Beach water	BP7/26/2004WK2-7	26-Jul-04	-	+
0026736802	04BK	G09	BKG09	Hamilton	Beach water	BP7/26/2004WK2-8	26-Jul-04	-	+
0036632720	04BK	G10	BKG10	Hamilton	Beach sand	BP4/5/2004SD2-2	05-Apr-04	-	+
0031940946	04BK	G11	BKG11	Hamilton	Beach water	BP6/7/2004WA2-7	07-Jun-04	-	+
0031942753	04BK	G12	BKG12	Hamilton	Beach water	BP6/7/2004WK1-3	07-Jun-04	-	+
0031924796	04BN	A01	BNA01	Toronto	Beach sand	KW17/12/2004SD1-10	12-Jul-04	-	+
0031924795	04BN	A02	BNA02	Toronto	Beach sand	KW17/12/2004SD1-11	12-Jul-04	-	+
0031924783	04BN	A03	BNA03	Toronto	Beach sand	KW17/12/2004SD1-12	12-Jul-04	-	+
0031924785	04BN	A04	BNA04	Toronto	Beach sand	KW27/12/2004SD1-2	12-Jul-04	-	+
0031924786	04BN	A05	BNA05	Toronto	Beach sand	KW27/12/2004SD1-3	12-Jul-04	-	+
0031924787	04BN	A06	BNA06	Toronto	Beach sand	KW27/12/2004SD1-4	12-Jul-04	-	+
0031924788	04BN	A07	BNA07	Toronto	Beach sand	KW27/12/2004SD1-5	12-Jul-04	-	+
0031924789	04BN	A08	BNA08	Toronto	Beach sand	KW27/12/2004SD1-6	12-Jul-04	-	+
0031924790	04BN	A09	BNA09	Toronto	Beach sand	KW27/12/2004SD1-7	12-Jul-04	-	+
0031924791	04BN	A10	BNA10	Toronto	Beach sand	KW27/12/2004SD1-8	12-Jul-04	-	+
0031924792	04BN	A11	BNA11	Toronto	Beach sand	KW27/12/2004SD1-9	12-Jul-04	-	+
0031924793	04BN	A12	BNA12	Toronto	Beach sand	KW27/12/2004SD1-10	12-Jul-04	-	+
0031924794	04BN	B01	BNB01	Toronto	Beach sand	KW27/12/2004SD1-11	12-Jul-04	-	+
0031924782	04BN	B02	BNB02	Toronto	Beach sand	KW27/12/2004SD1-12	12-Jul-04	-	+
0031924780	04BN	B03	BNB03	Toronto	Beach sand	KW37/12/2004SD1-2	12-Jul-04	+	-
0031924779	04BN	B04	BNB04	Toronto	Beach sand	KW37/12/2004SD1-3	12-Jul-04	+	-
0031924778	04BN	B05	BNB05	Toronto	Beach sand	KW37/12/2004SD1-4	12-Jul-04	-	+
0031924777	04BN	B06	BNB06	Toronto	Beach sand	KW37/12/2004SD1-5	12-Jul-04	-	+
0031924776	04BN	B07	BNB07	Toronto	Beach sand	KW37/12/2004SD1-6	12-Jul-04	+	-
0031924775	04BN	B08	BNB08	Toronto	Beach sand	KW37/12/2004SD1-7	12-Jul-04	-	+
0031924774	04BN	B09	BNB09	Toronto	Beach sand	KW37/12/2004SD1-8	12-Jul-04	-	+
0031924773	04BN	B10	BNB10	Toronto	Beach sand	KW37/12/2004SD1-9	12-Jul-04	-	+
0031924772	04BN	B11	BNB11	Toronto	Beach sand	KW37/12/2004SD1-10	12-Jul-04	-	+
0031924771	04BN	B12	BNB12	Toronto	Beach sand	KW37/12/2004SD1-11	12-Jul-04	-	+
0029895462	04BN	C01	BNC01	Toronto	Beach sand	CI18/23/2004SD2-11	23-Aug-04	-	+
0029895461	04BN	C02	BNC02	Toronto	Beach sand	CI18/23/2004SD2-12	23-Aug-04	-	+
0029894582	04BN	C03	BNC03	Toronto	Beach sand	CI28/23/2004SD1-2	23-Aug-04	-	+
0029895453	04BN	C04	BNC04	Toronto	Beach sand	CI28/23/2004SD1-8	23-Aug-04	-	+
0029895452	04BN	C05	BNC05	Toronto	Beach sand	CI28/23/2004SD1-9	23-Aug-04	-	+
0029894580	04BN	C06	BNC06	Toronto	Beach sand	CI28/23/2004SD1-10	23-Aug-04	-	+
0029895439	04BN	C07	BNC07	Toronto	Beach sand	CI28/23/2004SD1-11	23-Aug-04	-	+
0029895440	04BN	C08	BNC08	Toronto	Beach sand	CI28/23/2004SD1-12	23-Aug-04	-	+
0029895441	04BN	C09	BNC09	Toronto	Beach sand	CI28/23/2004SD2-1	23-Aug-04	-	+
0029895447	04BN	C10	BNC10	Toronto	Beach sand	CI28/23/2004SD2-7	23-Aug-04	-	+
0029895448	04BN	C11	BNC11	Toronto	Beach sand	CI28/23/2004SD2-8	23-Aug-04	-	+
0029895449	04BN	C12	BNC12	Toronto	Beach sand	CI28/23/2004SD2-9	23-Aug-04	-	+
0029895450	04BN	D01	BND01	Toronto	Beach sand	CI28/23/2004SD2-10	23-Aug-04	-	+
0029895437	04BN	D02	BND02	Toronto	Beach sand	CI28/23/2004SD2-12	23-Aug-04	-	+
0026733794	04BN	D03	BND03	Toronto	Beach sand	KW18/9/2004SD1-3	09-Aug-04	-	+
0026733792	04BN	D04	BND04	Toronto	Beach sand	KW18/9/2004SD1-5	09-Aug-04	-	+
0026733790	04BN	D05	BND05	Toronto	Beach sand	KW18/9/2004SD1-7	09-Aug-04	-	+
0026733499	04BN	D06	BND06	Toronto	Beach sand	KW18/9/2004SD1-11	09-Aug-04	-	+
0026733776	04BN	D07	BND07	Toronto	Beach sand	KW18/9/2004SD1-12	09-Aug-04	-	+
0026733440	04BN	D08	BND08	Toronto	Beach sand	KW18/9/2004SD2-2	09-Aug-04	-	+
0026733779	04BN	D09	BND09	Toronto	Beach sand	KW18/9/2004SD2-3	09-Aug-04	-	+
0026733780	04BN	D10	BND10	Toronto	Beach sand	KW18/9/2004SD2-4	09-Aug-04	-	+
0026733781	04BN	D11	BND11	Toronto	Beach sand	KW18/9/2004SD2-5	09-Aug-04	-	+
0026733782	04BN	D12	BND12	Toronto	Beach sand	KW18/9/2004SD2-6	09-Aug-04	-	+
0026733783	04BN	E01	BNE01	Toronto	Beach sand	KW18/9/2004SD2-7	09-Aug-04	-	+
0026733784	04BN	E02	BNE02	Toronto	Beach sand	KW18/9/2004SD2-8	09-Aug-04	-	+
0026733785	04BN	E03	BNE03	Toronto	Beach sand	KW18/9/2004SD2-9	09-Aug-04	-	+
0026733786	04BN	E04	BNE04	Toronto	Beach sand	KW18/9/2004SD2-10	09-Aug-04	-	+
0026733451	04BN	E05	BNE05	Toronto	Beach sand	KW18/9/2004SD2-11	09-Aug-04	-	+
0026733773	04BN	E06	BNE06	Toronto	Beach sand	KW18/9/2004SD2-12	09-Aug-04	-	+
0026733771	04BN	E07	BNE07	Toronto	Beach sand	KW28/9/2004SD1-2	09-Aug-04	-	+
0026733770	04BN	E08	BNE08	Toronto	Beach sand	KW28/9/2004SD1-3	09-Aug-04	-	+
0026733769	04BN	E09	BNE09	Toronto	Beach sand	KW28/9/2004SD1-4	09-Aug-04	-	+
0026733768	04BN	E10	BNE10	Toronto	Beach sand	KW28/9/2004SD1-5	09-Aug-04	-	+
0026733767	04BN	E11	BNE11	Toronto	Beach sand	KW28/9/2004SD1-6	09-Aug-04	-	+
0026733766	04BN	E12	BNE12	Toronto	Beach sand	KW28/9/2004SD1-7	09-Aug-04	-	+
0026733764	04BN	F01	BNF01	Toronto	Beach sand	KW28/9/2004SD1-9	09-Aug-04	-	+
0026733763	04BN	F02	BNF02	Toronto	Beach sand	KW28/9/2004SD1-10	09-Aug-04	-	+
0026733471	04BN	F03	BNF03	Toronto	Beach sand	KW28/9/2004SD1-11	09-Aug-04	-	+
0026733752	04BN	F04	BNF04	Toronto	Beach sand	KW28/9/2004SD1-12	09-Aug-04	-	+
0026733754	04BN	F05	BNF05	Toronto	Beach sand	KW28/9/2004SD2-2	09-Aug-04	-	+
0026733755	04BN	F06	BNF06	Toronto	Beach sand	KW28/9/2004SD2-3	09-Aug-04	-	+
0026733756	04BN	F07	BNF07	Toronto	Beach sand	KW28/9/2004SD2-4	09-Aug-04	-	+
0026733757	04BN	F08	BNF08	Toronto	Beach sand	KW28/9/2004SD2-5	09-Aug-04	-	+
0026733758	04BN	F09	BNF09	Toronto	Beach sand	KW28/9/2004SD2-6	09-Aug-04	-	+
0026733759	04BN	F10	BNF10	Toronto	Beach sand	KW28/9/2004SD2-7	09-Aug-04	-	+
0026733760	04BN	F11	BNF11	Toronto	Beach sand	KW28/9/2004SD2-8	09-Aug-04	-	+
0026733761	04BN	F12	BNF12	Toronto	Beach sand	KW28/9/2004SD2-9	09-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026733762	04BN	G01	BNG01	Toronto	Beach sand	KW28/9/2004SD2-10	09-Aug-04	-	+
0026733750	04BN	G02	BNG02	Toronto	Beach sand	KW28/9/2004SD2-11	09-Aug-04	-	+
0026733749	04BN	G03	BNG03	Toronto	Beach sand	KW28/9/2004SD2-12	09-Aug-04	-	+
0026733442	04BN	G04	BNG04	Toronto	Beach sand	KW38/9/2004SD1-2	09-Aug-04	-	+
0026733472	04BN	G05	BNG05	Toronto	Beach sand	KW38/9/2004SD1-3	09-Aug-04	-	+
0026733745	04BN	G06	BNG06	Toronto	Beach sand	KW38/9/2004SD1-4	09-Aug-04	-	+
0026733744	04BN	G07	BNG07	Toronto	Beach sand	KW38/9/2004SD1-5	09-Aug-04	-	+
0026733743	04BN	G08	BNG08	Toronto	Beach sand	KW38/9/2004SD1-6	09-Aug-04	-	+
0026733742	04BN	G09	BNG09	Toronto	Beach sand	KW38/9/2004SD1-7	09-Aug-04	-	+
0026733741	04BN	G10	BNG10	Toronto	Beach sand	KW38/9/2004SD1-8	09-Aug-04	-	+
0026733740	04BN	G11	BNG11	Toronto	Beach sand	KW38/9/2004SD1-9	09-Aug-04	-	+
0026733739	04BN	G12	BNG12	Toronto	Beach sand	KW38/9/2004SD1-10	09-Aug-04	-	+
0026739537	04BO	A01	BOA01	Hamilton	Beach sand	BP8/3/2004SD2-5	03-Aug-04	-	+
0026739538	04BO	A02	BOA02	Hamilton	Beach sand	BP8/3/2004SD2-6	03-Aug-04	-	+
0026739539	04BO	A03	BOA03	Hamilton	Beach sand	BP8/3/2004SD2-7	03-Aug-04	-	+
0026739540	04BO	A04	BOA04	Hamilton	Beach sand	BP8/3/2004SD2-8	03-Aug-04	-	+
0026739541	04BO	A05	BOA05	Hamilton	Beach sand	BP8/3/2004SD2-9	03-Aug-04	-	+
0029879136	04BO	A06	BOA06	Hamilton	Beach water	HH19/7/2004W1-8	07-Sep-04	-	+
0026739534	04BO	A07	BOA07	Hamilton	Beach water	HH18/3/2004W1-3	03-Aug-04	-	+
0026739528	04BO	A08	BOA08	Hamilton	Beach water	HH18/3/2004W1-9	03-Aug-04	-	+
0031942754	04BO	A09	BOA09	Hamilton	Beach water	BP6/7/2004WK1-4	07-Jun-04	-	+
0026739523	04BO	A10	BOA10	Hamilton	Beach water	HH18/3/2004W2-3	03-Aug-04	-	+
0026739511	04BO	A11	BOA11	Hamilton	Beach water	HH18/3/2004W2-4	03-Aug-04	-	+
0026739512	04BO	A12	BOA12	Hamilton	Beach water	HH18/3/2004W2-5	03-Aug-04	-	+
0026739513	04BO	B01	BOB01	Hamilton	Beach water	HH18/3/2004W2-6	03-Aug-04	-	+
0026739515	04BO	B02	BOB02	Hamilton	Beach water	HH18/3/2004W2-8	03-Aug-04	+	+
0026739518	04BO	B03	BOB03	Hamilton	Beach water	HH18/3/2004W2-11	03-Aug-04	-	+
0029879135	04BO	B04	BOB04	Hamilton	Beach water	HH19/7/2004W2-1	07-Sep-04	-	+
0026737963	04BO	B05	BOB05	Hamilton	Untreated CSO sewage	IN7/28/2004CSO1-1	28-Jul-04	-	+
0026738767	04BO	B06	BOB06	Hamilton	Untreated CSO sewage	IN7/28/2004CSO1-2	28-Jul-04	-	+
0026738775	04BO	B07	BOB07	Hamilton	Untreated CSO sewage	IN7/28/2004CSO2-2	28-Jul-04	-	+
0031940937	04BO	B08	BOB08	Hamilton	Beach water	BP6/7/2004WK1-5	07-Jun-04	-	+
0031940936	04BO	B09	BOB09	Hamilton	Beach water	BP6/7/2004WK1-6	07-Jun-04	-	+
0026738750	04BO	B10	BOB10	Hamilton	Untreated CSO sewage	EW7/31/2004CSO1-2	31-Jul-04	-	+
0031940935	04BO	B11	BOB11	Hamilton	Beach water	BP6/7/2004WK1-7	07-Jun-04	-	+
0031940950	04BO	B12	BOB12	Hamilton	Beach water	BP6/7/2004WK2-1	07-Jun-04	-	+
0026738738	04BO	C01	BOC01	Hamilton	Untreated CSO sewage	EW7/31/2004CSO2-2	31-Jul-04	-	+
0026739525	04BO	C02	BOC02	Hamilton	Beach water	HH18/3/2004W1-12	03-Aug-04	-	+
0026739517	04BO	C03	BOC03	Hamilton	Beach water	HH18/3/2004W2-10	03-Aug-04	-	+
0026739514	04BO	C04	BOC04	Hamilton	Beach water	HH18/3/2004W2-7	03-Aug-04	-	+
0026739526	04BO	C05	BOC05	Hamilton	Beach water	HH18/3/2004W1-11	03-Aug-04	-	+
0026739527	04BO	C06	BOC06	Hamilton	Beach water	HH18/3/2004W1-10	03-Aug-04	-	+
0026739529	04BO	C07	BOC07	Hamilton	Beach water	HH18/3/2004W1-8	03-Aug-04	-	+
0026739530	04BO	C08	BOC08	Hamilton	Beach water	HH18/3/2004W1-7	03-Aug-04	-	+
0031940951	04BO	C09	BOC09	Hamilton	Beach water	BP6/7/2004WK2-2	07-Jun-04	-	+
0026739532	04BO	C10	BOC10	Hamilton	Beach water	HH18/3/2004W1-5	03-Aug-04	-	+
0026739533	04BO	C11	BOC11	Hamilton	Beach water	HH18/3/2004W1-4	03-Aug-04	-	+
0029864713	04BO	C12	BOC12	Hamilton	Beach sand	BP8/9/2004SD1-1	09-Aug-04	-	+
0029864712	04BO	D01	BOD01	Hamilton	Beach sand	BP8/9/2004SD1-2	09-Aug-04	-	+
0031940952	04BO	D02	BOD02	Hamilton	Beach water	BP6/7/2004WK2-3	07-Jun-04	-	+
0029864710	04BO	D03	BOD03	Hamilton	Beach sand	BP8/9/2004SD1-4	09-Aug-04	-	+
0029864709	04BO	D04	BOD04	Hamilton	Beach sand	BP8/9/2004SD1-5	09-Aug-04	-	+
0031941980	04BO	D05	BOD05	Hamilton	Beach water	BP6/7/2004WK2-4	07-Jun-04	-	+
0029864707	04BO	D06	BOD06	Hamilton	Beach sand	BP8/9/2004SD1-7	09-Aug-04	-	+
0029864138	04BO	D07	BOD07	Hamilton	Beach sand	BP8/9/2004SD1-8	09-Aug-04	-	+
0031940954	04BO	D08	BOD08	Hamilton	Beach water	BP6/7/2004WK2-5	07-Jun-04	-	+
0029864700	04BO	D09	BOD09	Hamilton	Beach sand	BP8/9/2004SD2-1	09-Aug-04	-	+
0029864701	04BO	D10	BOD10	Hamilton	Beach sand	BP8/9/2004SD2-2	09-Aug-04	-	+
0029864702	04BO	D11	BOD11	Hamilton	Beach sand	BP8/9/2004SD2-3	09-Aug-04	-	+
0029864703	04BO	D12	BOD12	Hamilton	Beach sand	BP8/9/2004SD2-4	09-Aug-04	-	+
0029864704	04BO	E01	BOE01	Hamilton	Beach sand	BP8/9/2004SD2-5	09-Aug-04	-	+
0031940955	04BO	E02	BOE02	Hamilton	Beach water	BP6/7/2004WK2-6	07-Jun-04	-	+
0029864706	04BO	E03	BOE03	Hamilton	Beach sand	BP8/9/2004SD2-7	09-Aug-04	-	+
0029864126	04BO	E04	BOE04	Hamilton	Beach sand	BP8/9/2004SD2-8	09-Aug-04	-	+
0029864693	04BO	E05	BOE05	Hamilton	Beach sand	BP8/9/2004SD2-9	09-Aug-04	-	+
0029862052	04BO	E06	BOE06	Hamilton	Beach water	BP8/16/2004WK2-1	16-Aug-04	-	+
0029862051	04BO	E07	BOE07	Hamilton	Beach water	BP8/16/2004WK2-2	16-Aug-04	-	+
0029862050	04BO	E08	BOE08	Hamilton	Beach water	BP8/16/2004WK2-3	16-Aug-04	-	+
0029861674	04BO	E09	BOE09	Hamilton	Beach water	BP8/16/2004WK2-4	16-Aug-04	-	+
0029861691	04BO	E10	BOE10	Hamilton	Beach water	BP8/16/2004WK2-6	16-Aug-04	-	+
0029862047	04BO	E11	BOE11	Hamilton	Beach water	BP8/16/2004WK2-7	16-Aug-04	-	+
0029862046	04BO	E12	BOE12	Hamilton	Beach water	BP8/16/2004WK2-8	16-Aug-04	-	+
0029864323	04BO	F01	BOF01	Hamilton	Beach water	BP8/9/2004WA1-4	09-Aug-04	-	+
0029862894	04BO	F02	BOF02	Hamilton	Beach water	BP8/9/2004WA1-5	09-Aug-04	-	+
0029864312	04BO	F03	BOF03	Hamilton	Beach water	BP8/9/2004WA1-6	09-Aug-04	-	+
0029858646	04BO	F04	BOF04	Hamilton	Beach water	BP8/16/2004WA1-1	16-Aug-04	-	+
0029858645	04BO	F05	BOF05	Hamilton	Beach water	BP8/16/2004WA1-2	16-Aug-04	-	+
0029858644	04BO	F06	BOF06	Hamilton	Beach water	BP8/16/2004WA1-3	16-Aug-04	-	+
0029858643	04BO	F07	BOF07	Hamilton	Beach water	BP8/16/2004WA1-4	16-Aug-04	-	+
0029858642	04BO	F08	BOF08	Hamilton	Beach water	BP8/16/2004WA1-5	16-Aug-04	-	+
0029858641	04BO	F09	BOF09	Hamilton	Beach water	BP8/16/2004WA1-6	16-Aug-04	-	+
0029858640	04BO	F10	BOF10	Hamilton	Beach water	BP8/16/2004WA1-7	16-Aug-04	-	+
0029858623	04BO	F11	BOF11	Hamilton	Beach water	BP8/16/2004WA2-1	16-Aug-04	-	+
0029858624	04BO	F12	BOF12	Hamilton	Beach water	BP8/16/2004WA2-2	16-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0029858625	04BO	G01	BOG01	Hamilton	Beach water	BP8/16/2004WA2-3	16-Aug-04	-	+
0029858626	04BO	G02	BOG02	Hamilton	Beach water	BP8/16/2004WA2-4	16-Aug-04	-	+
0029858627	04BO	G03	BOG03	Hamilton	Beach water	BP8/16/2004WA2-5	16-Aug-04	-	+
0029858628	04BO	G04	BOG04	Hamilton	Beach water	BP8/16/2004WA2-6	16-Aug-04	-	+
0029858629	04BO	G05	BOG05	Hamilton	Beach water	BP8/16/2004WA2-7	16-Aug-04	-	+
0029858622	04BO	G06	BOG06	Hamilton	Beach water	BP8/16/2004WK1-1	16-Aug-04	-	+
0029858621	04BO	G07	BOG07	Hamilton	Beach water	BP8/16/2004WK1-2	16-Aug-04	-	+
0029858620	04BO	G08	BOG08	Hamilton	Beach water	BP8/16/2004WK1-3	16-Aug-04	-	+
0029878502	04BO	G09	BOG09	Hamilton	Beach water	BP9/7/2004WC1-10	07-Sep-04	-	+
0029858618	04BO	G10	BOG10	Hamilton	Beach water	BP8/16/2004WK1-5	16-Aug-04	-	+
0029858617	04BO	G11	BOG11	Hamilton	Beach water	BP8/16/2004WK1-6	16-Aug-04	-	+
0029858616	04BO	G12	BOG12	Hamilton	Beach water	BP8/16/2004WK1-7	16-Aug-04	-	+
0031925094	04BU	A01	BUA01	Toronto	Beach sand	KW37/19/2004SD1-7	19-Jul-04	-	+
0031925093	04BU	A02	BUA02	Toronto	Beach sand	KW37/19/2004SD1-8	19-Jul-04	-	+
0031925092	04BU	A03	BUA03	Toronto	Beach sand	KW37/19/2004SD1-9	19-Jul-04	-	+
0031925091	04BU	A04	BUA04	Toronto	Beach sand	KW37/19/2004SD1-10	19-Jul-04	-	+
0031925090	04BU	A05	BUA05	Toronto	Beach sand	KW37/19/2004SD1-11	19-Jul-04	-	+
0031925089	04BU	A06	BUA06	Toronto	Beach sand	KW37/19/2004SD1-12	19-Jul-04	-	+
0026738095	04BU	A07	BUA07	Toronto	Beach water	KW3E8/3/2004WC1-8	03-Aug-04	-	+
0031925087	04BU	A08	BUA08	Toronto	Beach sand	KW37/19/2004SD2-2	19-Jul-04	-	+
0031923611	04BU	A09	BUA09	Toronto	Beach sand	KW37/19/2004SD2-3	19-Jul-04	-	+
0031923625	04BU	A10	BUA10	Toronto	Beach sand	KW37/19/2004SD2-4	19-Jul-04	-	+
0031925084	04BU	A11	BUA11	Toronto	Beach sand	KW37/19/2004SD2-5	19-Jul-04	-	+
0031925083	04BU	A12	BUA12	Toronto	Beach sand	KW37/19/2004SD2-6	19-Jul-04	-	+
0031925071	04BU	B01	BUB01	Toronto	Beach sand	KW37/19/2004SD2-7	19-Jul-04	-	+
0031925072	04BU	B02	BUB02	Toronto	Beach sand	KW37/19/2004SD2-8	19-Jul-04	-	+
0031925073	04BU	B03	BUB03	Toronto	Beach sand	KW37/19/2004SD2-9	19-Jul-04	-	+
0031925074	04BU	B04	BUB04	Toronto	Beach sand	KW37/19/2004SD2-10	19-Jul-04	-	+
0031925075	04BU	B05	BUB05	Toronto	Beach sand	KW37/19/2004SD2-11	19-Jul-04	-	+
0031925076	04BU	B06	BUB06	Toronto	Beach sand	KW37/19/2004SD2-12	19-Jul-04	-	+
0031925077	04BU	B07	BUB07	Toronto	Beach sand	CI17/19/2004SD1-1	19-Jul-04	-	+
0031925078	04BU	B08	BUB08	Toronto	Beach sand	CI17/19/2004SD1-2	19-Jul-04	-	+
0031925079	04BU	B09	BUB09	Toronto	Beach sand	CI17/19/2004SD1-3	19-Jul-04	-	+
0031925080	04BU	B10	BUB10	Toronto	Beach sand	CI17/19/2004SD1-4	19-Jul-04	-	+
0031925081	04BU	B11	BUB11	Toronto	Beach sand	CI17/19/2004SD1-5	19-Jul-04	-	+
0031925082	04BU	B12	BUB12	Toronto	Beach sand	CI17/19/2004SD1-6	19-Jul-04	-	+
0031925070	04BU	C01	BUC01	Toronto	Beach sand	CI17/19/2004SD1-7	19-Jul-04	-	+
0031925069	04BU	C02	BUC02	Toronto	Beach sand	CI17/19/2004SD1-8	19-Jul-04	-	+
0031925068	04BU	C03	BUC03	Toronto	Beach sand	CI17/19/2004SD1-9	19-Jul-04	-	+
0031925067	04BU	C04	BUC04	Toronto	Beach sand	CI17/19/2004SD1-10	19-Jul-04	-	+
0031925066	04BU	C05	BUC05	Toronto	Beach sand	CI17/19/2004SD1-11	19-Jul-04	-	+
0031925065	04BU	C06	BUC06	Toronto	Beach sand	CI17/19/2004SD1-12	19-Jul-04	-	+
0031925064	04BU	C07	BUC07	Toronto	Beach sand	CI17/19/2004SD1-13	19-Jul-04	+	+
0031924730	04BU	C08	BUC08	Toronto	Beach sand	CI27/12/2004SD1-17	12-Jul-04	-	+
0031924731	04BU	C09	BUC09	Toronto	Beach sand	CI27/12/2004SD1-16	12-Jul-04	-	+
0031924732	04BU	C10	BUC10	Toronto	Beach sand	CI27/12/2004SD1-15	12-Jul-04	-	+
0031924733	04BU	C11	BUC11	Toronto	Beach sand	CI27/12/2004SD1-14	12-Jul-04	-	+
0031924734	04BU	C12	BUC12	Toronto	Beach sand	CI27/12/2004SD1-12	12-Jul-04	-	+
0031925047	04BU	D01	BUD01	Toronto	Beach sand	CI17/19/2004SD1-19	19-Jul-04	-	+
0031925048	04BU	D02	BUD02	Toronto	Beach sand	CI17/19/2004SD2-1	19-Jul-04	-	+
0031925049	04BU	D03	BUD03	Toronto	Beach sand	CI17/19/2004SD2-2	19-Jul-04	-	+
0031925050	04BU	D04	BUD04	Toronto	Beach sand	CI17/19/2004SD2-3	19-Jul-04	-	+
0031925051	04BU	D05	BUD05	Toronto	Beach sand	CI17/19/2004SD2-4	19-Jul-04	-	+
0031925052	04BU	D06	BUD06	Toronto	Beach sand	CI17/19/2004SD2-5	19-Jul-04	+	+
0031924741	04BU	D07	BUD07	Toronto	Beach sand	CI27/12/2004SD1-6	12-Jul-04	-	+
0031924740	04BU	D08	BUD08	Toronto	Beach sand	CI27/12/2004SD1-5	12-Jul-04	-	+
0031924739	04BU	D09	BUD09	Toronto	Beach sand	CI27/12/2004SD1-4	12-Jul-04	-	+
0031924738	04BU	D10	BUD10	Toronto	Beach sand	CI27/12/2004SD1-3	12-Jul-04	-	+
0031924737	04BU	D11	BUD11	Toronto	Beach sand	CI27/12/2004SD1-2	12-Jul-04	-	+
0031925058	04BU	D12	BUD12	Toronto	Beach sand	CI17/19/2004SD2-11	19-Jul-04	-	+
0031925046	04BU	E01	BUE01	Toronto	Beach sand	CI17/19/2004SD2-12	19-Jul-04	-	+
0031925045	04BU	E02	BUE02	Toronto	Beach sand	CI17/19/2004SD2-13	19-Jul-04	-	+
0031925044	04BU	E03	BUE03	Toronto	Beach sand	CI17/19/2004SD2-14	19-Jul-04	-	+
0031925043	04BU	E04	BUE04	Toronto	Beach sand	CI17/19/2004SD2-16	19-Jul-04	-	+
0031925042	04BU	E05	BUE05	Toronto	Beach sand	CI17/19/2004SD2-17	19-Jul-04	-	+
0031925041	04BU	E06	BUE06	Toronto	Beach sand	CI17/19/2004SD2-18	19-Jul-04	-	+
0031925040	04BU	E07	BUE07	Toronto	Beach sand	CI17/19/2004SD2-19	19-Jul-04	-	+
0031925039	04BU	E08	BUE08	Toronto	Beach sand	CI17/19/2004SD2-20	19-Jul-04	-	+
0031925038	04BU	E09	BUE09	Toronto	Beach sand	CI27/19/2004SD1-1	19-Jul-04	-	+
0031924736	04BU	E10	BUE10	Toronto	Beach sand	CI27/12/2004SD1-1	12-Jul-04	-	+
0031924735	04BU	E11	BUE11	Toronto	Beach sand	CI17/12/2004SD2-12	12-Jul-04	-	+
0031924747	04BU	E12	BUE12	Toronto	Beach sand	CI17/12/2004SD2-11	12-Jul-04	-	+
0031924754	04BU	F01	BUF01	Toronto	Beach sand	CI17/12/2004SD2-3	12-Jul-04	-	+
0031924748	04BU	F02	BUF02	Toronto	Beach sand	CI17/12/2004SD2-10	12-Jul-04	-	+
0031925025	04BU	F03	BUF03	Toronto	Beach sand	CI27/19/2004SD1-8	19-Jul-04	-	+
0031925026	04BU	F04	BUF04	Toronto	Beach sand	CI27/19/2004SD1-9	19-Jul-04	-	+
0031925027	04BU	F05	BUF05	Toronto	Beach sand	CI27/19/2004SD1-10	19-Jul-04	-	+
0031925028	04BU	F06	BUF06	Toronto	Beach sand	CI27/19/2004SD1-12	19-Jul-04	-	+
0031925029	04BU	F07	BUF07	Toronto	Beach sand	CI27/19/2004SD1-13	19-Jul-04	-	+
0031925030	04BU	F08	BUF08	Toronto	Beach sand	CI27/19/2004SD1-14	19-Jul-04	-	+
0031925031	04BU	F09	BUF09	Toronto	Beach sand	CI27/19/2004SD1-15	19-Jul-04	-	+
0031925032	04BU	F10	BUF10	Toronto	Beach sand	CI27/19/2004SD1-16	19-Jul-04	-	+
0031925033	04BU	F11	BUF11	Toronto	Beach sand	CI27/19/2004SD1-17	19-Jul-04	-	+
0031925034	04BU	F12	BUF12	Toronto	Beach sand	CI27/19/2004SD1-18	19-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0031925022	04BU	G01	BUG01	Toronto	Beach sand	CI27/19/2004SD1-19	19-Jul-04	-	+
0031925021	04BU	G02	BUG02	Toronto	Beach sand	CI27/19/2004SD1-20	19-Jul-04	-	+
0031924755	04BU	G03	BUG03	Toronto	Beach sand	CI17/12/2004SD1-19	12-Jul-04	-	+
0031924756	04BU	G04	BUG04	Toronto	Beach sand	CI17/12/2004SD1-18	12-Jul-04	-	+
0031924757	04BU	G05	BUG05	Toronto	Beach sand	CI17/12/2004SD1-17	12-Jul-04	-	+
0031924758	04BU	G06	BUG06	Toronto	Beach sand	CI17/12/2004SD1-16	12-Jul-04	-	+
0031924770	04BU	G07	BUG07	Toronto	Beach sand	CI17/12/2004SD1-14	12-Jul-04	-	-
0031925015	04BU	G08	BUG08	Toronto	Beach sand	CI27/19/2004SD2-6	19-Jul-04	-	+
0031925014	04BU	G09	BUG09	Toronto	Beach sand	CI27/19/2004SD2-7	19-Jul-04	-	+
0031925013	04BU	G10	BUG10	Toronto	Beach sand	CI27/19/2004SD2-8	19-Jul-04	-	+
0031924769	04BU	G11	BUG11	Toronto	Beach sand	CI17/12/2004SD1-13	12-Jul-04	-	+
0031924768	04BU	G12	BUG12	Toronto	Beach sand	CI17/12/2004SD1-12	12-Jul-04	-	+
0031939223	04BW	A01	BWA01	Hamilton	Beach water	BB7/19/2004WA1-7	19-Jul-04	-	+
0031939224	04BW	A02	BWA02	Hamilton	Beach water	BB7/19/2004WA1-8	19-Jul-04	-	+
0031939225	04BW	A03	BWA03	Hamilton	Beach water	BB7/19/2004WA1-9	19-Jul-04	-	+
0031939226	04BW	A04	BWA04	Hamilton	Beach water	BB7/19/2004WA1-10	19-Jul-04	-	+
0031939763	04BW	A05	BWA05	Hamilton	Beach water	BB7/19/2004WA1-11	19-Jul-04	-	+
0031939228	04BW	A06	BWA06	Hamilton	Beach water	BB7/19/2004WA1-12	19-Jul-04	-	+
0031939229	04BW	A07	BWA07	Hamilton	Beach water	BB7/19/2004WA2-2	19-Jul-04	-	+
0031939230	04BW	A08	BWA08	Hamilton	Beach water	BB7/19/2004WA2-3	19-Jul-04	-	+
0031939242	04BW	A09	BWA09	Hamilton	Beach water	BB7/19/2004WA2-6	19-Jul-04	-	+
0031939241	04BW	A10	BWA10	Hamilton	Beach water	BB7/19/2004WA2-12	19-Jul-04	-	+
0031939240	04BW	A11	BWA11	Hamilton	Beach water	BB7/19/2004WK1-1	19-Jul-04	-	+
0031939239	04BW	A12	BWA12	Hamilton	Beach water	BB7/19/2004WK1-2	19-Jul-04	-	+
0031939238	04BW	B01	BWB01	Hamilton	Beach water	BB7/19/2004WK1-3	19-Jul-04	-	+
0031939237	04BW	B02	BWB02	Hamilton	Beach water	BB7/19/2004WK1-4	19-Jul-04	-	+
0031939236	04BW	B03	BWB03	Hamilton	Beach water	BB7/19/2004WK1-7	19-Jul-04	-	+
0031939235	04BW	B04	BWB04	Hamilton	Beach water	BB7/19/2004WK1-8	19-Jul-04	-	+
0031939234	04BW	B05	BWB05	Hamilton	Beach water	BB7/19/2004WK1-9	19-Jul-04	-	+
0031939233	04BW	B06	BWB06	Hamilton	Beach water	BB7/19/2004WK1-10	19-Jul-04	-	+
0031939232	04BW	B07	BWB07	Hamilton	Beach water	BB7/19/2004WK1-11	19-Jul-04	-	+
0031939231	04BW	B08	BWB08	Hamilton	Beach water	BB7/19/2004WK2-3	19-Jul-04	-	+
0031939243	04BW	B09	BWB09	Hamilton	Beach water	BB7/19/2004WK2-4	19-Jul-04	-	+
0031939244	04BW	B10	BWB10	Hamilton	Beach water	BB7/19/2004WK2-5	19-Jul-04	+	+
0031939245	04BW	B11	BWB11	Hamilton	Beach water	BB7/19/2004WK2-7	19-Jul-04	-	+
0031939246	04BW	B12	BWB12	Hamilton	Beach water	BB7/19/2004WK2-8	19-Jul-04	-	+
0031939247	04BW	C01	BWC01	Hamilton	Beach water	BB7/19/2004WK2-9	19-Jul-04	-	+
0031939248	04BW	C02	BWC02	Hamilton	Beach water	BB7/19/2004WK2-12	19-Jul-04	-	+
0034437431	04BW	C03	BWC03	Hamilton	Beach sand	bb7/19/2004SD1-1	19-Jul-04	-	+
0034437432	04BW	C04	BWC04	Hamilton	Beach sand	bb7/19/2004SD1-2	19-Jul-04	-	+
0034437433	04BW	C05	BWC05	Hamilton	Beach sand	BB7/19/2004SD1-3	19-Jul-04	-	+
0034437434	04BW	C06	BWC06	Hamilton	Beach sand	BB7/19/2004SD1-4	19-Jul-04	-	+
0034437435	04BW	C07	BWC07	Hamilton	Beach sand	BB7/19/2004SD1-5	19-Jul-04	-	+
0034437436	04BW	C08	BWC08	Hamilton	Beach sand	BB7/19/2004SD1-6	19-Jul-04	-	+
0034437437	04BW	C09	BWC09	Hamilton	Beach sand	BB7/19/2004SD1-7	19-Jul-04	-	+
0034437438	04BW	C10	BWC10	Hamilton	Beach sand	BB7/19/2004SD1-8	19-Jul-04	-	+
0034436087	04BW	C11	BWC11	Hamilton	Beach sand	BB7/19/2004SD1-9	19-Jul-04	-	+
0034437449	04BW	C12	BWC12	Hamilton	Beach sand	BB7/19/2004SD1-10	19-Jul-04	+	+
0034437448	04BW	D01	BWD01	Hamilton	Beach sand	BB7/19/2004SD1-12	19-Jul-04	-	+
0034437447	04BW	D02	BWD02	Hamilton	Beach sand	BB7/19/2004SD2-1	19-Jul-04	-	+
0034437446	04BW	D03	BWD03	Hamilton	Beach sand	BB7/19/2004SD2-2	19-Jul-04	-	+
0034437445	04BW	D04	BWD04	Hamilton	Beach sand	BB7/19/2004SD2-3	19-Jul-04	-	+
0034437444	04BW	D05	BWD05	Hamilton	Beach sand	BB7/19/2004SD2-4	19-Jul-04	-	+
0034437443	04BW	D06	BWD06	Hamilton	Beach sand	BB7/19/2004SD2-5	19-Jul-04	-	+
0034437442	04BW	D07	BWD07	Hamilton	Beach sand	BB7/19/2004SD2-6	19-Jul-04	-	+
0034437441	04BW	D08	BWD08	Hamilton	Beach sand	BB7/19/2004SD2-8	19-Jul-04	-	+
0034437440	04BW	D09	BWD09	Hamilton	Beach sand	BB7/19/2004SD2-9	19-Jul-04	-	+
0034437439	04BW	D10	BWD10	Hamilton	Beach sand	BB7/19/2004SD2-10	19-Jul-04	-	+
0034437451	04BW	D11	BWD11	Hamilton	Beach sand	BB7/19/2004SD2-11	19-Jul-04	-	+
0034437452	04BW	D12	BWD12	Hamilton	Beach sand	BB7/19/2004SD2-12	19-Jul-04	-	+
0031939220	04BW	E01	BWE01	Hamilton	Beach water	BB7/19/2004WA1-3	19-Jul-04	-	+
0031939221	04BW	E02	BWE02	Hamilton	Beach water	BB7/19/2004WA1-4	19-Jul-04	-	+
0031939222	04BW	E03	BWE03	Hamilton	Beach water	BB7/19/2004WA1-5	19-Jul-04	-	+
0034437380	04BW	E04	BWE04	Hamilton	Beach water	BB7/26/2004WA1-1	26-Jul-04	-	+
0034437381	04BW	E05	BWE05	Hamilton	Beach water	BB7/26/2004WA1-2	26-Jul-04	-	+
0034437382	04BW	E06	BWE06	Hamilton	Beach water	BB7/26/2004WA1-3	26-Jul-04	-	+
0034437383	04BW	E07	BWE07	Hamilton	Beach water	BB7/26/2004WA1-4	26-Jul-04	-	+
0034437384	04BW	E08	BWE08	Hamilton	Beach water	BB7/26/2004WA1-5	26-Jul-04	-	+
0034437385	04BW	E09	BWE09	Hamilton	Beach water	BB7/26/2004WA1-6	26-Jul-04	-	+
0034437386	04BW	E10	BWE10	Hamilton	Beach water	BB7/26/2004WA1-7	26-Jul-04	-	+
0034437387	04BW	E11	BWE11	Hamilton	Beach water	BB7/26/2004WA1-8	26-Jul-04	+	+
0034437388	04BW	E12	BWE12	Hamilton	Beach water	BB7/26/2004WA1-9	26-Jul-04	+	+
0034437389	04BW	F01	BWF01	Hamilton	Beach water	BB7/26/2004WA1-10	26-Jul-04	-	+
0034437390	04BW	F02	BWF02	Hamilton	Beach water	BB7/26/2004WA1-11	26-Jul-04	-	+
0034437627	04BW	F03	BWF03	Hamilton	Beach water	BB7/26/2004WA1-12	26-Jul-04	-	+
0034437401	04BW	F04	BWF04	Hamilton	Beach water	BB7/26/2004WA2-1	26-Jul-04	-	+
0034437400	04BW	F05	BWF05	Hamilton	Beach water	BB7/26/2004WA2-2	26-Jul-04	-	+
0034437399	04BW	F06	BWF06	Hamilton	Beach water	BB7/26/2004WA2-3	26-Jul-04	-	+
0034437398	04BW	F07	BWF07	Hamilton	Beach water	BB7/26/2004WA2-4	26-Jul-04	-	+
0034437397	04BW	F08	BWF08	Hamilton	Beach water	BB7/26/2004WA2-6	26-Jul-04	-	+
0034437396	04BW	F09	BWF09	Hamilton	Beach water	BB7/26/2004WA2-7	26-Jul-04	+	+
0034437395	04BW	F10	BWF10	Hamilton	Beach water	BB7/26/2004WA2-8	26-Jul-04	-	+
0034437394	04BW	F11	BWF11	Hamilton	Beach water	BB7/26/2004WA2-9	26-Jul-04	-	+
0034437393	04BW	F12	BWF12	Hamilton	Beach water	BB7/26/2004WA2-10	26-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026739495	04BW	G01	BWG01	Hamilton	Beach water	BB8/3/2004WK1-5	03-Aug-04	-	+
0026739492	04BW	G02	BWG02	Hamilton	Beach water	BB8/3/2004WK1-2	03-Aug-04	-	+
0026739491	04BW	G03	BWG03	Hamilton	Beach water	BB8/3/2004WK1-1	03-Aug-04	-	+
0026739504	04BW	G04	BWG04	Hamilton	Beach water	BB8/3/2004WA2-3	03-Aug-04	-	+
0026739508	04BW	G05	BWG05	Hamilton	Beach water	BB8/3/2004WA1-7	03-Aug-04	-	+
0026739509	04BW	G06	BWG06	Hamilton	Beach water	BB8/3/2004WA1-6	03-Aug-04	-	+
0026739510	04BW	G07	BWG07	Hamilton	Beach water	BB8/3/2004WA1-5	03-Aug-04	-	-
0029864137	04BW	G08	BWG08	Hamilton	Beach water	BB8/9/2004WA1-3	09-Aug-04	-	+
0029864741	04BW	G09	BWG09	Hamilton	Beach water	BB8/9/2004WA1-4	09-Aug-04	-	+
0029864162	04BW	G10	BWG10	Hamilton	Beach water	BB8/9/2004WA1-5	09-Aug-04	-	+
0029864739	04BW	G11	BWG11	Hamilton	Beach water	BB8/9/2004WA1-6	09-Aug-04	-	+
0029864147	04BW	G12	BWG12	Hamilton	Beach water	BB8/9/2004WA1-7	09-Aug-04	-	+
0031924400	04CC	A01	CCA01	Toronto	Beach water	KW17/26/2004WA1-12	26-Jul-04	-	+
0031924401	04CC	A02	CCA02	Toronto	Beach water	KW17/26/2004WA2-2	26-Jul-04	-	+
0031924402	04CC	A03	CCA03	Toronto	Beach water	KW17/26/2004WA2-3	26-Jul-04	-	+
0031924403	04CC	A04	CCA04	Toronto	Beach water	KW17/26/2004WA2-6	26-Jul-04	-	+
0031924404	04CC	A05	CCA05	Toronto	Beach water	KW17/26/2004WA2-7	26-Jul-04	-	+
0031924405	04CC	A06	CCA06	Toronto	Beach water	KW17/26/2004WA2-8	26-Jul-04	-	+
0031924406	04CC	A07	CCA07	Toronto	Beach water	KW17/26/2004WA2-9	26-Jul-04	-	+
0031924407	04CC	A08	CCA08	Toronto	Beach water	KW17/26/2004WA2-10	26-Jul-04	-	+
0031924408	04CC	A09	CCA09	Toronto	Beach water	KW17/26/2004WA2-11	26-Jul-04	-	+
0031924410	04CC	A10	CCA10	Toronto	Beach water	KW17/26/2004WK1-2	26-Jul-04	-	+
0031924398	04CC	A11	CCA11	Toronto	Beach water	KW17/26/2004WK1-3	26-Jul-04	-	+
0031924397	04CC	A12	CCA12	Toronto	Beach water	KW17/26/2004WK1-4	26-Jul-04	-	+
0031924396	04CC	B01	CCB01	Toronto	Beach water	KW17/26/2004WK1-5	26-Jul-04	-	+
0031924395	04CC	B02	CCB02	Toronto	Beach water	KW17/26/2004WK1-6	26-Jul-04	-	+
0031924394	04CC	B03	CCB03	Toronto	Beach water	KW17/26/2004WK1-7	26-Jul-04	-	+
0031924393	04CC	B04	CCB04	Toronto	Beach water	KW17/26/2004WK1-8	26-Jul-04	-	+
0031924392	04CC	B05	CCB05	Toronto	Beach water	KW17/26/2004WK1-9	26-Jul-04	-	+
0031924391	04CC	B06	CCB06	Toronto	Beach water	KW17/26/2004WK1-10	26-Jul-04	-	+
0031924390	04CC	B07	CCB07	Toronto	Beach water	KW17/26/2004WK1-11	26-Jul-04	-	+
0031924388	04CC	B08	CCB08	Toronto	Beach water	KW17/26/2004WK2-2	26-Jul-04	-	+
0031924387	04CC	B09	CCB09	Toronto	Beach water	KW17/26/2004WK2-3	26-Jul-04	-	+
0031924375	04CC	B10	CCB10	Toronto	Beach water	KW17/26/2004WK2-4	26-Jul-04	-	+
0031924376	04CC	B11	CCB11	Toronto	Beach water	KW17/26/2004WK2-5	26-Jul-04	-	+
0031924377	04CC	B12	CCB12	Toronto	Beach water	KW17/26/2004WK2-6	26-Jul-04	-	+
0031924378	04CC	C01	CCC01	Toronto	Beach water	KW17/26/2004WK2-7	26-Jul-04	-	+
0031924379	04CC	C02	CCC02	Toronto	Beach water	KW17/26/2004WK2-9	26-Jul-04	-	+
0031924380	04CC	C03	CCC03	Toronto	Beach water	KW17/26/2004WK2-10	26-Jul-04	-	+
0031924381	04CC	C04	CCC04	Toronto	Beach water	KW17/26/2004WK2-11	26-Jul-04	-	+
0031924382	04CC	C05	CCC05	Toronto	Beach water	KW17/26/2004WK2-12	26-Jul-04	-	+
0031924384	04CC	C06	CCC06	Toronto	Beach water	KW27/26/2004WA1-2	26-Jul-04	-	+
0031924385	04CC	C07	CCC07	Toronto	Beach water	KW27/26/2004WA1-3	26-Jul-04	-	+
0031924386	04CC	C08	CCC08	Toronto	Beach water	KW27/26/2004WA1-4	26-Jul-04	-	+
0031924374	04CC	C09	CCC09	Toronto	Beach water	KW27/26/2004WA1-5	26-Jul-04	-	+
0031924373	04CC	C10	CCC10	Toronto	Beach water	KW27/26/2004WA1-6	26-Jul-04	-	+
0031924372	04CC	C11	CCC11	Toronto	Beach water	KW27/26/2004WA1-7	26-Jul-04	-	+
0031924371	04CC	C12	CCC12	Toronto	Beach water	KW27/26/2004WA1-8	26-Jul-04	-	+
0031924370	04CC	D01	CCD01	Toronto	Beach water	KW27/26/2004WA1-9	26-Jul-04	-	+
0031924369	04CC	D02	CCD02	Toronto	Beach water	KW27/26/2004WA1-10	26-Jul-04	-	+
0031924368	04CC	D03	CCD03	Toronto	Beach water	KW27/26/2004WA1-11	26-Jul-04	-	+
0031924367	04CC	D04	CCD04	Toronto	Beach water	KW27/26/2004WA1-12	26-Jul-04	-	+
0031924365	04CC	D05	CCD05	Toronto	Beach water	KW27/26/2004WA2-2	26-Jul-04	-	+
0031924364	04CC	D06	CCD06	Toronto	Beach water	KW27/26/2004WA2-3	26-Jul-04	-	+
0031924363	04CC	D07	CCD07	Toronto	Beach water	KW27/26/2004WA2-5	26-Jul-04	-	+
0031924351	04CC	D08	CCD08	Toronto	Beach water	KW27/26/2004WA2-6	26-Jul-04	-	+
0031924352	04CC	D09	CCD09	Toronto	Beach water	KW27/26/2004WA2-7	26-Jul-04	-	+
0031924353	04CC	D10	CCD10	Toronto	Beach water	KW27/26/2004WA2-8	26-Jul-04	-	+
0031924354	04CC	D11	CCD11	Toronto	Beach water	KW27/26/2004WA2-9	26-Jul-04	-	+
0031924355	04CC	D12	CCD12	Toronto	Beach water	KW27/26/2004WA2-10	26-Jul-04	-	+
0031924356	04CC	E01	CCE01	Toronto	Beach water	KW27/26/2004WA2-11	26-Jul-04	-	+
0031924357	04CC	E02	CCE02	Toronto	Beach water	KW27/26/2004WA2-12	26-Jul-04	-	+
0031924344	04CC	E03	CCE03	Toronto	Beach water	KW27/26/2004WK1-12	26-Jul-04	-	+
0031924342	04CC	E04	CCE04	Toronto	Beach water	KW27/26/2004WK2-2	26-Jul-04	-	+
0031924341	04CC	E05	CCE05	Toronto	Beach water	KW27/26/2004WK2-3	26-Jul-04	-	+
0026734044	04CC	E06	CCE06	Toronto	Beach water	KW18/9/2004WC1-2	09-Aug-04	-	+
0026734045	04CC	E07	CCE07	Toronto	Beach water	KW18/9/2004WC1-3	09-Aug-04	-	+
0026734048	04CC	E08	CCE08	Toronto	Beach water	KW18/9/2004WC2-2	09-Aug-04	-	+
0026734049	04CC	E09	CCE09	Toronto	Beach water	KW18/9/2004WC2-3	09-Aug-04	-	+
0026734050	04CC	E10	CCE10	Toronto	Beach water	KW18/9/2004WC2-4	09-Aug-04	+	+
0026734038	04CC	E11	CCE11	Toronto	Beach water	KW18/9/2004WC2-5	09-Aug-04	-	+
0026734037	04CC	E12	CCE12	Toronto	Beach water	KW18/9/2004WC2-6	09-Aug-04	-	+
0026734036	04CC	F01	CCF01	Toronto	Beach water	KW18/9/2004WC2-7	09-Aug-04	-	+
0026734032	04CC	F02	CCF02	Toronto	Beach water	KW38/9/2004WC1-3	09-Aug-04	-	+
0026734031	04CC	F03	CCF03	Toronto	Beach water	KW38/9/2004WC1-4	09-Aug-04	-	+
0026734030	04CC	F04	CCF04	Toronto	Beach water	KW38/9/2004WC1-5	09-Aug-04	-	+
0026734029	04CC	F05	CCF05	Toronto	Beach water	KW38/9/2004WC1-6	09-Aug-04	-	+
0026734027	04CC	F06	CCF06	Toronto	Beach water	KW38/9/2004WC1-8	09-Aug-04	-	-
0026734015	04CC	F07	CCF07	Toronto	Beach water	KW38/9/2004WC1-9	09-Aug-04	-	+
0026734016	04CC	F08	CCF08	Toronto	Beach water	KW38/9/2004WC1-10	09-Aug-04	-	+
0026734017	04CC	F09	CCF09	Toronto	Beach water	KW38/9/2004WC1-11	09-Aug-04	-	+
0026734018	04CC	F10	CCF10	Toronto	Beach water	KW38/9/2004WC1-12	09-Aug-04	-	+
0026734020	04CC	F11	CCF11	Toronto	Beach water	KW38/9/2004WC2-3	09-Aug-04	-	+
0026734046	04CC	F12	CCF12	Toronto	Beach water	KW18/9/2004WC1-4	09-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026734022	04CC	G01	CCG01	Toronto	Beach water	KW38/9/2004WC2-5	09-Aug-04	-	+
0026734023	04CC	G02	CCG02	Toronto	Beach water	KW38/9/2004WC2-6	09-Aug-04	-	+
0026734024	04CC	G03	CCG03	Toronto	Beach water	KW38/9/2004WC2-7	09-Aug-04	-	+
0026734025	04CC	G04	CCG04	Toronto	Beach water	KW38/9/2004WC2-8	09-Aug-04	-	+
0026734026	04CC	G05	CCG05	Toronto	Beach water	KW38/9/2004WC2-9	09-Aug-04	-	+
0026734014	04CC	G06	CCG06	Toronto	Beach water	KW38/9/2004WC2-10	09-Aug-04	-	+
0026734013	04CC	G07	CCG07	Toronto	Beach water	KW38/9/2004WC2-11	09-Aug-04	-	+
0026734012	04CC	G08	CCG08	Toronto	Beach water	KW38/9/2004WC2-12	09-Aug-04	-	+
0031924471	04CC	G09	CCG09	Toronto	Beach water	CI17/12/2004WC2-7	12-Jul-04	-	+
0031924472	04CC	G10	CCG10	Toronto	Beach water	CI17/12/2004WC2-8	12-Jul-04	-	+
0031924473	04CC	G11	CCG11	Toronto	Beach water	CI17/12/2004WC2-9	12-Jul-04	-	+
0031924474	04CC	G12	CCG12	Toronto	Beach water	CI17/12/2004WC2-10	12-Jul-04	-	+
0031940030	04CD	A01	CDA01	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG8-1	26-Jul-04	-	+
0031940029	04CD	A02	CDA02	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG8-2	26-Jul-04	-	+
0031940028	04CD	A03	CDA03	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG8-3	26-Jul-04	-	+
0031940027	04CD	A04	CDA04	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG10-1	26-Jul-04	-	+
0031940026	04CD	A05	CDA05	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG10-3	26-Jul-04	-	+
0031940025	04CD	A06	CDA06	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG11-1	26-Jul-04	-	+
0031940024	04CD	A07	CDA07	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG16-3	26-Jul-04	-	+
0031905250	04CD	A08	CDA08	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG17-2	26-Jul-04	-	+
0031940035	04CD	A09	CDA09	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG17-3	26-Jul-04	-	+
0031905242	04CD	A10	CDA10	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG18-1	26-Jul-04	-	+
0031940037	04CD	A11	CDA11	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG18-2	26-Jul-04	-	+
0031940038	04CD	A12	CDA12	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG18-3	26-Jul-04	-	+
0031940039	04CD	B01	CDB01	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG19-2	26-Jul-04	-	+
0031940040	04CD	B02	CDB02	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG20-1	26-Jul-04	-	+
0031940041	04CD	B03	CDB03	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG20-2	26-Jul-04	-	+
0031940042	04CD	B04	CDB04	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG20-3	26-Jul-04	-	+
0031940043	04CD	B05	CDB05	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG21-1	26-Jul-04	-	+
0031940044	04CD	B06	CDB06	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG21-2	26-Jul-04	-	+
0031940045	04CD	B07	CDB07	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG21-3	26-Jul-04	-	+
0031940046	04CD	B08	CDB08	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG22-1	26-Jul-04	-	+
0031940058	04CD	B09	CDB09	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG22-2	26-Jul-04	-	+
0031905241	04CD	B10	CDB10	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG22-3	26-Jul-04	-	+
0031940056	04CD	B11	CDB11	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG24-1	26-Jul-04	-	+
0031940055	04CD	B12	CDB12	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG24-2	26-Jul-04	-	+
0031940054	04CD	C01	CDC01	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG24-3	26-Jul-04	-	+
0031940053	04CD	C02	CDC02	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG25-1	26-Jul-04	-	+
0031940052	04CD	C03	CDC03	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG25-2	26-Jul-04	-	-
0031940051	04CD	C04	CDC04	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG25-3	26-Jul-04	-	-
0031940050	04CD	C05	CDC05	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG26-1	26-Jul-04	-	+
0031940049	04CD	C06	CDC06	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG26-2	26-Jul-04	-	+
0031940178	04CD	C07	CDC07	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG26-3	26-Jul-04	-	+
0031940177	04CD	C08	CDC08	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG27-1	26-Jul-04	-	+
0031940176	04CD	C09	CDC09	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG27-2	26-Jul-04	-	+
0031940175	04CD	C10	CDC10	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG27-3	26-Jul-04	-	+
0031940174	04CD	C11	CDC11	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG28-1	26-Jul-04	-	+
0031940173	04CD	C12	CDC12	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG28-2	26-Jul-04	-	+
0031940172	04CD	D01	CDD01	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG28-3	26-Jul-04	-	+
0031940171	04CD	D02	CDD02	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG32-2	26-Jul-04	-	+
0031940170	04CD	D03	CDD03	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG32-3	26-Jul-04	-	+
0031940169	04CD	D04	CDD04	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG33-1	26-Jul-04	-	+
0031940168	04CD	D05	CDD05	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG34-1	26-Jul-04	-	-
0031940167	04CD	D06	CDD06	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG34-2	26-Jul-04	-	+
0031940179	04CD	D07	CDD07	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG35-2	26-Jul-04	-	+
0031940180	04CD	D08	CDD08	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG36-1	26-Jul-04	-	+
0031940181	04CD	D09	CDD09	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG36-2	26-Jul-04	-	+
0031940182	04CD	D10	CDD10	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG36-3	26-Jul-04	-	+
0031940183	04CD	D11	CDD11	Toronto	Gull (Larus delawarensis)	AH7/26/2004G1-1	26-Jul-04	-	+
0031940184	04CD	D12	CDD12	Toronto	Gull (Larus delawarensis)	AH7/26/2004G1-2	26-Jul-04	-	+
0031940185	04CD	E01	CDE01	Toronto	Gull (Larus delawarensis)	AH7/26/2004G1-3	26-Jul-04	-	+
0031940186	04CD	E02	CDE02	Toronto	Mallard duck (Anas platyrhynchos)	AH7/26/2004D1-1	26-Jul-04	-	+
0031940187	04CD	E03	CDE03	Toronto	Mallard duck (Anas platyrhynchos)	AH7/26/2004D1-2	26-Jul-04	-	+
0031940188	04CD	E04	CDE04	Toronto	Mallard duck (Anas platyrhynchos)	AH7/26/2004D1-3	26-Jul-04	-	+
0031940189	04CD	E05	CDE05	Toronto	Dog (Canis lupus familiaris)	AH7/26/2004DG1-1	26-Jul-04	-	+
0031940201	04CD	E06	CDE06	Toronto	Dog (Canis lupus familiaris)	AH7/26/2004DG1-4	26-Jul-04	-	+
0031940200	04CD	E07	CDE07	Toronto	Dog (Canis lupus familiaris)	AH7/26/2004DG1-5	26-Jul-04	-	+
0031940199	04CD	E08	CDE08	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG1-1	26-Jul-04	-	+
0031940198	04CD	E09	CDE09	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG1-3	26-Jul-04	-	+
0031940197	04CD	E10	CDE10	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG1-4	26-Jul-04	-	+
0031940196	04CD	E11	CDE11	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG1-5	26-Jul-04	-	+
0031939728	04CD	E12	CDE12	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG2-3	26-Jul-04	-	+
0031940194	04CD	F01	CDF01	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG2-4	26-Jul-04	-	+
0031940193	04CD	F02	CDF02	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG2-5	26-Jul-04	-	+
0031940192	04CD	F03	CDF03	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG3-1	26-Jul-04	-	+
0031940191	04CD	F04	CDF04	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG3-2	26-Jul-04	-	+
0031940203	04CD	F05	CDF05	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG3-3	26-Jul-04	-	+
0031940204	04CD	F06	CDF06	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG3-4	26-Jul-04	-	+
0031940205	04CD	F07	CDF07	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG3-5	26-Jul-04	-	+
0031940206	04CD	F08	CDF08	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG4-1	26-Jul-04	-	+
0031940207	04CD	F09	CDF09	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG4-2	26-Jul-04	-	+
0031939726	04CD	F10	CDF10	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG4-3	26-Jul-04	-	+
0031940209	04CD	F11	CDF11	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG4-4	26-Jul-04	+	+
0031940210	04CD	F12	CDF12	Toronto	Dog (Canis lupus familiaris)	SPCA7/26/2004DG4-5	26-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0031924422	04CD	G01	CDG01	Toronto	Dog (Canis lupus familiaris)	KW7/26/2004DG1-3	26-Jul-04	-	+
0031924421	04CD	G02	CDG02	Toronto	Dog (Canis lupus familiaris)	KW7/26/2004DG2-3	26-Jul-04	-	+
0031924420	04CD	G03	CDG03	Toronto	Dog (Canis lupus familiaris)	KW7/26/2004DG2-4	26-Jul-04	-	+
0031924418	04CD	G04	CDG04	Toronto	Beach water	KW17/26/2004WA1-2	26-Jul-04	-	+
0031924417	04CD	G05	CDG05	Toronto	Beach water	KW17/26/2004WA1-4	26-Jul-04	-	+
0031924416	04CD	G06	CDG06	Toronto	Beach water	KW17/26/2004WA1-5	26-Jul-04	-	-
0031924415	04CD	G07	CDG07	Toronto	Beach water	KW17/26/2004WA1-6	26-Jul-04	-	+
0031924414	04CD	G08	CDG08	Toronto	Beach water	KW17/26/2004WA1-7	26-Jul-04	-	+
0031924413	04CD	G09	CDG09	Toronto	Beach water	KW17/26/2004WA1-8	26-Jul-04	-	+
0031924412	04CD	G10	CDG10	Toronto	Beach water	KW17/26/2004WA1-9	26-Jul-04	-	+
0031924411	04CD	G11	CDG11	Toronto	Beach water	KW17/26/2004WA1-10	26-Jul-04	-	+
0031924399	04CD	G12	CDG12	Toronto	Beach water	KW17/26/2004WA1-11	26-Jul-04	-	+
0031940233	04CF	A01	CFA01	Toronto	Canada Goose (Branta canadensis)	CI27/26/2004CG6-1	26-Jul-04	-	+
0031940236	04CF	A02	CFA02	Toronto	Canada Goose (Branta canadensis)	CI27/26/2004CG7-1	26-Jul-04	-	+
0031939737	04CF	A03	CFA03	Toronto	Canada Goose (Branta canadensis)	CI27/26/2004CG8-1	26-Jul-04	-	+
0031904891	04CF	A04	CFA04	Toronto	Sewage plant final effluent	AF27/26/2004FE1-2	26-Jul-04	-	+
0029881817	04CF	A05	CFA05	Toronto	Untreated CSO sewage	af98/23/2004CSO1-17	23-Aug-04	-	+
0029881802	04CF	A06	CFA06	Toronto	Untreated CSO sewage	af38/24/2004CSO1-3	24-Aug-04	-	+
0029881801	04CF	A07	CFA07	Toronto	Untreated CSO sewage	af38/24/2004CSO1-4	24-Aug-04	-	+
0029881787	04CF	A08	CFA08	Toronto	Untreated CSO sewage	af38/24/2004CSO1-15	24-Aug-04	+	+
0029881788	04CF	A09	CFA09	Toronto	Untreated CSO sewage	af38/24/2004CSO1-16	24-Aug-04	-	+
0029881793	04CF	A10	CFA10	Toronto	Untreated CSO sewage	af48/24/2004CSO1-1	24-Aug-04	-	+
0029881757	04CF	A11	CFA11	Toronto	Untreated CSO sewage	af68/24/2004CSO1-15	24-Aug-04	-	+
0029881756	04CF	A12	CFA12	Toronto	Untreated CSO sewage	af68/24/2004CSO1-16	24-Aug-04	-	+
0029895494	04CF	B01	CFB01	Toronto	Beach water	CI28/17/2004WC2-4	17-Aug-04	-	+
0029880628	04CF	B02	CFB02	Toronto	Beach water	CI17/26/2004WA1-1	26-Jul-04	-	+
0029880627	04CF	B03	CFB03	Toronto	Beach water	CI17/26/2004WA1-2	26-Jul-04	-	+
0029880626	04CF	B04	CFB04	Toronto	Beach water	CI17/26/2004WA1-3	26-Jul-04	-	+
0029880609	04CF	B05	CFB05	Toronto	Beach water	CI17/26/2004WA2-1	26-Jul-04	-	+
0029880610	04CF	B06	CFB06	Toronto	Beach water	CI17/26/2004WA2-2	26-Jul-04	-	+
0029880611	04CF	B07	CFB07	Toronto	Beach water	CI17/26/2004WA2-3	26-Jul-04	-	+
0029880604	04CF	B08	CFB08	Toronto	Beach water	CI17/26/2004WK1-1	26-Jul-04	-	+
0029880603	04CF	B09	CFB09	Toronto	Beach water	CI17/26/2004WK1-2	26-Jul-04	-	+
0029880589	04CF	B10	CFB10	Toronto	Beach water	CI17/26/2004WK1-3	26-Jul-04	-	+
0029879330	04CF	B11	CFB11	Toronto	Beach water	CI17/26/2004WK2-1	26-Jul-04	-	+
0029879329	04CF	B12	CFB12	Toronto	Beach water	CI17/26/2004WK2-2	26-Jul-04	-	+
0029879328	04CF	C01	CFC01	Toronto	Beach water	CI17/26/2004WK2-3	26-Jul-04	-	+
0029879314	04CF	C02	CFC02	Toronto	Beach water	CI27/26/2004WA1-1	26-Jul-04	-	+
0029879315	04CF	C03	CFC03	Toronto	Beach water	CI27/26/2004WA1-2	26-Jul-04	-	+
0029879316	04CF	C04	CFC04	Toronto	Beach water	CI27/26/2004WA1-3	26-Jul-04	-	+
0029879307	04CF	C05	CFC05	Toronto	Beach water	CI27/26/2004WA2-1	26-Jul-04	-	+
0029879306	04CF	C06	CFC06	Toronto	Beach water	CI27/26/2004WA2-2	26-Jul-04	-	+
0029879305	04CF	C07	CFC07	Toronto	Beach water	CI27/26/2004WA2-3	26-Jul-04	-	+
0029879290	04CF	C08	CFC08	Toronto	Beach water	CI27/26/2004WK1-1	26-Jul-04	-	+
0029879291	04CF	C09	CFC09	Toronto	Beach water	CI27/26/2004WK1-2	26-Jul-04	-	+
0029879292	04CF	C10	CFC10	Toronto	Beach water	CI27/26/2004WK1-3	26-Jul-04	-	+
0029879284	04CF	C11	CFC11	Toronto	Beach water	CI27/26/2004WK2-1	26-Jul-04	-	+
0029879283	04CF	C12	CFC12	Toronto	Beach water	CI27/26/2004WK2-2	26-Jul-04	-	+
0029879282	04CF	D01	CFD01	Toronto	Beach water	CI27/26/2004WK2-3	26-Jul-04	-	+
0031939538	04CF	D02	CFD02	Toronto	Sewage plant final effluent	AF 17/19/2004FE1-2	19-Jul-04	-	+
0031904914	04CF	D03	CFD03	Toronto	Beach water	CI27/26/2004WC2-1	26-Jul-04	-	+
0031904915	04CF	D04	CFD04	Toronto	Beach water	CI27/26/2004WC2-2	26-Jul-04	-	+
0026738087	04CF	D05	CFD05	Toronto	Beach water	CI18/3/2004WC1-3	03-Aug-04	-	+
0026738086	04CF	D06	CFD06	Toronto	Beach water	CI18/3/2004WC1-4	03-Aug-04	-	+
0029862577	04CF	D07	CFD07	Toronto	Beach water	CI28/17/2004WA1-1	17-Aug-04	-	+
0029862576	04CF	D08	CFD08	Toronto	Beach water	CI28/17/2004WA1-2	17-Aug-04	-	+
0029862575	04CF	D09	CFD09	Toronto	Beach water	CI28/17/2004WA1-3	17-Aug-04	-	-
0029862561	04CF	D10	CFD10	Toronto	Beach water	CI28/17/2004WA2-1	17-Aug-04	-	+
0029862557	04CF	D11	CFD11	Toronto	Beach water	CI28/17/2004WK1-1	17-Aug-04	-	+
0029862556	04CF	D12	CFD12	Toronto	Beach water	CI28/17/2004WK1-2	17-Aug-04	-	+
0029862555	04CF	E01	CFE01	Toronto	Beach water	CI28/17/2004WK1-3	17-Aug-04	-	+
0026738089	04CF	E02	CFE02	Toronto	Beach water	CI18/3/2004WC1-1	03-Aug-04	-	+
0026738088	04CF	E03	CFE03	Toronto	Beach water	CI18/3/2004WC1-2	03-Aug-04	-	+
0029878113	04CF	E04	CFE04	Toronto	Beach water	CI17/26/2004WC1-1	26-Jul-04	-	+
0029878114	04CF	E05	CFE05	Toronto	Beach water	CI17/26/2004WC1-2	26-Jul-04	-	+
0029878115	04CF	E06	CFE06	Toronto	Beach water	CI17/26/2004WC1-3	26-Jul-04	-	+
0029878116	04CF	E07	CFE07	Toronto	Beach water	CI17/26/2004WC1-4	26-Jul-04	-	+
0029878109	04CF	E08	CFE08	Toronto	Beach water	CI17/26/2004WC2-1	26-Jul-04	-	+
0029878108	04CF	E09	CFE09	Toronto	Beach water	CI17/26/2004WC2-2	26-Jul-04	-	+
0029878107	04CF	E10	CFE10	Toronto	Beach water	CI17/26/2004WC2-3	26-Jul-04	-	+
0029878106	04CF	E11	CFE11	Toronto	Beach water	CI17/26/2004WC2-4	26-Jul-04	-	+
0026732665	04CF	E12	CFE12	Toronto	Beach water	CI18/23/2004WC1-1	23-Aug-04	-	+
0026738984	04CF	F01	CFF01	Toronto	Beach water	CI18/23/2004WC1-2	23-Aug-04	-	+
0026739055	04CF	F02	CFF02	Toronto	Beach water	CI18/23/2004WC1-3	23-Aug-04	-	+
0026732534	04CF	F03	CFF03	Toronto	Beach water	CI18/23/2004WC1-4	23-Aug-04	-	+
0026738076	04CF	F04	CFF04	Toronto	Beach water	CI18/3/2004WC2-1	03-Aug-04	-	+
0026738077	04CF	F05	CFF05	Toronto	Beach water	CI18/3/2004WC2-2	03-Aug-04	-	+
0026738078	04CF	F06	CFF06	Toronto	Beach water	CI18/3/2004WC2-3	03-Aug-04	-	+
0026738079	04CF	F07	CFF07	Toronto	Beach water	CI18/3/2004WC2-4	03-Aug-04	-	+
0026711648	04CF	F08	CFF08	Toronto	Beach water	CI18/9/2004WA1-1	09-Aug-04	-	+
0026711649	04CF	F09	CFF09	Toronto	Beach water	CI18/9/2004WA1-2	09-Aug-04	-	+
0026711650	04CF	F10	CFF10	Toronto	Beach water	CI18/9/2004WA1-3	09-Aug-04	-	+
0026711645	04CF	F11	CFF11	Toronto	Beach water	CI18/9/2004WA2-1	09-Aug-04	-	+
0026711644	04CF	F12	CFF12	Toronto	Beach water	CI18/9/2004WA2-2	09-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026733579	04CF	G01	CFG01	Toronto	Beach water	CI28/9/2004WA1-1	09-Aug-04	-	+
0026733578	04CF	G02	CFG02	Toronto	Beach water	CI28/9/2004WA1-2	09-Aug-04	-	+
0026733577	04CF	G03	CFG03	Toronto	Beach water	CI28/9/2004WA1-3	09-Aug-04	-	+
0026733562	04CF	G04	CFG04	Toronto	Beach water	CI28/9/2004WK1-1	09-Aug-04	-	+
0026733563	04CF	G05	CFG05	Toronto	Beach water	CI28/9/2004WK1-2	09-Aug-04	-	+
0026733564	04CF	G06	CFG06	Toronto	Beach water	CI28/9/2004WK1-3	09-Aug-04	-	+
0026733555	04CF	G07	CFG07	Toronto	Beach water	CI18/9/2004WC1-1	09-Aug-04	-	+
0026733554	04CF	G08	CFG08	Toronto	Beach water	CI18/9/2004WC1-2	09-Aug-04	-	+
0026733553	04CF	G09	CFG09	Toronto	Beach water	CI18/9/2004WC1-3	09-Aug-04	-	+
0026733552	04CF	G10	CFG10	Toronto	Beach water	CI18/9/2004WC1-4	09-Aug-04	-	+
0026733538	04CF	G11	CFG11	Toronto	Beach water	CI18/9/2004WC2-1	09-Aug-04	-	+
0026733539	04CF	G12	CFG12	Toronto	Beach water	CI18/9/2004WC2-2	09-Aug-04	-	+
0036634194	04CI	A01	CIA01	Toronto	Beach sand	KW17/26/2004SD2-11	26-Jul-04	-	+
0036633787	04CI	A02	CIA02	Toronto	Beach sand	KW17/26/2004SD2-12	26-Jul-04	-	+
0029896167	04CI	A03	CIA03	Toronto	Beach sand	KW38/17/2004SD1-7	17-Aug-04	-	+
0036633789	04CI	A04	CIA04	Toronto	Beach sand	KW27/26/2004SD1-2	26-Jul-04	-	+
0036634190	04CI	A05	CIA05	Toronto	Beach sand	KW27/26/2004SD1-3	26-Jul-04	-	+
0036634189	04CI	A06	CIA06	Toronto	Beach sand	KW27/26/2004SD1-4	26-Jul-04	-	+
0036634188	04CI	A07	CIA07	Toronto	Beach sand	KW27/26/2004SD1-5	26-Jul-04	-	+
0036634187	04CI	A08	CIA08	Toronto	Beach sand	KW27/26/2004SD1-6	26-Jul-04	-	+
0036634186	04CI	A09	CIA09	Toronto	Beach sand	KW27/26/2004SD1-7	26-Jul-04	-	+
0036634185	04CI	A10	CIA10	Toronto	Beach sand	KW27/26/2004SD1-8	26-Jul-04	-	+
0036634184	04CI	A11	CIA11	Toronto	Beach sand	KW27/26/2004SD1-9	26-Jul-04	-	+
0036634183	04CI	A12	CIA12	Toronto	Beach sand	KW27/26/2004SD1-10	26-Jul-04	-	+
0036634195	04CI	B01	CIB01	Toronto	Beach sand	KW27/26/2004SD1-11	26-Jul-04	-	+
0036634196	04CI	B02	CIB02	Toronto	Beach sand	KW27/26/2004SD1-12	26-Jul-04	-	+
0031904866	04CI	B03	CIB03	Toronto	Beach sand	KW17/26/2004SD1-2	26-Jul-04	-	+
0036634198	04CI	B04	CIB04	Toronto	Beach sand	KW27/26/2004SD2-2	26-Jul-04	-	+
0036634199	04CI	B05	CIB05	Toronto	Beach sand	KW27/26/2004SD2-3	26-Jul-04	+	+
0036634200	04CI	B06	CIB06	Toronto	Beach sand	KW27/26/2004SD2-4	26-Jul-04	-	+
0036634201	04CI	B07	CIB07	Toronto	Beach sand	KW27/26/2004SD2-6	26-Jul-04	-	+
0036634202	04CI	B08	CIB08	Toronto	Beach sand	KW27/26/2004SD2-7	26-Jul-04	-	+
0036634203	04CI	B09	CIB09	Toronto	Beach sand	KW27/26/2004SD2-8	26-Jul-04	-	+
0036634204	04CI	B10	CIB10	Toronto	Beach sand	KW27/26/2004SD2-9	26-Jul-04	-	+
0036634205	04CI	B11	CIB11	Toronto	Beach sand	KW27/26/2004SD2-10	26-Jul-04	-	+
0036633788	04CI	B12	CIB12	Toronto	Beach sand	KW27/26/2004SD2-11	26-Jul-04	-	+
0036634218	04CI	C01	CIC01	Toronto	Beach sand	KW27/26/2004SD2-12	26-Jul-04	-	+
0034437140	04CI	C02	CIC02	Toronto	Beach sand	CI5/3/2004SD1-3	03-May-04	-	+
0036634216	04CI	C03	CIC03	Toronto	Beach sand	KW37/26/2004SD1-2	26-Jul-04	-	+
0036634215	04CI	C04	CIC04	Toronto	Beach sand	KW37/26/2004SD1-3	26-Jul-04	-	+
0036634214	04CI	C05	CIC05	Toronto	Beach sand	KW37/26/2004SD1-4	26-Jul-04	-	+
0036634213	04CI	C06	CIC06	Toronto	Beach sand	KW37/26/2004SD1-5	26-Jul-04	-	+
0036634212	04CI	C07	CIC07	Toronto	Beach sand	KW37/26/2004SD1-6	26-Jul-04	-	+
0036634211	04CI	C08	CIC08	Toronto	Beach sand	KW37/26/2004SD1-7	26-Jul-04	-	+
0036634210	04CI	C09	CIC09	Toronto	Beach sand	KW37/26/2004SD1-8	26-Jul-04	-	+
0036634209	04CI	C10	CIC10	Toronto	Beach sand	KW37/26/2004SD1-9	26-Jul-04	-	+
0036634208	04CI	C11	CIC11	Toronto	Beach sand	KW37/26/2004SD1-10	26-Jul-04	-	+
0036634207	04CI	C12	CIC12	Toronto	Beach sand	KW37/26/2004SD1-11	26-Jul-04	-	+
0036634219	04CI	D01	CID01	Toronto	Beach sand	KW37/26/2004SD1-12	26-Jul-04	-	+
0034437141	04CI	D02	CID02	Toronto	Beach sand	CI5/3/2004SD1-2	03-May-04	-	+
0036634221	04CI	D03	CID03	Toronto	Beach sand	KW37/26/2004SD2-2	26-Jul-04	-	+
0036634222	04CI	D04	CID04	Toronto	Beach sand	KW37/26/2004SD2-3	26-Jul-04	-	+
0036634223	04CI	D05	CID05	Toronto	Beach sand	KW37/26/2004SD2-4	26-Jul-04	-	+
0036634224	04CI	D06	CID06	Toronto	Beach sand	KW37/26/2004SD2-5	26-Jul-04	+	+
0036634225	04CI	D07	CID07	Toronto	Beach sand	KW37/26/2004SD2-6	26-Jul-04	-	+
0036634226	04CI	D08	CID08	Toronto	Beach sand	KW37/26/2004SD2-7	26-Jul-04	+	+
0036634227	04CI	D09	CID09	Toronto	Beach sand	KW37/26/2004SD2-8	26-Jul-04	-	+
0036634228	04CI	D10	CID10	Toronto	Beach sand	KW37/26/2004SD2-9	26-Jul-04	-	+
0036634229	04CI	D11	CID11	Toronto	Beach sand	KW37/26/2004SD2-10	26-Jul-04	-	+
0036634230	04CI	D12	CID12	Toronto	Beach sand	KW37/26/2004SD2-11	26-Jul-04	-	+
0036634242	04CI	E01	CIE01	Toronto	Beach sand	KW37/26/2004SD2-12	26-Jul-04	-	+
0034437142	04CI	E02	CIE02	Toronto	Beach sand	CI5/3/2004SD1-1	03-May-04	-	+
0036634240	04CI	E03	CIE03	Toronto	Beach sand	CI17/26/2004SD1-2	26-Jul-04	-	+
0031924767	04CI	E04	CIE04	Toronto	Beach sand	CI17/12/2004SD1-11	12-Jul-04	-	+
0036634238	04CI	E05	CIE05	Toronto	Beach sand	CI17/26/2004SD1-5	26-Jul-04	-	+
0031924766	04CI	E06	CIE06	Toronto	Beach sand	CI17/12/2004SD1-10	12-Jul-04	-	+
0036634236	04CI	E07	CIE07	Toronto	Beach sand	CI17/26/2004SD1-9	26-Jul-04	-	+
0036634235	04CI	E08	CIE08	Toronto	Beach sand	CI17/26/2004SD1-10	26-Jul-04	-	+
0036634234	04CI	E09	CIE09	Toronto	Beach sand	CI17/26/2004SD1-11	26-Jul-04	-	+
0036634233	04CI	E10	CIE10	Toronto	Beach sand	CI17/26/2004SD2-1	26-Jul-04	-	+
0036634232	04CI	E11	CIE11	Toronto	Beach sand	CI17/26/2004SD2-2	26-Jul-04	-	+
0036634231	04CI	E12	CIE12	Toronto	Beach sand	CI17/26/2004SD2-3	26-Jul-04	-	+
0036634243	04CI	F01	CIF01	Toronto	Beach sand	CI17/26/2004SD2-4	26-Jul-04	+	+
0036634244	04CI	F02	CIF02	Toronto	Beach sand	CI17/26/2004SD2-5	26-Jul-04	-	+
0036634245	04CI	F03	CIF03	Toronto	Beach sand	CI17/26/2004SD2-6	26-Jul-04	-	+
0036634246	04CI	F04	CIF04	Toronto	Beach sand	CI17/26/2004SD2-7	26-Jul-04	-	+
0036634247	04CI	F05	CIF05	Toronto	Beach sand	CI17/26/2004SD2-8	26-Jul-04	-	+
0036634248	04CI	F06	CIF06	Toronto	Beach sand	CI17/26/2004SD2-9	26-Jul-04	-	+
0036634249	04CI	F07	CIF07	Toronto	Beach sand	CI17/26/2004SD2-10	26-Jul-04	-	+
0036634250	04CI	F08	CIF08	Toronto	Beach sand	CI17/26/2004SD2-11	26-Jul-04	+	+
0036634251	04CI	F09	CIF09	Toronto	Beach sand	CI17/26/2004SD2-12	26-Jul-04	-	+
0036634252	04CI	F10	CIF10	Toronto	Beach sand	CI27/26/2004SD1-1	26-Jul-04	-	+
0036634253	04CI	F11	CIF11	Toronto	Beach sand	CI27/26/2004SD1-2	26-Jul-04	-	+
0036634254	04CI	F12	CIF12	Toronto	Beach sand	CI27/26/2004SD1-3	26-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0036634266	04CI	G01	CIG01	Toronto	Beach sand	CI27/26/2004SD1-4	26-Jul-04	-	+
0036634265	04CI	G02	CIG02	Toronto	Beach sand	CI27/26/2004SD1-5	26-Jul-04	-	+
0036634264	04CI	G03	CIG03	Toronto	Beach sand	CI27/26/2004SD1-6	26-Jul-04	-	+
0036634263	04CI	G04	CIG04	Toronto	Beach sand	CI27/26/2004SD1-7	26-Jul-04	-	+
0036634262	04CI	G05	CIG05	Toronto	Beach sand	CI27/26/2004SD1-8	26-Jul-04	-	+
0036634261	04CI	G06	CIG06	Toronto	Beach sand	CI27/26/2004SD1-9	26-Jul-04	-	+
0036634260	04CI	G07	CIG07	Toronto	Beach sand	CI27/26/2004SD1-10	26-Jul-04	-	+
0036634259	04CI	G08	CIG08	Toronto	Beach sand	CI27/26/2004SD1-11	26-Jul-04	+	+
0036634258	04CI	G09	CIG09	Toronto	Beach sand	CI27/26/2004SD1-12	26-Jul-04	-	+
0036634257	04CI	G10	CIG10	Toronto	Beach sand	CI27/26/2004SD2-1	26-Jul-04	-	+
0031924760	04CI	G11	CIG11	Toronto	Beach sand	CI17/12/2004SD1-3	12-Jul-04	+	+
0031924759	04CI	G12	CIG12	Toronto	Beach sand	KW37/12/2004SD1-12	12-Jul-04	-	-
0026722599	04CM	A01	CMA01	Toronto	Beach water	KW38/3/2004WC1-6	03-Aug-04	-	+
0026722600	04CM	A02	CMA02	Toronto	Beach water	KW38/3/2004WC1-7	03-Aug-04	-	+
0026722601	04CM	A03	CMA03	Toronto	Beach water	KW38/3/2004WC1-8	03-Aug-04	-	+
0031915757	04CM	A04	CMA04	Toronto	Beach water	KW37/19/2004WA1-2	19-Jul-04	-	+
0031915756	04CM	A05	CMA05	Toronto	Beach water	KW37/19/2004WA1-3	19-Jul-04	-	+
0031915755	04CM	A06	CMA06	Toronto	Beach water	KW37/19/2004WA1-4	19-Jul-04	-	+
0031915754	04CM	A07	CMA07	Toronto	Beach water	KW37/19/2004WA1-5	19-Jul-04	+	+
0031915753	04CM	A08	CMA08	Toronto	Beach water	KW37/19/2004WA1-6	19-Jul-04	-	+
0031915752	04CM	A09	CMA09	Toronto	Beach water	KW37/19/2004WA1-7	19-Jul-04	-	+
0031915751	04CM	A10	CMA10	Toronto	Beach water	KW37/19/2004WA1-8	19-Jul-04	-	+
0031915750	04CM	A11	CMA11	Toronto	Beach water	KW37/19/2004WA1-9	19-Jul-04	-	+
0031915749	04CM	A12	CMA12	Toronto	Beach water	KW37/19/2004WA1-11	19-Jul-04	-	+
0031915748	04CM	B01	CMB01	Toronto	Beach water	KW37/19/2004WA1-10	19-Jul-04	-	+
0031923633	04CM	B02	CMB02	Toronto	Beach water	KW37/19/2004WA1-12	19-Jul-04	-	+
0026720954	04CM	B03	CMB03	Toronto	Beach water	KW38/3/2004WC1-9	03-Aug-04	-	+
0031915736	04CM	B04	CMB04	Toronto	Beach water	KW37/19/2004WA2-2	19-Jul-04	-	+
0031915737	04CM	B05	CMB05	Toronto	Beach water	KW37/19/2004WA2-3	19-Jul-04	-	+
0031915738	04CM	B06	CMB06	Toronto	Beach water	KW37/19/2004WA2-4	19-Jul-04	-	+
0031915739	04CM	B07	CMB07	Toronto	Beach water	KW37/19/2004WA2-5	19-Jul-04	-	+
0031915740	04CM	B08	CMB08	Toronto	Beach water	KW37/19/2004WA2-6	19-Jul-04	-	+
0031915741	04CM	B09	CMB09	Toronto	Beach water	KW37/19/2004WA2-7	19-Jul-04	-	+
0031915742	04CM	B10	CMB10	Toronto	Beach water	KW37/19/2004WA2-8	19-Jul-04	-	+
0031915743	04CM	B11	CMB11	Toronto	Beach water	KW37/19/2004WA2-9	19-Jul-04	-	+
0031915744	04CM	B12	CMB12	Toronto	Beach water	KW37/19/2004WA2-10	19-Jul-04	-	+
0031915745	04CM	C01	CMC01	Toronto	Beach water	KW37/19/2004WA2-11	19-Jul-04	-	+
0031923634	04CM	C02	CMC02	Toronto	Beach water	KW37/19/2004WA2-12	19-Jul-04	-	+
0026722590	04CM	C03	CMC03	Toronto	Beach water	KW38/3/2004WC1-10	03-Aug-04	-	+
0031915733	04CM	C04	CMC04	Toronto	Beach water	KW37/19/2004WK1-2	19-Jul-04	-	+
0031915732	04CM	C05	CMC05	Toronto	Beach water	KW37/19/2004WK1-3	19-Jul-04	-	+
0031915731	04CM	C06	CMC06	Toronto	Beach water	KW37/19/2004WK1-4	19-Jul-04	-	+
0031915730	04CM	C07	CMC07	Toronto	Beach water	KW37/19/2004WK1-5	19-Jul-04	-	+
0031915729	04CM	C08	CMC08	Toronto	Beach water	KW37/19/2004WK1-6	19-Jul-04	-	+
0031915728	04CM	C09	CMC09	Toronto	Beach water	KW37/19/2004WK1-7	19-Jul-04	-	+
0031915727	04CM	C10	CMC10	Toronto	Beach water	KW37/19/2004WK1-8	19-Jul-04	-	+
0031915726	04CM	C11	CMC11	Toronto	Beach water	KW37/19/2004WK1-9	19-Jul-04	-	+
0031915725	04CM	C12	CMC12	Toronto	Beach water	KW37/19/2004WK1-10	19-Jul-04	-	+
0031915724	04CM	D01	CMD01	Toronto	Beach water	KW37/19/2004WK1-11	19-Jul-04	-	+
0031923637	04CM	D02	CMD02	Toronto	Beach water	KW37/19/2004WK1-12	19-Jul-04	-	+
0026722589	04CM	D03	CMD03	Toronto	Beach water	KW38/3/2004WC1-11	03-Aug-04	-	+
0031915712	04CM	D04	CMD04	Toronto	Beach water	KW37/19/2004WK2-2	19-Jul-04	-	+
0031915713	04CM	D05	CMD05	Toronto	Beach water	KW37/19/2004WK2-3	19-Jul-04	-	+
0031915714	04CM	D06	CMD06	Toronto	Beach water	KW37/19/2004WK2-4	19-Jul-04	-	+
0031915715	04CM	D07	CMD07	Toronto	Beach water	KW37/19/2004WK2-5	19-Jul-04	-	+
0031915716	04CM	D08	CMD08	Toronto	Beach water	KW37/19/2004WK2-6	19-Jul-04	-	+
0031915717	04CM	D09	CMD09	Toronto	Beach water	KW37/19/2004WK2-7	19-Jul-04	-	+
0031915718	04CM	D10	CMD10	Toronto	Beach water	KW37/19/2004WK2-8	19-Jul-04	-	+
0031915719	04CM	D11	CMD11	Toronto	Beach water	KW37/19/2004WK2-9	19-Jul-04	-	+
0031915720	04CM	D12	CMD12	Toronto	Beach water	KW37/19/2004WK2-10	19-Jul-04	-	+
0031915721	04CM	E01	CME01	Toronto	Beach water	KW37/19/2004WK2-11	19-Jul-04	-	+
0031915722	04CM	E02	CME02	Toronto	Beach water	KW37/19/2004WK2-12	19-Jul-04	-	+
0026722588	04CM	E03	CME03	Toronto	Beach water	KW38/3/2004WC1-12	03-Aug-04	-	+
0026722586	04CM	E04	CME04	Toronto	Beach water	KW38/3/2004WC2-2	03-Aug-04	-	+
0026722585	04CM	E05	CME05	Toronto	Beach water	KW38/3/2004WC2-3	03-Aug-04	-	+
0026722584	04CM	E06	CME06	Toronto	Beach water	KW38/3/2004WC2-4	03-Aug-04	-	+
0026722583	04CM	E07	CME07	Toronto	Beach water	KW38/3/2004WC2-5	03-Aug-04	-	+
0026722581	04CM	E08	CME08	Toronto	Beach water	KW38/3/2004WC2-7	03-Aug-04	-	+
0026738118	04CM	E09	CME09	Toronto	Beach water	KW38/3/2004WC2-8	03-Aug-04	-	+
0031938271	04CM	E10	CME10	Toronto	Beach water	KW37/19/2004WC1-6	19-Jul-04	-	+
0031939505	04CM	E11	CME11	Toronto	Beach water	KW37/19/2004WC1-7	19-Jul-04	-	+
0031938293	04CM	E12	CME12	Toronto	Beach water	KW37/19/2004WC1-8	19-Jul-04	-	+
0031939503	04CM	F01	CMF01	Toronto	Beach water	KW37/19/2004WC1-9	19-Jul-04	-	+
0031939502	04CM	F02	CMF02	Toronto	Beach water	KW37/19/2004WC1-10	19-Jul-04	-	+
0031939501	04CM	F03	CMF03	Toronto	Beach water	KW37/19/2004WC1-11	19-Jul-04	-	+
0031939500	04CM	F04	CMF04	Toronto	Beach water	KW37/19/2004WC1-12	19-Jul-04	-	+
0031939498	04CM	F05	CMF05	Toronto	Beach water	KW37/19/2004WC2-2	19-Jul-04	-	+
0031938283	04CM	F06	CMF06	Toronto	Beach water	KW37/19/2004WC2-3	19-Jul-04	-	+
0031938279	04CM	F07	CMF07	Toronto	Beach water	KW37/19/2004WC2-5	19-Jul-04	-	+
0031939495	04CM	F08	CMF08	Toronto	Beach water	KW37/19/2004WC2-6	19-Jul-04	-	+
0031939507	04CM	F09	CMF09	Toronto	Beach water	KW37/19/2004WC2-7	19-Jul-04	-	+
0031939508	04CM	F10	CMF10	Toronto	Beach water	KW37/19/2004WC2-8	19-Jul-04	-	+
0031939509	04CM	F11	CMF11	Toronto	Beach water	KW37/19/2004WC2-9	19-Jul-04	-	+
0031939510	04CM	F12	CMF12	Toronto	Beach water	KW37/19/2004WC2-10	19-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0031939511	04CM	G01	CMG01	Toronto	Beach water	KW37/19/2004WC2-12	19-Jul-04	-	+
0031940016	04CM	G02	CMG02	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG2-3	26-Jul-04	+	+
0031940017	04CM	G03	CMG03	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG3-2	26-Jul-04	-	+
0031940018	04CM	G04	CMG04	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG4-1	26-Jul-04	-	+
0031940019	04CM	G05	CMG05	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG4-3	26-Jul-04	-	+
0031940020	04CM	G06	CMG06	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG5-1	26-Jul-04	-	+
0031940021	04CM	G07	CMG07	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG5-3	26-Jul-04	-	+
0031905248	04CM	G08	CMG08	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG6-1	26-Jul-04	-	+
0031940034	04CM	G09	CMG09	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG6-2	26-Jul-04	-	+
0031940033	04CM	G10	CMG10	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG7-1	26-Jul-04	-	+
0031940032	04CM	G11	CMG11	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG7-2	26-Jul-04	-	+
0031905239	04CM	G12	CMG12	Toronto	Canada Goose (Branta canadensis)	AH7/26/2004CG7-3	26-Jul-04	-	+
0026733727	04CO	A01	COA01	Toronto	Beach sand	KW38/9/2004SD1-11	09-Aug-04	-	+
0026733728	04CO	A02	COA02	Toronto	Beach sand	KW38/9/2004SD1-12	09-Aug-04	-	+
0026733730	04CO	A03	COA03	Toronto	Beach sand	KW38/9/2004SD2-2	09-Aug-04	-	+
0026733731	04CO	A04	COA04	Toronto	Beach sand	KW38/9/2004SD2-3	09-Aug-04	-	+
0026733732	04CO	A05	COA05	Toronto	Beach sand	KW38/9/2004SD2-4	09-Aug-04	-	+
0026733733	04CO	A06	COA06	Toronto	Beach sand	KW38/9/2004SD2-5	09-Aug-04	-	+
0026733734	04CO	A07	COA07	Toronto	Beach sand	KW38/9/2004SD2-6	09-Aug-04	-	+
0026733735	04CO	A08	COA08	Toronto	Beach sand	KW38/9/2004SD2-7	09-Aug-04	-	+
0026733736	04CO	A09	COA09	Toronto	Beach sand	KW38/9/2004SD2-8	09-Aug-04	-	+
0026733737	04CO	A10	COA10	Toronto	Beach sand	KW38/9/2004SD2-9	09-Aug-04	-	+
0026733738	04CO	A11	COA11	Toronto	Beach sand	KW38/9/2004SD2-10	09-Aug-04	+	+
0026733719	04CO	A12	COA12	Toronto	Beach sand	CI18/9/2004SD1-6	09-Aug-04	-	+
0026733726	04CO	B01	COB01	Toronto	Beach sand	KW38/9/2004SD2-11	09-Aug-04	-	+
0026733725	04CO	B02	COB02	Toronto	Beach sand	KW38/9/2004SD2-12	09-Aug-04	-	+
0026733724	04CO	B03	COB03	Toronto	Beach sand	CI18/9/2004SD1-1	09-Aug-04	-	+
0026733723	04CO	B04	COB04	Toronto	Beach sand	CI18/9/2004SD1-2	09-Aug-04	-	+
0026733722	04CO	B05	COB05	Toronto	Beach sand	CI18/9/2004SD1-3	09-Aug-04	-	+
0026733721	04CO	B06	COB06	Toronto	Beach sand	CI18/9/2004SD1-4	09-Aug-04	-	+
0026733720	04CO	B07	COB07	Toronto	Beach sand	CI18/9/2004SD1-5	09-Aug-04	-	+
0026733718	04CO	B08	COB08	Toronto	Beach sand	CI18/9/2004SD1-7	09-Aug-04	-	+
0026733717	04CO	B09	COB09	Toronto	Beach sand	CI18/9/2004SD1-8	09-Aug-04	-	+
0036633836	04CO	B10	COB10	Toronto	Dog (Canis lupus familiaris)	KW7/19/2004DG1-1	19-Jul-04	-	+
0031940242	04CO	B11	COB11	Toronto	Dog (Canis lupus familiaris)	KW7/26/2004DG1-1	26-Jul-04	-	+
0029895636	04CO	B12	COB12	Toronto	Cat (Felis catus)	SPCA8/16/2004C3-3	16-Aug-04	-	+
0029895637	04CO	C01	COC01	Toronto	Cat (Felis catus)	SPCA8/16/2004C3-4	16-Aug-04	-	+
0029895638	04CO	C02	COC02	Toronto	Cat (Felis catus)	SPCA8/16/2004C3-5	16-Aug-04	-	+
0029895639	04CO	C03	COC03	Toronto	Cat (Felis catus)	SPCA8/16/2004C3-6	16-Aug-04	-	+
0029895640	04CO	C04	COC04	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-1	16-Aug-04	-	+
0029895634	04CO	C05	COC05	Toronto	Cat (Felis catus)	SPCA8/16/2004C3-1	16-Aug-04	-	+
0029895635	04CO	C06	COC06	Toronto	Cat (Felis catus)	SPCA8/16/2004C3-2	16-Aug-04	-	+
0029860321	04CO	C07	COC07	Toronto	Beach sand	KW16/7/2004SD2-7	07-Jun-04	-	+
0029895628	04CO	C08	COC08	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-6	16-Aug-04	-	+
0029895630	04CO	C09	COC09	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-4	16-Aug-04	-	+
0029895629	04CO	C10	COC10	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-5	16-Aug-04	-	+
0029895641	04CO	C11	COC11	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-2	16-Aug-04	-	+
0029895642	04CO	C12	COC12	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-3	16-Aug-04	+	+
0029895624	04CO	D01	COD01	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-10	16-Aug-04	-	+
0029895627	04CO	D02	COD02	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-7	16-Aug-04	-	+
0029895626	04CO	D03	COD03	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-8	16-Aug-04	-	+
0029895625	04CO	D04	COD04	Toronto	Cat (Felis catus)	SPCA8/16/2004C10-9	16-Aug-04	-	+
0026731701	04CO	D05	COD05	Toronto	Beach sand	CI18/9/2004SD1-9	09-Aug-04	+	+
0026731722	04CO	D06	COD06	Toronto	Beach sand	CI18/9/2004SD1-10	09-Aug-04	-	+
0026731702	04CO	D07	COD07	Toronto	Beach sand	CI18/9/2004SD1-11	09-Aug-04	-	+
0026731703	04CO	D08	COD08	Toronto	Beach sand	CI18/9/2004SD1-12	09-Aug-04	-	+
0026732949	04CO	D09	COD09	Toronto	Beach sand	KW28/3/2004SD2-9	03-Aug-04	-	+
0026732950	04CO	D10	COD10	Toronto	Beach sand	KW28/3/2004SD2-8	03-Aug-04	-	+
0026732951	04CO	D11	COD11	Toronto	Beach sand	KW28/3/2004SD2-7	03-Aug-04	-	+
0026732952	04CO	D12	COD12	Toronto	Beach sand	KW28/3/2004SD2-6	03-Aug-04	-	+
0026732953	04CO	E01	COE01	Toronto	Beach sand	KW28/3/2004SD2-5	03-Aug-04	-	+
0026732954	04CO	E02	COE02	Toronto	Beach sand	KW28/3/2004SD2-4	03-Aug-04	-	+
0026732955	04CO	E03	COE03	Toronto	Beach sand	KW28/3/2004SD2-3	03-Aug-04	-	-
0026732956	04CO	E04	COE04	Toronto	Beach sand	KW28/3/2004SD2-2	03-Aug-04	-	+
0026732958	04CO	E05	COE05	Toronto	Beach sand	KW28/3/2004SD1-12	03-Aug-04	-	+
0026731781	04CO	E06	COE06	Toronto	Beach sand	KW28/3/2004SD1-11	03-Aug-04	-	+
0026732968	04CO	E07	COE07	Toronto	Beach sand	KW28/3/2004SD1-9	03-Aug-04	-	+
0026732967	04CO	E08	COE08	Toronto	Beach sand	KW28/3/2004SD1-8	03-Aug-04	-	-
0026732966	04CO	E09	COE09	Toronto	Beach sand	KW28/3/2004SD1-7	03-Aug-04	-	+
0026732965	04CO	E10	COE10	Toronto	Beach sand	KW28/3/2004SD1-6	03-Aug-04	-	+
0026732964	04CO	E11	COE11	Toronto	Beach sand	KW28/3/2004SD1-5	03-Aug-04	-	+
0026732963	04CO	E12	COE12	Toronto	Beach sand	KW28/3/2004SD1-4	03-Aug-04	-	-
0026732962	04CO	F01	COF01	Toronto	Beach sand	KW28/3/2004SD1-3	03-Aug-04	-	+
0026732961	04CO	F02	COF02	Toronto	Beach sand	KW28/3/2004SD1-2	03-Aug-04	-	+
0026731751	04CO	F03	COF03	Toronto	Beach sand	CI18/3/2004SD1-9	03-Aug-04	-	+
0026731743	04CO	F04	COF04	Toronto	Beach sand	CI18/3/2004SD1-10	03-Aug-04	-	+
0026731742	04CO	F05	COF05	Toronto	Beach sand	CI18/3/2004SD1-11	03-Aug-04	-	+
0026733027	04CO	F06	COF06	Toronto	Beach sand	CI18/3/2004SD1-12	03-Aug-04	-	+
0026731738	04CO	F07	COF07	Toronto	Beach sand	CI18/3/2004SD2-1	03-Aug-04	-	+
0026733020	04CO	F08	COF08	Toronto	Beach sand	CI18/3/2004SD2-7	03-Aug-04	-	+
0026731780	04CO	F09	COF09	Toronto	Beach sand	CI18/3/2004SD2-8	03-Aug-04	-	+
0026733007	04CO	F10	COF10	Toronto	Beach sand	CI18/3/2004SD2-9	03-Aug-04	-	+
0026733008	04CO	F11	COF11	Toronto	Beach sand	CI18/3/2004SD2-10	03-Aug-04	-	+
0026733009	04CO	F12	COF12	Toronto	Beach sand	CI18/3/2004SD2-11	03-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026733010	04CO	G01	COG01	Toronto	Beach sand	CI18/3/2004SD2-12	03-Aug-04	-	+
0026731767	04CO	G02	COG02	Toronto	Beach sand	CI28/3/2004SD1-1	03-Aug-04	-	+
0026732959	04CO	G03	COG03	Toronto	Beach sand	KW18/3/2004SD2-12	03-Aug-04	-	+
0026732971	04CO	G04	COG04	Toronto	Beach sand	KW18/3/2004SD2-11	03-Aug-04	-	+
0026733006	04CO	G05	COG05	Toronto	Beach sand	CI28/3/2004SD1-10	03-Aug-04	-	+
0026733005	04CO	G06	COG06	Toronto	Beach sand	CI28/3/2004SD1-11	03-Aug-04	-	+
0026733004	04CO	G07	COG07	Toronto	Beach sand	CI28/3/2004SD1-12	03-Aug-04	-	+
0026733003	04CO	G08	COG08	Toronto	Beach sand	CI28/3/2004SD2-1	03-Aug-04	-	+
0026733002	04CO	G09	COG09	Toronto	Beach sand	CI28/3/2004SD2-2	03-Aug-04	-	+
0026732996	04CO	G10	COG10	Toronto	Beach sand	CI28/3/2004SD2-9	03-Aug-04	-	+
0026732995	04CO	G11	COG11	Toronto	Beach sand	CI28/3/2004SD2-10	03-Aug-04	-	+
0026731750	04CO	G12	COG12	Toronto	Beach sand	CI28/3/2004SD2-11	03-Aug-04	-	+
0026733222	04CU	A01	CUA01	Toronto	Beach water	KW28/3/2004WA1-9	03-Aug-04	-	+
0026733295	04CU	A02	CUA02	Toronto	Beach water	KW28/3/2004WA1-10	03-Aug-04	-	+
0026733308	04CU	A03	CUA03	Toronto	Beach water	KW28/3/2004WA1-11	03-Aug-04	-	+
0026733296	04CU	A04	CUA04	Toronto	Beach water	KW28/3/2004WA1-12	03-Aug-04	-	+
0026732019	04CU	A05	CUA05	Toronto	Beach water	KW18/3/2004WK1-2	03-Aug-04	+	+
0026733217	04CU	A06	CUA06	Toronto	Beach water	KW28/3/2004WA2-2	03-Aug-04	-	+
0026733216	04CU	A07	CUA07	Toronto	Beach water	KW28/3/2004WA2-3	03-Aug-04	-	+
0026733215	04CU	A08	CUA08	Toronto	Beach water	KW28/3/2004WA2-4	03-Aug-04	-	+
0026732020	04CU	A09	CUA09	Toronto	Beach water	KW18/3/2004WK1-1	03-Aug-04	-	+
0026733213	04CU	A10	CUA10	Toronto	Beach water	KW28/3/2004WA2-6	03-Aug-04	-	+
0026733309	04CU	A11	CUA11	Toronto	Beach water	KW28/3/2004WA2-7	03-Aug-04	-	+
0026733211	04CU	A12	CUA12	Toronto	Beach water	KW28/3/2004WA2-8	03-Aug-04	+	+
0026733199	04CU	B01	CUB01	Toronto	Beach water	KW28/3/2004WK1-9	03-Aug-04	-	+
0026733200	04CU	B02	CUB02	Toronto	Beach water	KW28/3/2004WK1-10	03-Aug-04	+	+
0026733201	04CU	B03	CUB03	Toronto	Beach water	KW28/3/2004WK1-11	03-Aug-04	-	+
0026733202	04CU	B04	CUB04	Toronto	Beach water	KW28/3/2004WK1-12	03-Aug-04	-	+
0026733203	04CU	B05	CUB05	Toronto	Beach water	KW28/3/2004WK2-9	03-Aug-04	-	+
0026733204	04CU	B06	CUB06	Toronto	Beach water	KW28/3/2004WK2-10	03-Aug-04	-	+
0026733205	04CU	B07	CUB07	Toronto	Beach water	KW28/3/2004WK2-11	03-Aug-04	-	+
0026733206	04CU	B08	CUB08	Toronto	Beach water	KW28/3/2004WK2-12	03-Aug-04	-	+
0026733214	04CU	B09	CUB09	Toronto	Beach water	KW28/3/2004WA2-5	03-Aug-04	-	+
0026733208	04CU	B10	CUB10	Toronto	Beach water	KW28/3/2004WK1-2	03-Aug-04	-	+
0026733209	04CU	B11	CUB11	Toronto	Beach water	KW28/3/2004WK1-3	03-Aug-04	-	+
0026733210	04CU	B12	CUB12	Toronto	Beach water	KW28/3/2004WK1-4	03-Aug-04	-	+
0026733198	04CU	C01	CUC01	Toronto	Beach water	KW28/3/2004WA2-9	03-Aug-04	-	+
0026733197	04CU	C02	CUC02	Toronto	Beach water	KW28/3/2004WA2-10	03-Aug-04	-	+
0026733196	04CU	C03	CUC03	Toronto	Beach water	KW28/3/2004WA2-11	03-Aug-04	-	+
0026733195	04CU	C04	CUC04	Toronto	Beach water	KW28/3/2004WA2-12	03-Aug-04	-	+
0026732021	04CU	C05	CUC05	Toronto	Beach water	KW18/3/2004WA2-12	03-Aug-04	-	+
0026733193	04CU	C06	CUC06	Toronto	Beach water	KW28/3/2004WK2-2	03-Aug-04	-	+
0026733192	04CU	C07	CUC07	Toronto	Beach water	KW28/3/2004WK2-3	03-Aug-04	-	+
0026733191	04CU	C08	CUC08	Toronto	Beach water	KW28/3/2004WK2-4	03-Aug-04	-	+
0026733190	04CU	C09	CUC09	Toronto	Beach water	KW28/3/2004WK2-5	03-Aug-04	-	+
0026733189	04CU	C10	CUC10	Toronto	Beach water	KW28/3/2004WK2-6	03-Aug-04	-	+
0026733188	04CU	C11	CUC11	Toronto	Beach water	KW28/3/2004WK2-7	03-Aug-04	-	+
0026733187	04CU	C12	CUC12	Toronto	Beach water	KW28/3/2004WK2-8	03-Aug-04	-	+
0026733175	04CU	D01	CUD01	Toronto	Beach water	KW28/3/2004WK1-5	03-Aug-04	-	+
0026733176	04CU	D02	CUD02	Toronto	Beach water	KW28/3/2004WK1-6	03-Aug-04	-	+
0026733177	04CU	D03	CUD03	Toronto	Beach water	KW28/3/2004WK1-7	03-Aug-04	-	+
0026733178	04CU	D04	CUD04	Toronto	Beach water	KW28/3/2004WK1-8	03-Aug-04	-	+
0036633824	04CU	D05	CUD05	Toronto	Mallard duck (Anas platyrhynchos)	CI7/19/2004D5-2	19-Jul-04	-	+
0026733180	04CU	D06	CUD06	Toronto	Beach water	KW38/3/2004WA1-2	03-Aug-04	-	+
0026733181	04CU	D07	CUD07	Toronto	Beach water	KW38/3/2004WA1-3	03-Aug-04	-	+
0026733182	04CU	D08	CUD08	Toronto	Beach water	KW38/3/2004WA1-4	03-Aug-04	-	+
0026733183	04CU	D09	CUD09	Toronto	Beach water	KW38/3/2004WA1-5	03-Aug-04	-	+
0026733184	04CU	D10	CUD10	Toronto	Beach water	KW38/3/2004WA1-6	03-Aug-04	-	+
0026733185	04CU	D11	CUD11	Toronto	Beach water	KW38/3/2004WA1-7	03-Aug-04	-	+
0026733186	04CU	D12	CUD12	Toronto	Beach water	KW38/3/2004WA1-8	03-Aug-04	-	+
0026733174	04CU	E01	CUE01	Toronto	Beach water	KW38/3/2004WA1-9	03-Aug-04	-	+
0026733173	04CU	E02	CUE02	Toronto	Beach water	KW38/3/2004WA1-10	03-Aug-04	-	+
0026733172	04CU	E03	CUE03	Toronto	Beach water	KW38/3/2004WA1-11	03-Aug-04	-	+
0026733171	04CU	E04	CUE04	Toronto	Beach water	KW38/3/2004WA1-12	03-Aug-04	-	+
0026738043	04CU	E05	CUE05	Toronto	Beach water	CI28/3/2004WC2-11	03-Aug-04	-	+
0026733169	04CU	E06	CUE06	Toronto	Beach water	KW38/3/2004WA2-2	03-Aug-04	-	+
0026733168	04CU	E07	CUE07	Toronto	Beach water	KW38/3/2004WA2-3	03-Aug-04	-	+
0026733167	04CU	E08	CUE08	Toronto	Beach water	KW38/3/2004WA2-4	03-Aug-04	-	+
0026733166	04CU	E09	CUE09	Toronto	Beach water	KW38/3/2004WA2-5	03-Aug-04	-	+
0026733165	04CU	E10	CUE10	Toronto	Beach water	KW38/3/2004WA2-6	03-Aug-04	-	+
0026733164	04CU	E11	CUE11	Toronto	Beach water	KW38/3/2004WA2-7	03-Aug-04	-	+
0026733163	04CU	E12	CUE12	Toronto	Beach water	KW38/3/2004WA2-8	03-Aug-04	-	+
0026733151	04CU	F01	CUF01	Toronto	Beach water	KW38/3/2004WA2-9	03-Aug-04	-	+
0026733152	04CU	F02	CUF02	Toronto	Beach water	KW38/3/2004WA2-10	03-Aug-04	-	+
0026733153	04CU	F03	CUF03	Toronto	Beach water	KW38/3/2004WA2-11	03-Aug-04	-	+
0026733154	04CU	F04	CUF04	Toronto	Beach water	KW38/3/2004WA2-12	03-Aug-04	-	+
0026738042	04CU	F05	CUF05	Toronto	Beach water	CI28/3/2004WC2-12	03-Aug-04	-	+
0026733156	04CU	F06	CUF06	Toronto	Beach water	KW38/3/2004WK1-2	03-Aug-04	-	+
0026733157	04CU	F07	CUF07	Toronto	Beach water	KW38/3/2004WK1-3	03-Aug-04	-	+
0026733158	04CU	F08	CUF08	Toronto	Beach water	KW38/3/2004WK1-4	03-Aug-04	-	+
0026733159	04CU	F09	CUF09	Toronto	Beach water	KW38/3/2004WK1-5	03-Aug-04	-	+
0026733160	04CU	F10	CUF10	Toronto	Beach water	KW38/3/2004WK1-6	03-Aug-04	-	+
0026733161	04CU	F11	CUF11	Toronto	Beach water	KW38/3/2004WK1-7	03-Aug-04	-	+
0026733162	04CU	F12	CUF12	Toronto	Beach water	KW38/3/2004WK1-8	03-Aug-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0026733150	04CU	G01	CUG01	Toronto	Beach water	KW38/3/2004WK1-9	03-Aug-04	-	+
0026733149	04CU	G02	CUG02	Toronto	Beach water	KW38/3/2004WK1-10	03-Aug-04	-	+
0026733148	04CU	G03	CUG03	Toronto	Beach water	KW38/3/2004WK1-11	03-Aug-04	-	+
0026733147	04CU	G04	CUG04	Toronto	Beach water	KW38/3/2004WK1-12	03-Aug-04	-	+
0036633823	04CU	G05	CUG05	Toronto	Mallard duck (Anas platyrhynchos)	CI7/19/2004D5-3	19-Jul-04	-	+
0026733145	04CU	G06	CUG06	Toronto	Beach water	KW38/3/2004WK2-2	03-Aug-04	-	+
0026733144	04CU	G07	CUG07	Toronto	Beach water	KW38/3/2004WK2-3	03-Aug-04	-	+
0026733143	04CU	G08	CUG08	Toronto	Beach water	KW38/3/2004WK2-4	03-Aug-04	-	+
0026733142	04CU	G09	CUG09	Toronto	Beach water	KW38/3/2004WK2-5	03-Aug-04	-	+
0026733271	04CU	G10	CUG10	Toronto	Beach water	KW38/3/2004WK2-6	03-Aug-04	-	+
0026732040	04CU	G11	CUG11	Toronto	Beach water	KW18/3/2004WA1-5	03-Aug-04	-	+
0026732039	04CU	G12	CUG12	Toronto	Beach water	KW18/3/2004WA1-6	03-Aug-04	-	+
0029859232	04DX	A01	DXA01	Hamilton	Beach water	HH17/5/2004W2-10	05-Jul-04	-	+
0029859233	04DX	A02	DXA02	Hamilton	Beach water	HH17/5/2004W2-11	05-Jul-04	-	+
0031940315	04DX	A03	DXA03	Hamilton	Beach water	HH16/21/2004W1-12	21-Jun-04	-	+
0029895319	04DX	A04	DXA04	Hamilton	Beach water	BP8/23/2004WK1-8	23-Aug-04	-	+
0031940313	04DX	A05	DXA05	Hamilton	Beach water	HH16/21/2004W2-3	21-Jun-04	-	+
0029895324	04DX	A06	DXA06	Hamilton	Beach water	BP8/23/2004WK2-1	23-Aug-04	-	+
0031939752	04DX	A07	DXA07	Hamilton	Beach water	HH16/21/2004W2-6	21-Jun-04	-	+
0029895325	04DX	A08	DXA08	Hamilton	Beach water	BP8/23/2004WK2-2	23-Aug-04	-	-
0031940324	04DX	A09	DXA09	Hamilton	Beach water	HH16/21/2004W2-8	21-Jun-04	-	+
0031940325	04DX	A10	DXA10	Hamilton	Beach water	HH16/21/2004W2-9	21-Jun-04	-	+
0031940326	04DX	A11	DXA11	Hamilton	Beach water	HH16/21/2004W2-10	21-Jun-04	-	+
0031940327	04DX	A12	DXA12	Hamilton	Beach water	HH16/21/2004W2-11	21-Jun-04	-	+
0031939743	04DX	B01	DXB01	Hamilton	Beach water	LL6/16/2004W1-1	16-Jun-04	-	+
0031940345	04DX	B02	DXB02	Hamilton	Beach water	LL6/15/2004W1-2	15-Jun-04	-	+
0031924674	04DX	B03	DXB03	Hamilton	Beach water	BP7/12/2004WA1-1	12-Jul-04	-	+
0031924662	04DX	B04	DXB04	Hamilton	Beach water	BP7/12/2004WA1-2	12-Jul-04	-	+
0031924661	04DX	B05	DXB05	Hamilton	Beach water	BP7/12/2004WA1-3	12-Jul-04	-	+
0031924660	04DX	B06	DXB06	Hamilton	Beach water	BP7/12/2004WA1-4	12-Jul-04	-	+
0031924659	04DX	B07	DXB07	Hamilton	Beach water	BP7/12/2004WA1-5	12-Jul-04	-	+
0031924658	04DX	B08	DXB08	Hamilton	Beach water	BP7/12/2004WA1-6	12-Jul-04	-	+
0031924657	04DX	B09	DXB09	Hamilton	Beach water	BP7/12/2004WA1-7	12-Jul-04	-	+
0031924639	04DX	B10	DXB10	Hamilton	Beach water	BP7/12/2004WA2-6	12-Jul-04	-	+
0031924651	04DX	B11	DXB11	Hamilton	Beach water	BP7/12/2004WA2-4	12-Jul-04	+	+
0031924640	04DX	B12	DXB12	Hamilton	Beach water	BP7/12/2004WA2-7	12-Jul-04	-	+
0031924646	04DX	C01	DXC01	Hamilton	Beach water	BP7/12/2004WK1-2	12-Jul-04	+	+
0031924647	04DX	C02	DXC02	Hamilton	Beach water	BP7/12/2004WK1-3	12-Jul-04	-	+
0031924648	04DX	C03	DXC03	Hamilton	Beach water	BP7/12/2004WK1-4	12-Jul-04	-	+
0031924649	04DX	C04	DXC04	Hamilton	Beach water	BP7/12/2004WK1-5	12-Jul-04	-	+
0031924650	04DX	C05	DXC05	Hamilton	Beach water	BP7/12/2004WK1-6	12-Jul-04	-	+
0031924638	04DX	C06	DXC06	Hamilton	Beach water	BP7/12/2004WK1-7	12-Jul-04	+	+
0031924637	04DX	C07	DXC07	Hamilton	Beach water	BP7/12/2004WK1-8	12-Jul-04	-	+
0031924632	04DX	C08	DXC08	Hamilton	Beach water	BP7/12/2004WK2-1	12-Jul-04	-	+
0031924631	04DX	C09	DXC09	Hamilton	Beach water	BP7/12/2004WK2-2	12-Jul-04	-	+
0031924630	04DX	C10	DXC10	Hamilton	Beach water	BP7/12/2004WK2-3	12-Jul-04	-	+
0031924629	04DX	C11	DXC11	Hamilton	Beach water	BP7/12/2004WK2-4	12-Jul-04	-	+
0036634578	04DX	C12	DXC12	Hamilton	Beach water	BP7/12/2004WK2-5	12-Jul-04	-	+
0036634577	04DX	D01	DXD01	Hamilton	Beach water	BP7/12/2004WK2-6	12-Jul-04	-	+
0036634576	04DX	D02	DXD02	Hamilton	Beach water	BP7/12/2004WK2-7	12-Jul-04	-	+
0036634575	04DX	D03	DXD03	Hamilton	Beach water	BP7/12/2004WK2-8	12-Jul-04	-	+
0036634571	04DX	D04	DXD04	Hamilton	Beach sand	BP7/12/2004SD1-1	12-Jul-04	-	+
0036634570	04DX	D05	DXD05	Hamilton	Beach sand	BP7/12/2004SD1-2	12-Jul-04	-	+
0029895326	04DX	D06	DXD06	Hamilton	Beach water	BP8/23/2004WK2-3	23-Aug-04	-	+
0029895327	04DX	D07	DXD07	Hamilton	Beach water	BP8/23/2004WK2-4	23-Aug-04	-	+
0029895328	04DX	D08	DXD08	Hamilton	Beach water	BP8/23/2004WK2-5	23-Aug-04	-	+
0036634579	04DX	D09	DXD09	Hamilton	Beach sand	BP7/12/2004SD1-6	12-Jul-04	-	+
0029895329	04DX	D10	DXD10	Hamilton	Beach water	BP8/23/2004WK2-6	23-Aug-04	-	+
0036634581	04DX	D11	DXD11	Hamilton	Beach sand	BP7/12/2004SD1-8	12-Jul-04	-	+
0036634582	04DX	D12	DXD12	Hamilton	Beach sand	BP7/12/2004SD1-9	12-Jul-04	-	+
0029895318	04DX	E01	DXE01	Hamilton	Beach water	BP8/23/2004WK2-8	23-Aug-04	-	+
0036634587	04DX	E02	DXE02	Hamilton	Beach sand	BP7/12/2004SD2-2	12-Jul-04	-	+
0036634588	04DX	E03	DXE03	Hamilton	Beach sand	BP7/12/2004SD2-3	12-Jul-04	-	+
0036634589	04DX	E04	DXE04	Hamilton	Beach sand	BP7/12/2004SD2-4	12-Jul-04	-	+
0029880182	04DX	E05	DXE05	Hamilton	Beach water	HH8/23/2004W1-1	23-Aug-04	-	+
0036634602	04DX	E06	DXE06	Hamilton	Beach sand	BP7/12/2004SD2-6	12-Jul-04	-	+
0036634601	04DX	E07	DXE07	Hamilton	Beach sand	BP7/12/2004SD2-7	12-Jul-04	-	+
0036634600	04DX	E08	DXE08	Hamilton	Beach sand	BP7/12/2004SD2-8	12-Jul-04	-	+
0036634599	04DX	E09	DXE09	Hamilton	Beach sand	BP7/12/2004SD2-9	12-Jul-04	-	+
0036630587	04DX	E10	DXE10	Hamilton	Beach water	BP7/13/2004WC1-1	13-Jul-04	-	+
0036630586	04DX	E11	DXE11	Hamilton	Beach water	BP7/13/2004WC1-2	13-Jul-04	-	+
0036630585	04DX	E12	DXE12	Hamilton	Beach water	BP7/13/2004WC2-1	13-Jul-04	-	+
0036630584	04DX	F01	DXF01	Hamilton	Beach water	BP7/13/2004WC2-2	13-Jul-04	-	+
0036630583	04DX	F02	DXF02	Hamilton	Beach water	BP7/13/2004WC2-3	13-Jul-04	-	+
0036630595	04DX	F03	DXF03	Hamilton	Beach water	BP7/13/2004WC2-4	13-Jul-04	-	+
0036630596	04DX	F04	DXF04	Hamilton	Beach water	BP7/13/2004WC2-5	13-Jul-04	-	+
0036630597	04DX	F05	DXF05	Hamilton	Beach water	BP7/13/2004WC2-6	13-Jul-04	-	+
0036630598	04DX	F06	DXF06	Hamilton	Beach water	BP7/13/2004WC2-7	13-Jul-04	-	+
0036630599	04DX	F07	DXF07	Hamilton	Beach water	BP7/13/2004WC2-8	13-Jul-04	-	+
0036630600	04DX	F08	DXF08	Hamilton	Beach water	BP7/13/2004WC2-9	13-Jul-04	-	+
0036630601	04DX	F09	DXF09	Hamilton	Beach water	HH17/13/2004W1-1	13-Jul-04	-	+
0036630602	04DX	F10	DXF10	Hamilton	Beach water	HH17/13/2004W1-2	13-Jul-04	-	+
0036630603	04DX	F11	DXF11	Hamilton	Beach water	HH17/13/2004W2-1	13-Jul-04	+	+
0036630604	04DX	F12	DXF12	Hamilton	Beach water	HH17/13/2004W2-2	13-Jul-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0036630605	04DX	G01	DXG01	Hamilton	Beach water	HH17/13/2004W2-3	13-Jul-04	-	+
0036630606	04DX	G02	DXG02	Hamilton	Beach water	HH17/13/2004W2-4	13-Jul-04	-	+
0036630618	04DX	G03	DXG03	Hamilton	Beach water	HH17/13/2004W2-5	13-Jul-04	-	+
0031939214	04DX	G04	DXG04	Hamilton	Beach water	BP7/19/2004WK2-1	19-Jul-04	-	+
0031939764	04DX	G05	DXG05	Hamilton	Beach water	BP7/19/2004WK2-2	19-Jul-04	-	+
0031939780	04DX	G06	DXG06	Hamilton	Beach water	BP7/19/2004WK2-3	19-Jul-04	-	+
0031939781	04DX	G07	DXG07	Hamilton	Beach water	BP7/19/2004WK2-4	19-Jul-04	-	+
0031939762	04DX	G08	DXG08	Hamilton	Beach water	BP7/19/2004WK2-5	19-Jul-04	-	+
0031939761	04DX	G09	DXG09	Hamilton	Beach water	BP7/19/2004WK2-7	19-Jul-04	-	+
0036633854	04DX	G10	DXG10	Hamilton	Beach water	BP7/19/2004WA1-1	19-Jul-04	-	+
0036633853	04DX	G11	DXG11	Hamilton	Beach water	BP7/19/2004WA1-2	19-Jul-04	-	+
0036633852	04DX	G12	DXG12	Hamilton	Beach water	BP7/19/2004WA1-3	19-Jul-04	-	+
0031940967	04EC	A01	ECA01	Bayfront Park beach	Beach water	BP6/7/2004WC1-1	07-Jun-04	-	+
0031940974	04EC	A02	ECA02	Bayfront Park beach	Beach water	BP6/7/2004WC2-1	07-Jun-04	-	+
0031940976	04EC	A03	ECA03	Bayfront Park beach	Beach water	BP6/7/2004WC2-3	07-Jun-04	-	+
0031941018	04EC	A04	ECA04	Hamilton Harbour	Beach water	HH16/7/2004W1-1	07-Jun-04	+	+
0031940284	04EC	A05	ECA05	Bayfront Park beach	Beach water	BP6/21/2004WC1-1	21-Jun-04	-	+
0031940298	04EC	A06	ECA06	Bayfront Park beach	Beach water	BP6/21/2004WC1-4	21-Jun-04	-	+
0031940300	04EC	A07	ECA07	Bayfront Park beach	Beach water	BP6/21/2004WC2-5	21-Jun-04	-	+
0031940308	04EC	A08	ECA08	Hamilton Harbour	Beach water	HH16/21/2004W1-2	21-Jun-04	-	+
0029860757	04EC	A09	ECA09	Bayfront Park beach	Beach water	BP7/5/2004WC1-1	05-Jul-04	-	+
0029860745	04EC	A10	ECA10	Hamilton Harbour	Beach water	HH17/5/2004W1-1	05-Jul-04	-	+
0029860732	04EC	A11	ECA11	Hamilton Harbour	Beach water	HH17/5/2004W1-5	05-Jul-04	-	+
0029859223	04EC	A12	ECA12	Hamilton Harbour	Beach water	HH17/5/2004W2-1	05-Jul-04	-	+
0029860898	04EC	B01	ECB01	Hamilton Harbour	Beach water	HH17/5/2004W2-5	05-Jul-04	-	+
0031941426	04EC	B02	ECB02	Hamilton Harbour	Beach water	HH16/7/2004W2-1	07-Jun-04	+	+
0029861908	04EC	B03	ECB03	Bayfront Park beach	Beach water	BP6/14/2004WC1-1	14-Jun-04	-	+
0029861903	04EC	B04	ECB04	Bayfront Park beach	Beach water	BP6/14/2004WC2-1	14-Jun-04	-	+
0029861050	04EC	B05	ECB05	Bayfront Park beach	Beach water	BP6/14/2004WC2-5	14-Jun-04	-	+
0029861885	04EC	B06	ECB06	Hamilton Harbour	Beach water	HH16/14/2004W2-1	14-Jun-04	-	+
0034436093	04EC	B07	ECB07	Bayfront Park beach	Beach sand	BP7/19/2004SD2-4	19-Jul-04	-	+
0026739524	04EC	B08	ECB08	Hamilton Harbour	Beach water	HH18/3/2004W2-1	03-Aug-04	-	+
0026739531	04EC	B09	ECB09	Hamilton Harbour	Beach water	HH18/3/2004W1-6	03-Aug-04	-	+
0026736797	04EC	B10	ECB10	Bayfront Park beach	Beach sand	BP7/26/2004SD1-6	26-Jul-04	-	+
0026738535	04EC	B11	ECB11	Bayfront Park beach	Beach water	BP8/3/2004WC2-1	03-Aug-04	-	+
0026738542	04EC	B12	ECB12	Bayfront Park beach	Beach water	BP8/3/2004WC1-1	03-Aug-04	-	+
0026739546	04EC	C01	ECC01	Hamilton Harbour	Beach water	HH18/3/2004W1-2	03-Aug-04	-	+
0029880167	04EC	C02	ECC02	Bayfront Park beach	Beach water	BP8/23/2004WC1-1	23-Aug-04	-	+
0029880158	04EC	C03	ECC03	Bayfront Park beach	Beach water	BP8/23/2004WC2-1	23-Aug-04	-	+
0029880162	04EC	C04	ECC04	Bayfront Park beach	Beach water	BP8/23/2004WC2-5	23-Aug-04	-	+
0029877779	04EC	C05	ECC05	Hamilton Harbour	Beach water	HH18/18/2004W1-5	18-Aug-04	-	+
0029879426	04EC	C06	ECC06	Hamilton Harbour	Beach water	HH18/18/2004W2-2	18-Aug-04	-	+
0029879422	04EC	C07	ECC07	Hamilton Harbour	Beach water	HH18/18/2004W2-6	18-Aug-04	-	-
0029879412	04EC	C08	ECC08	Bayfront Park beach	Beach water	BP8/18/2004WC1-3	18-Aug-04	+	+
0029879403	04EC	C09	ECC09	Bayfront Park beach	Beach water	BP8/18/2004WC2-1	18-Aug-04	-	+
0031940314	04EC	C10	ECC10	Hamilton Harbour	Beach water	HH16/21/2004W2-1	21-Jun-04	-	+
0031940312	04EC	C11	ECC11	Hamilton Harbour	Beach water	HH16/21/2004W2-4	21-Jun-04	-	+
0031940323	04EC	C12	ECC12	Hamilton Harbour	Beach water	HH16/21/2004W2-7	21-Jun-04	-	+
0036633747	04EC	D01	ECD01	Bayfront Park beach	Beach sand	BP7/12/2004SD1-5	12-Jul-04	-	+
0029879354	04EC	D02	ECD02	Bayfront Park beach	Beach sand	BP8/23/2004SD2-4	23-Aug-04	-	+
0029877481	04EC	D03	ECD03	Bayfront Park beach	Beach sand	BP8/23/2004SD2-7	23-Aug-04	-	+
0029898945	04EC	D04	ECD04	Bayfront Park beach	Beach sand	BP8/30/2004SD2-6	30-Aug-04	-	+
0029881155	04EC	D05	ECD05	Bayfront Park beach	Beach sand	BP5/10/2004SD1-6	10-May-04	-	+
0031915556	04EC	D06	ECD06	Hamilton Harbour	Beach water	HH16/1/2004W2-2	01-Jun-04	-	+
0031924316	04EC	D07	ECD07	Hamilton Harbour	Beach water	HH15/11/2004W1-1	11-May-04	-	+
0031924305	04EC	D08	ECD08	Hamilton Harbour	Beach water	HH15/11/2004W1-6	11-May-04	+	+
0031923559	04EC	D09	ECD09	Hamilton Harbour	Beach water	HH15/11/2004W2-1	11-May-04	-	+
0031942523	04EC	D10	ECD10	Bayfront Park beach	Beach water	BP5/17/2004WC1-1	17-May-04	-	+
0031941742	04EC	D11	ECD11	Bayfront Park beach	Beach water	BP5/17/2004WC1-5	17-May-04	-	+
0031921866	04EC	D12	ECD12	Bayfront Park beach	Beach water	BP5/11/2004WC1-1	11-May-04	-	+
0031921854	04EC	E01	ECE01	Bayfront Park beach	Beach water	BP5/11/2004WC1-2	11-May-04	-	+
0031921845	04EC	E02	ECE02	Bayfront Park beach	Beach water	BP5/11/2004WC2-1	11-May-04	-	+
0031942471	04EC	E03	ECE03	Bayfront Park beach	Beach sand	BP5/17/2004SD2-5	17-May-04	-	+
0029861894	04EC	E04	ECE04	Hamilton Harbour	Beach water	HH16/14/2004W1-1	14-Jun-04	-	+
0031942479	04EC	E05	ECE05	Bayfront Park beach	Beach sand	BP5/17/2004SD1-3	17-May-04	-	+
0031942477	04EC	E06	ECE06	Bayfront Park beach	Beach sand	BP5/17/2004SD1-5	17-May-04	-	+
0029880214	04EC	E07	ECE07	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS8/27/2004DG3-3	27-Aug-04	-	+
0029864520	04EC	E08	ECE08	Dundas sewage treatment plant	Sewage plant final effluent	DS8/9/2004FE1-4	09-Aug-04	-	+
0029880526	04EC	E09	ECE09	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO1-7	14-Sep-04	-	+
0029880511	04EC	E10	ECE10	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO1-11	14-Sep-04	-	+
0029878064	04EC	E11	ECE11	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO2-6	14-Sep-04	-	+
0034435732	04EC	E12	ECE12	Main and King	Untreated CSO sewage	MK5/24/2004CSO1-9	24-May-04	+	-
0029878061	04EC	F01	ECF01	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO2-18	14-Sep-04	-	+
0029878029	04EC	F02	ECF02	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO3-11	14-Sep-04	-	+
0029878026	04EC	F03	ECF03	Bayfront Park beach	Untreated CSO sewage	BP9/14/2004CSO3-25	14-Sep-04	-	+
0031915925	04EC	F04	ECF04	Hamilton sewage treatment plant	Sewage plant final effluent	HS5/6/2004FE1-2	06-May-04	-	+
0034435744	04EC	F05	ECF05	Main and King	Untreated CSO sewage	MK5/23/2004CSO1-5	23-May-04	-	+
0034435743	04EC	F06	ECF06	Main and King	Untreated CSO sewage	MK5/23/2004CSO1-7	23-May-04	-	+
0031921899	04EC	F07	ECF07	Hamilton sewage treatment plant	Sewage plant final effluent	HS5/3/2004FE1-3	03-May-04	-	+
0031921896	04EC	F08	ECF08	Hamilton sewage treatment plant	Sewage plant final effluent	HS5/3/2004FE1-6	03-May-04	-	+
0031921856	04EC	F09	ECF09	Eastwood	Untreated CSO sewage	EW5/2/2004CSO2-10	02-May-04	+	+
0031924296	04EC	F10	ECF10	Bayfront Park beach	Gull (Larus delawarensis)	BP5/17/2004G1-2	17-May-04	-	+
0031924260	04EC	F11	ECF11	Bayfront Park beach	Canada Goose (Branta canadensis)	BP5/17/2004CG2-1	17-May-04	-	-
0034434819	04EC	F12	ECF12	Bayfront Park beach	Beach sand	BP5/3/2004SD1-2	03-May-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0031942599	04EC	G01	ECG01	Bayfront Park beach	Beach sand	BP5/25/2004SD1-2	25-May-04	-	+
0031942598	04EC	G02	ECG02	Bayfront Park beach	Beach sand	BP5/25/2004SD1-3	25-May-04	-	+
0031942595	04EC	G03	ECG03	Bayfront Park beach	Beach sand	BP5/25/2004SD1-6	25-May-04	-	+
0031942592	04EC	G04	ECG04	Bayfront Park beach	Beach sand	BP5/25/2004SD1-9	25-May-04	-	+
0031942612	04EC	G05	ECG05	Bayfront Park beach	Beach sand	BP5/25/2004SD2-8	25-May-04	-	+
0034435427	04EC	G06	ECG06	Main and King	Untreated CSO sewage	MKCSO1-6	12-May-04	-	+
0034435096	04EC	G07	ECG07	Main and King	Untreated CSO sewage	MKCSO2-7	12-May-04	-	+
0034435374	04EC	G08	ECG08	Bayfront Park beach	Canada Goose (Branta canadensis)	BP5/25/2004CG3-1	25-May-04	+	+
0031942531	04EC	G09	ECG09	Bayfront Park beach	Beach water	BP5/17/2004WC2-1	17-May-04	+	+
0031942534	04EC	G10	ECG10	Bayfront Park beach	Beach water	BP5/17/2004WC2-4	17-May-04	-	+
0031942535	04EC	G11	ECG11	Hamilton Harbour	Beach water	HH15/17/2004W1-3	17-May-04	-	+
0029878518	04EC	G12	ECG12	Hamilton SPCA Animal Shelter	Dog (Canis lupus familiaris)	AS9/7/2004DG6-1	07-Sep-04	-	+
0031942553	04EC	H01	ECH01	Hamilton Harbour	Beach water	HH15/17/2004W2-1	17-May-04	-	-
0031942549	04EC	H02	ECH02	Hamilton Harbour	Beach water	HH15/17/2004W2-6	17-May-04	-	+
0031915585	04EC	H03	ECH03	Bayfront Park beach	Beach water	BP6/1/2004WC1-1	01-Jun-04	-	+
0031915570	04EC	H04	ECH04	Bayfront Park beach	Beach water	BP6/1/2004WC2-1	01-Jun-04	-	+
0031915566	04EC	H05	ECH05	Hamilton Harbour	Beach water	HH16/1/2004W1-3	01-Jun-04	-	+
0031937639	04EC	H06	ECH06	Bayfront Park beach	Beach sand	BP5/31/2004SD1-1	31-May-04	-	+
0031937640	04EC	H07	ECH07	Bayfront Park beach	Beach sand	BP5/31/2004SD1-2	31-May-04	-	+
0031937641	04EC	H08	ECH08	Bayfront Park beach	Beach sand	BP5/31/2004SD1-3	31-May-04	-	+
0029879166	04EC	H09	ECH09	Dundas sewage treatment plant	Sewage plant final effluent	DS9/8/2004FE33-5	08-Sep-04	-	+
0036634603	04EC	H10	ECH10	Bayfront Park beach	Gull (Larus delawarensis)	BP7/12/2004G3-1	12-Jul-04	-	+
0036634633	04EC	H11	ECH11	Bayfront Park beach	Canada Goose (Branta canadensis)	BP7/12/2004CG3-3	12-Jul-04	-	+
0031942593	04EC	H12	ECH12	Bayfront Park beach	Beach sand	BP5/25/2004SD1-8	25-May-04	-	+
0029877507	04EK	A01	EKA01	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-3	14-Sep-04	-	+
0029878499	04EK	A02	EKA02	Hamilton	Beach water	BP9/7/2004WC1-7	07-Sep-04	-	+
0029878500	04EK	A03	EKA03	Hamilton	Beach water	BP9/7/2004WC1-8	07-Sep-04	-	+
0031915557	04EK	A04	EKA04	Hamilton	Beach water	HH16/1/2004W1-12	01-Jun-04	-	+
0029898095	04EK	A05	EKA05	Hamilton	Beach water	BP8/30/2004WA1-1	30-Aug-04	-	+
0029878050	04EK	A06	EKA06	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-1	14-Sep-04	-	+
0029878038	04EK	A07	EKA07	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-2	14-Sep-04	-	+
0029898094	04EK	A08	EKA08	Hamilton	Beach water	BP8/30/2004WA1-2	30-Aug-04	+	+
0029898093	04EK	A09	EKA09	Hamilton	Beach water	BP8/30/2004WA1-3	30-Aug-04	-	+
0031921886	04EK	A10	EKA10	Hamilton	Untreated CSO sewage	MK5/4/2004CSO1-3	04-May-04	-	-
0029898092	04EK	A11	EKA11	Hamilton	Beach water	BP8/30/2004WA1-4	30-Aug-04	-	+
0031921859	04EK	A12	EKA12	Hamilton	Untreated CSO sewage	EW5/4/2004CSO3-1	04-May-04	-	+
0031921860	04EK	B01	EKB01	Hamilton	Untreated CSO sewage	EW5/4/2004CSO3-2	04-May-04	-	+
0031921861	04EK	B02	EKB02	Hamilton	Untreated CSO sewage	EW5/4/2004CSO3-3	04-May-04	-	+
0031921870	04EK	B03	EKB03	Hamilton	Untreated CSO sewage	EW5/2/2004CSO2-1	02-May-04	-	+
0031921891	04EK	B04	EKB04	Hamilton	Untreated CSO sewage	MK5/2/2004CSO1-1	02-May-04	-	+
0031924325	04EK	B05	EKB05	Hamilton	Beach water	BP5/11/2004WC2-4	11-May-04	-	+
0031924324	04EK	B06	EKB06	Hamilton	Beach water	BP5/11/2004WC2-5	11-May-04	-	+
0031924323	04EK	B07	EKB07	Hamilton	Beach water	BP5/11/2004WC2-6	11-May-04	-	+
0031924322	04EK	B08	EKB08	Hamilton	Beach water	BP5/11/2004WC2-7	11-May-04	-	+
0031924321	04EK	B09	EKB09	Hamilton	Beach water	BP5/11/2004WC2-8	11-May-04	-	+
0031924320	04EK	B10	EKB10	Hamilton	Beach water	BP5/11/2004WC2-9	11-May-04	-	+
0031924319	04EK	B11	EKB11	Hamilton	Beach water	BP5/11/2004WC2-10	11-May-04	-	+
0031923583	04EK	B12	EKB12	Hamilton	Beach water	BP5/11/2004WC2-11	11-May-04	+	+
0031923606	04EK	C01	EKC01	Hamilton	Beach water	BP5/11/2004WC2-12	11-May-04	+	+
0029898071	04EK	C02	EKC02	Hamilton	Beach water	BP8/30/2004WK1-1	30-Aug-04	-	+
0031924315	04EK	C03	EKC03	Hamilton	Beach water	HH15/11/2004W1-2	11-May-04	-	+
0031924303	04EK	C04	EKC04	Hamilton	Beach water	HH15/11/2004W1-3	11-May-04	-	+
0031924304	04EK	C05	EKC05	Hamilton	Beach water	HH15/11/2004W1-4	11-May-04	+	+
0029898070	04EK	C06	EKC06	Hamilton	Beach water	BP8/30/2004WK1-2	30-Aug-04	-	+
0031924306	04EK	C07	EKC07	Hamilton	Beach water	HH15/11/2004W1-7	11-May-04	-	+
0031924307	04EK	C08	EKC08	Hamilton	Beach water	HH15/11/2004W1-8	11-May-04	-	+
0031924308	04EK	C09	EKC09	Hamilton	Beach water	HH15/11/2004W1-9	11-May-04	-	+
0031924309	04EK	C10	EKC10	Hamilton	Beach water	HH15/11/2004W1-10	11-May-04	-	+
0031924310	04EK	C11	EKC11	Hamilton	Beach water	HH15/11/2004W1-12	11-May-04	-	+
0029898069	04EK	C12	EKC12	Hamilton	Beach water	BP8/30/2004WK1-3	30-Aug-04	-	+
0031923582	04EK	D01	EKD01	Hamilton	Beach water	HH15/11/2004W2-2	11-May-04	-	+
0031924313	04EK	D02	EKD02	Hamilton	Beach water	HH15/11/2004W2-4	11-May-04	-	+
0031924314	04EK	D03	EKD03	Hamilton	Beach water	HH15/11/2004W2-5	11-May-04	-	+
0031924302	04EK	D04	EKD04	Hamilton	Beach water	HH15/11/2004W2-6	11-May-04	-	+
0031924301	04EK	D05	EKD05	Hamilton	Beach water	HH15/11/2004W2-9	11-May-04	+	+
0031924300	04EK	D06	EKD06	Hamilton	Beach water	HH15/11/2004W2-10	11-May-04	+	+
0031924298	04EK	D07	EKD07	Hamilton	Beach water	HH15/11/2004W2-12	11-May-04	-	+
0031924326	04EK	D08	EKD08	Hamilton	Beach water	BP5/11/2004WC2-3	11-May-04	-	+
0029899014	04EK	D09	EKD09	Hamilton	Beach water	BP8/30/2004WK1-4	30-Aug-04	-	+
0031942507	04EK	D10	EKD10	Hamilton	Beach water	BP5/17/2004WK1-5	17-May-04	-	+
0029898633	04EK	D11	EKD11	Hamilton	Beach water	BP8/30/2004WK1-5	30-Aug-04	-	+
0031942509	04EK	D12	EKD12	Hamilton	Beach water	BP5/17/2004WK1-7	17-May-04	-	+
0031942520	04EK	E01	EKE01	Hamilton	Beach water	BP5/17/2004WC1-4	17-May-04	-	+
0031942510	04EK	E02	EKE02	Hamilton	Beach water	BP5/17/2004WK1-8	17-May-04	-	+
0029898638	04EK	E03	EKE03	Hamilton	Beach water	BP8/30/2004WK1-6	30-Aug-04	-	+
0031942522	04EK	E04	EKE04	Hamilton	Beach water	BP5/17/2004WC1-2	17-May-04	-	+
0031942521	04EK	E05	EKE05	Hamilton	Beach water	BP5/17/2004WC1-3	17-May-04	-	+
0029899011	04EK	E06	EKE06	Hamilton	Beach water	BP8/30/2004WK1-7	30-Aug-04	-	+
0031942481	04EK	E07	EKE07	Hamilton	Beach sand	BP5/17/2004SD1-1	17-May-04	-	+
0031942480	04EK	E08	EKE08	Hamilton	Beach sand	BP5/17/2004SD1-2	17-May-04	-	+
0029898962	04EK	E09	EKE09	Hamilton	Beach sand	BP8/30/2004SD1-8	30-Aug-04	-	+
0031942478	04EK	E10	EKE10	Hamilton	Beach sand	BP5/17/2004SD1-4	17-May-04	-	+
0029898957	04EK	E11	EKE11	Hamilton	Beach sand	BP8/30/2004SD2-1	30-Aug-04	-	+
0031941735	04EK	E12	EKE12	Hamilton	Beach sand	BP5/17/2004SD1-6	17-May-04	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0034435423	04EK	F01	EKF01	Hamilton	Untreated CSO sewage	MKCSO1-2	12-May-04	-	+
0034435424	04EK	F02	EKF02	Hamilton	Untreated CSO sewage	MKCSO1-3	12-May-04	-	+
0034435432	04EK	F03	EKF03	Hamilton	Untreated CSO sewage	MKCSO2-3	12-May-04	-	+
0031921874	04EK	F04	EKF04	Hamilton	Untreated CSO sewage	EW5/4/2004CSO1-1	04-May-04	-	+
0029899010	04EK	F05	EKF05	Hamilton	Beach water	BP8/30/2004WK1-8	30-Aug-04	-	+
0029899005	04EK	F06	EKF06	Hamilton	Beach water	BP8/30/2004WK2-1	30-Aug-04	+	+
0031921853	04EK	F07	EKF07	Hamilton	Beach water	BP5/11/2004WC1-3	11-May-04	+	-
0031921852	04EK	F08	EKF08	Hamilton	Beach water	BP5/11/2004WC1-4	11-May-04	+	+
0031921851	04EK	F09	EKF09	Hamilton	Beach water	BP5/11/2004WC1-5	11-May-04	+	+
0031921850	04EK	F10	EKF10	Hamilton	Beach water	BP5/11/2004WC1-6	11-May-04	-	+
0031921849	04EK	F11	EKF11	Hamilton	Beach water	BP5/11/2004WC1-7	11-May-04	-	+
0031921848	04EK	F12	EKF12	Hamilton	Beach water	BP5/11/2004WC1-8	11-May-04	-	+
0031921847	04EK	G01	EKG01	Hamilton	Beach water	BP5/11/2004WC1-9	11-May-04	-	+
0029898631	04EK	G02	EKG02	Hamilton	Beach water	BP8/30/2004WK2-2	30-Aug-04	-	+
0029864325	04EK	G03	EKG03	Hamilton	Beach water	BP8/9/2004WA1-2	09-Aug-04	-	+
0031942475	04EK	G04	EKG04	Hamilton	Beach sand	BP5/17/2004SD1-7	17-May-04	-	+
0029897478	04EK	G05	EKG05	Hamilton	Beach water	BP8/30/2004WK2-3	30-Aug-04	-	+
0029898991	04EK	G06	EKG06	Hamilton	Beach water	BP8/30/2004WK2-4	30-Aug-04	-	+
0031941745	04EK	G07	EKG07	Hamilton	Beach sand	BP5/17/2004SD2-8	17-May-04	-	+
0031942484	04EK	G08	EKG08	Hamilton	Beach water	BP5/17/2004WA1-1	17-May-04	-	+
0031942485	04EK	G09	EKG09	Hamilton	Beach water	BP5/17/2004WA1-2	17-May-04	-	+
0034434930	04EK	G10	EKG10	Hamilton	Beach water	HH15/17/2004W2-11	17-May-04	-	+
0034434929	04EK	G11	EKG11	Hamilton	Beach water	HH15/17/2004W2-12	17-May-04	-	+
0031942486	04EK	G12	EKG12	Hamilton	Beach water	BP5/17/2004WA1-3	17-May-04	-	+
0036634454	04FE	A01	FEA01	Toronto	Beach water	WB25/9/2005WA1-1	09-May-05	-	+
0036634453	04FE	A02	FEA02	Toronto	Beach water	WB25/9/2005WA1-2	09-May-05	-	+
0036634452	04FE	A03	FEA03	Toronto	Beach water	WB25/9/2005WA1-3	09-May-05	-	+
0036634451	04FE	A04	FEA04	Toronto	Beach water	WB25/9/2005WA1-4	09-May-05	+	+
0036634450	04FE	A05	FEA05	Toronto	Beach water	WB25/9/2005WA1-5	09-May-05	-	+
0036633326	04FE	A06	FEA06	Toronto	Beach water	WB35/9/2005WA1-1	09-May-05	-	+
0036633325	04FE	A07	FEA07	Toronto	Beach water	WB35/9/2005WA1-3	09-May-05	-	+
0036633324	04FE	A08	FEA08	Toronto	Beach water	WB35/9/2005WA1-4	09-May-05	-	+
0036633323	04FE	A09	FEA09	Toronto	Beach water	WB35/9/2005WA1-5	09-May-05	-	-
0036633322	04FE	A10	FEA10	Toronto	Beach water	WB35/9/2005WA1-6	09-May-05	-	+
0034434440	04FE	A11	FEA11	Toronto	Canada Goose (Branta canadensis)	WB26/16/2005CG3-5	16-Jun-05	-	+
0034506391	04FE	A12	FEA12	Toronto	Beach water	WB36/16/2005WA1-1	16-Jun-05	-	+
0029898222	04FE	B01	FEB01	Hamilton	Mallard duck (Anas platyrhynchos)	BP9/7/2004D8-4	07-Sep-04	-	+
0029898221	04FE	B02	FEB02	Hamilton	Mallard duck (Anas platyrhynchos)	BP9/7/2004D8-5	07-Sep-04	-	+
0029898220	04FE	B03	FEB03	Hamilton	Mallard duck (Anas platyrhynchos)	BP9/7/2004D9-1	07-Sep-04	-	+
0029898219	04FE	B04	FEB04	Hamilton	Mallard duck (Anas platyrhynchos)	BP9/7/2004D9-2	07-Sep-04	-	+
0029895240	04FE	B05	FEB05	Toronto	Cat (Felis catus)	SPCA8/23/2004C9-5	23-Aug-04	-	+
0029898217	04FE	B06	FEB06	Hamilton	Mallard duck (Anas platyrhynchos)	BP9/7/2004D9-4	07-Sep-04	-	+
0029898216	04FE	B07	FEB07	Hamilton	Mallard duck (Anas platyrhynchos)	BP9/7/2004D9-5	07-Sep-04	-	+
0029898804	04FE	B08	FEB08	Hamilton	Mallard duck (Anas platyrhynchos)	LA8/30/2004D2-3	30-Aug-04	-	+
0029878039	04FE	B09	FEB09	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-30	14-Sep-04	-	+
0029878040	04FE	B10	FEB10	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-31	14-Sep-04	-	+
0029878041	04FE	B11	FEB11	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-32	14-Sep-04	-	+
0029878042	04FE	B12	FEB12	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-33	14-Sep-04	-	+
0029878043	04FE	C01	FEC01	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-34	14-Sep-04	-	+
0029878044	04FE	C02	FEC02	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-35	14-Sep-04	-	+
0029878045	04FE	C03	FEC03	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-36	14-Sep-04	-	+
0029878046	04FE	C04	FEC04	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-37	14-Sep-04	-	+
0029878047	04FE	C05	FEC05	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-38	14-Sep-04	+	+
0029878048	04FE	C06	FEC06	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-39	14-Sep-04	-	+
0029878049	04FE	C07	FEC07	Hamilton	Untreated CSO sewage	BP9/14/2004CSO2-40	14-Sep-04	+	+
0031927945	04FE	C08	FEC08	Toronto	Beach water	CI27/26/2004WC1-5	26-Jul-04	-	+
0031927961	04FE	C09	FEC09	Toronto	Beach water	CI27/26/2004WC1-6	26-Jul-04	-	+
0029862653	04FE	C10	FEC10	Toronto	Mallard duck (Anas platyrhynchos)	CI8/9/2004D7-1	09-Aug-04	-	+
0029878010	04FE	C11	FEC11	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-30	14-Sep-04	-	+
0029878008	04FE	C12	FEC12	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-32	14-Sep-04	-	+
0029878009	04FE	D01	FED01	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-31	14-Sep-04	-	+
0029878007	04FE	D02	FED02	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-33	14-Sep-04	-	+
0029878006	04FE	D03	FED03	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-34	14-Sep-04	-	+
0029878005	04FE	D04	FED04	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-35	14-Sep-04	-	+
0029878004	04FE	D05	FED05	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-36	14-Sep-04	-	+
0029878003	04FE	D06	FED06	Hamilton	Untreated CSO sewage	BP9/14/2004CSO3-37	14-Sep-04	-	+
0034435754	04FE	D07	FED07	Misc	Untreated CSO sewage	UNKN5/26/2004CSO1-	26-May-04	-	+
0034435755	04FE	D08	FED08	Misc	Untreated CSO sewage	UNKN5/26/2004CSO1-	26-May-04	-	+
0034435756	04FE	D09	FED09	Misc	Untreated CSO sewage	UNKN5/26/2004CSO1-	26-May-04	-	+
0043661956	04FE	D10	FED10	Toronto	Beach water	KW36/21/2004WC1-1	21-Jun-04	-	+
0034434946	04FE	D11	FED11	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG1-1	17-May-04	-	+
0034434945	04FE	D12	FED12	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG1-2	17-May-04	-	+
0034434944	04FE	E01	FEE01	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG2-1	17-May-04	-	+
0034434943	04FE	E02	FEE02	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG2-2	17-May-04	-	+
0034434755	04FE	E03	FEE03	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG2-3	17-May-04	-	+
0034434956	04FE	E04	FEE04	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG4-1	17-May-04	-	+
0034434957	04FE	E05	FEE05	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG4-2	17-May-04	-	+
0034434958	04FE	E06	FEE06	Toronto	Dog (Canis lupus familiaris)	KW5/17/2004DG4-3	17-May-04	-	+
0034506406	04FE	E07	FEE07	Toronto	Beach sand	WB26/16/2005SD1-18	16-Jun-05	-	+
0034506405	04FE	E08	FEE08	Toronto	Beach sand	WB26/16/2005SD1-19	16-Jun-05	-	+
0034506404	04FE	E09	FEE09	Toronto	Beach sand	WB26/16/2005SD1-20	16-Jun-05	-	+
0034506403	04FE	E10	FEE10	Toronto	Beach sand	WB26/16/2005SD1-21	16-Jun-05	-	+
0034506392	04FE	E11	FEE11	Toronto	Beach water	WB36/16/2005WA1-2	16-Jun-05	-	+
0034506393	04FE	E12	FEE12	Toronto	Beach water	WB36/16/2005WA1-3	16-Jun-05	-	+

Appendix C (*continued*).

Tracker	Rack	Well	StrainName	LocationFull	TypeFull	Unique Code	Date	Suc^{++}	Catalase
0036633138	04FE	F01	FEF01	Toronto	Beach water	WB15/31/2005WA1-1	31-May-05	-	+
0036633137	04FE	F02	FEF02	Toronto	Beach water	WB15/31/2005WA1-2	31-May-05	-	+
0036633136	04FE	F03	FEF03	Toronto	Beach water	WB15/31/2005WA1-3	31-May-05	-	+
0036633135	04FE	F04	FEF04	Toronto	Beach water	WB15/31/2005WA1-4	31-May-05	-	+
0036633134	04FE	F05	FEF05	Toronto	Beach water	WB15/31/2005WA1-5	31-May-05	-	+
0036632408	04FE	F06	FEF06	Toronto	Beach water	WB15/31/2005WK1-1	31-May-05	-	+
0036633139	04FE	F07	FEF07	Toronto	Beach water	WB15/31/2005WK1-2	31-May-05	-	+
0036633140	04FE	F08	FEF08	Toronto	Beach water	WB15/31/2005WK1-3	31-May-05	-	+
0036633141	04FE	F09	FEF09	Toronto	Beach water	WB15/31/2005WK1-4	31-May-05	-	+
0036633142	04FE	F10	FEF10	Toronto	Beach water	WB15/31/2005WK1-5	31-May-05	-	+
0036633143	04FE	F11	FEF11	Toronto	Beach water	WB15/31/2005WK1-6	31-May-05	-	+
0036633144	04FE	F12	FEF12	Toronto	Beach water	WB15/31/2005WK1-7	31-May-05	-	+
0036633149	04FE	G01	FEG01	Toronto	Beach water	WB15/31/2005WC1-1	31-May-05	-	+
0036632407	04FE	G02	FEG02	Toronto	Beach water	WB15/31/2005WC1-2	31-May-05	-	+
0036633162	04FE	G03	FEG03	Toronto	Beach water	WB15/31/2005WC1-3	31-May-05	-	+
0036633161	04FE	G04	FEG04	Toronto	Beach water	WB15/31/2005WC1-4	31-May-05	-	+
0036633160	04FE	G05	FEG05	Toronto	Beach water	WB15/31/2005WC1-5	31-May-05	-	+
0036633159	04FE	G06	FEG06	Toronto	Beach water	WB15/31/2005WC1-6	31-May-05	-	+
0036633157	04FE	G07	FEG07	Toronto	Beach water	WB15/31/2005WC1-8	31-May-05	-	+
0036633152	04FE	G08	FEG08	Toronto	Beach water	WB25/31/2005WA1-2	31-May-05	-	+
0036633151	04FE	G09	FEG09	Toronto	Beach water	WB25/31/2005WA1-3	31-May-05	-	+
0036633163	04FE	G10	FEG10	Toronto	Beach water	WB25/31/2005WA1-4	31-May-05	-	+
0029862902	04FE	G11	FEG11	Toronto	Mallard duck (Anas platyrhynchos)	CI8/9/2004D13-3	09-Aug-04	-	+
0029862879	04FE	G12	FEG12	Toronto	Mallard duck (Anas platyrhynchos)	CI8/9/2004D14-1	09-Aug-04	-	+

Appendix D. Mutations within the *rpoS* gene of environmental isolates.

Codon	14 Asp	27 Glu	28 Lys	31 Val	33 Glu	41 Ala	44 Glu	48 Gln	49 Arg	55 Leu	68 Ser	79 Phe	82 Arg	85 Arg	88 Val	89 Ala	90 Ser	91 Arg	92 Arg	95 Ile	96 Glu	99 Leu	102 Val	103 Val	104 Lys	115 Ala	116 Leu	117 Leu
Consensus	GAT	GAA	AAG	GTA	GAG	GCC	GAA	CAG	GGA	TTG	TCA	TTT	CGC	CGT	GTC	GCC	TCT	CGC	CGC	ATC	GAG	TTG	GTG	GTA	AAA	GCG	TTG	CTG
ECE10 [5]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	..C	...	...	...	...	...	...	...	...	...	...	...
↳ECE12	...	...	...	...	...	...	...	...	...	...	...	...	...	5bp deletion (frameshift, short protein)														
ABC08	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	..T	...	...
ECA08	...	...	...	...	...	...	...	..A	..G	...	...	...	...	..A	..T	...	..C	...	...	...	...	...	...	...	...	..T	...	...
ECC08	...	..G	..A	..T	...	..T	..G	..A	..G	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...
BNB03 [3]	..T	..G	..A	..T	...	..T	..G	..A	..G	...	..G	..C	...	...	...	...	..A	..T	...	..T	..A	C..	..C	..G	..G	...	C..	T..
ABH11 [2]	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABC10	...	...	...	...	...	...	...	...	...	C..	...	...	..A	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...
ABD09	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ECC07 [2]	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABB10	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ECH01	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
AZB10	...	...	...	..T	...	...	..G	...	..T	C..	..G	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...
ASC02	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABF02	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O157:H7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ECG08	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABA01	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ECA03 [4]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABC01	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ECB07	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABC03 [6]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
K-12	...	...	...	...	C.. (Gln)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
ABA03 [7]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
EKF07	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
AZB07	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Mutations in *rpoS* are indicated as differences from the consensus *rpoS* sequence, where the consensus refers to the most frequently identified base at each nucleotide position among the 45 isolates and laboratory K-12 *rpoS*. Periods represent a conserved sequence to the consensus.

Isolates are listed in the same order as shown in Figure 3.1. Isolates with the same *rpoS* sequence are collapsed into one row, and number of isolates represented are indicated in square brackets.

K-12 (NC_000913, geneID:49175990) and O157:H7 (NC_002695, geneID:914683) sequences were retrieved from NCBI and are included for reference.

Appendix D (*continued*).

Codon	118 Asp	119 Leu	122 Glu	123 Gly	124 Asn	125 Leu	126 Gly	129 Arg	131 Val	143 Ser	147 Thr	148 Trp	150 Ile	154 Ile	164 Thr	169 Ile	181 Thr	183 Arg	188 Lys	190 Asp	191 His	193 Pro	194 Ser	197 Glu	198 Ile	201 Gln	222 Val	228 Gly
Consensus	GAC	CTT	GAG	GGC	AAC	CTG	GGG	CGC	GTA	TCA	ACC	TGG	ATT	ATT	ACT	ATT	ACC	CGT	AAG	GAC	CAT	CCA	AGT	GAG	ATC	CAA	GTA	GGT
ECE10 [5]	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	..C	..A	…	..C	…	…	…	…	…	…	…
↳ECE12																												
ABC08	..T	…	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	..C	..A	…	…	…	…	…	…	…	…	…
ECA08	..T	…	…	…	..	T..	…	…	…	…	…	…	…	..C	…	…	…	..C	..A	…	..C	…	…	…	…	..G	…	..A
ECC08	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
BNB03 [3]	…	..G	…	…	…	…	1bp deletion (frameshift, short protein)																					
ABH11 [2]	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	..C	…	…	…	…	…	…	…
ABC10	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	..C	…	…	…	…	…	…	…
ABD09	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	..C	…	…	…	…	…	…	…
ECC07 [2]	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	..C	…	…	…	…	…	…	…
ABB10	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	..C	…	…	…	…	…	…	…
ECH01	…	..G	…	…	..	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	..C	…	…	…	…	…	…	…
AZB10	..T	…	…	..T	..	…	…	…	..T	…	..A	…	…	..C	..G	..C	…	…	…	..T	..C	..G	..C	..A	…	…	…	…
ASC02	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABF02	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..T	…	…	…
O157:H7	…	…	…	…	…	…	…	..T	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	…	…	…	…	…	…
ECG08	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABA01	…	..G	…	…	..	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..T	…
ECA03 [4]	…	…	…	…	…	…	…	…	…	..G	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABC01	…	…	A.. (Lys)	…	…	…	…	…	…	..G	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ECB07	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABC03 [6]	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
K-12	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABA03 [7]	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
EKF07	…	…	…	…	T.. (Tyr)	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
AZB07	…	…	…	…	..	…	…	…	…	…	…	.A. (Stop)	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

Appendix D (*continued*).

Codon	233 Ala	240 Asp	243 Glu	244 Asn	245 Gly	248 Asp	253 Asp	256 Lys	257 Gln	259 Ile	265 Glu	269 Lys	273 Val	283 Tyr	291 Val	295 Ile	298 Thr	299 Arg	309 Gly	313 Leu	314 Arg	315 Glu	316 Ile	317 Leu	318 Gln	319 Thr	324 Ile	330 Glu
Consensus	GCG	GAT	GAG	AAC	GGT	GAT	GAC	AAG	CAG	ATC	GAG	AAA	GTG	TAC	GTA	ATT	ACC	CGT	GGC	TTG	CGC	GAA	ATC	CTG	CAA	ACG	ATC	GAG
ECE10 [5]	…	…	…	…	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
↳ECE12																												
ABC08	…	…	…	…	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A
ECA08	…	…	..A	…	..C	…	…	..A	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A
ECC08	…	…	…	…	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	..T	…	…	…	…	…	…	…	..A	..A
BNB03 [3]																												
ABH11 [2]	…	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABC10	…	…	…	..T	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABD09	…	…	…	..T	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A
ECC07 [2]	…	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A
ABB10	..A	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A
ECH01	..A	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	T.. (Ser)	…	…	…	…	…	…	…	…	…	…	..A
AZB10	…	..C	…	…	…	..C	…	…	…	…	..A	..G	…	..T	…	..A	..A	..A	…	C..	..T	..G	..T	T..	..G	..T	…	..A
ASC02	…	…	…	…	…	…	…	…	…	1329bp insertion & 9bp duplication (short protein)																		
ABF02	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..C	…	…	…	…	…	…	…	…	…	…	…	…	..A
O157:H7	…	…	…	…	…	…	…	…	…	…	…	…	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ECG08	…	…	…	…	…	…	…	…	…	…	..A	…	..A	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A
ABA01	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ECA03 [4]	…	…	…	…	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..A	…	…	…	…
ABC01	…	…	…	…	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ECB07	…	…	…	…	…	…	..T	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABC03 [6]	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
K-12	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
ABA03 [7]	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..T	…	…	…	…	…	…	…
EKF07	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..T	…	…	…	…	…	…	…
AZB07	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	..T	…	…	…	…	…	…	…

Appendix E. Standard Operating Procedures

E. 1. M9 Minimal Medium

Reference: (Sambrook & Russell, 2001)

Note: Use autoclaved reagents when preparing 2× M9 minimal medium to ensure sterility. Carbon source should be filter sterilized.

2× M9 minimal medium:

10× M9 salts[1]	100 ml
1M $MgSO_4$	2 ml
5% carbon source	100 ml (Final percentage in plates = 0.5% glucose[2])
1M $CaCl_2$	0.1 ml
ddH_2O	to 500 ml

Combine equal parts 2× M9 minimal medium with 2× agar (for plates) or ddH_2O (for liquid culture). Add 50 µl of 1% thiamine, a vitamin required in trace amounts.

[1] **10× M9 salts:**

$Na_2HPO_4 \cdot 7H_20$	128 g
KH_2PO_4	30 g
NaCl	5 g
NH_4Cl	10 g
ddH_2O	to 1 L

[2] For other carbon sources, 1% may be used. Refer to methods in (Chiang *et al.*, 2011) for percentage used for select carbon sources. Adjust pH of carbon to 7.0.

E. 2. Selection for RpoS Mutants on Succinate Minimal Plates

Reference: (Chen *et al.*, 2004)

Note: This method was used to select for RpoS mutants from environmental *E. coli* isolates in (Chiang *et al.*, 2011).

1. Inoculate a single bacterial colony into 10 ml of 1× LB in a sterile 50 ml Erlenmeyer flask, and incubate the culture overnight at 37^{o}C with shaking (200 rpm).
2. Transfer the overnight culture to a sterile 10 ml polypropylene tube (record OD_{600}), and centrifuge 4,000 × *g* for 10 min. Discard supernatant.
3. Resuspend cells in an equal volume of 1× M9 salts, and centrifuge 4,000 × *g* for 10 min. Discard supernatant.
4. Resuspend cells in 1 ml of 1× M9 salts.
5. Plate ~10^9 cells (based on OD_{600} recorded previously in step 2) on succinate minimal plates, and incubate plates at 37^{o}C.
6. After 48-72 h incubation, fast-growing mutants (Suc^{++}) can be picked, and streaked onto LB plates for purity.
7. Streak for purity 3× in total.
8. Check for RpoS activity by catalase and/or succinate growth tests, and sequence *rpoS* to identify mutation.

E. 3. Screen Environmental *E. coli* for RpoS Mutants

Reference: (Chiang *et al.*, 2011)

1. Inoculate single colonies of environmental *E. coli* into 200 μl of 1× LB in a sterile 96-well microtitre plate, and incubate plate at 37°C overnight without shaking.
2. Replica plate overnight cultures into 200 μl of 1× M9 salts to wash cells of excess 1× LB.
3. From the inoculated 1× M9 salts, replica plate cells onto succinate minimal medium plates, glucose minimal medium plates, and LB plates.

Growth on succinate minimal plates:

4. Incubate succinate and glucose minimal medium plates at 37°C for 24-48 h, checking growth after each 24 h period.
5. Record isolates that grow on succinate minimal medium plates after 24 h (glucose minimal medium plates are used as a control).

Catalase activity:

6. Incubate LB plates at 37°C for 24 h.
7. Apply 2-5 μl of 30% hydrogen peroxide onto patches. Record isolates that have a severe lag in bubbling time.

Putative RpoS mutants:

8. Isolates that were recorded with growth after 24 h on succinate minimal medium plates and with a severe lag in bubbling time after addition of hydrogen peroxide are considered putative RpoS mutants. Sequence *rpoS* and/or detect RpoS protein by Western blot to confirm loss of RpoS protein.

E. 4. Polymerase Chain Reaction

Reference: (Sambrook & Russell, 2001)

Note: Whole colony PCR procedure described here was developed by Tao Dong. If sequencing, the final PCR product must be cleaned to remove primer dimers. It is also preferable to use proofreading *Taq* for amplification. Confirm mutations by sequencing with both the forward and reverse primers.

Whole colony PCR:

1. Suspend a single colony of bacteria in 10 µl of ddH_2O in a sterile PCR tube.
2. Incubate the tube at 95°C for 5 min.
3. Aliquot 2 µl of boiled colony to PCR mixture as template.

PCR mixture:

4. Combine the reagents below to make 25 µl PCR reactions, making sure to keep everything on ice. For a negative control, add ddH_2O instead of template.

Reagent	Volume (µl)	Final concentration
ddH_2O	17	---
10× ThermoPol buffer	2.5	1×
10 mM dNTPs	0.5	0.2 mM each dNTP
25 µM forward primer	1	1 µM
25 µM reverse primer	1	1 µM
1U/µl Taq	1	1 unit
Template	2	---

PCR program:

5. Place tubes in thermocycler and run the following program.

PCR step	Temperature (°C)	Duration (min)	No. of cycles
Initial denaturation	95	3	1
Denaturation	95	0.5	30
Annealing	~5°C below primer melting temperature	0.5	
Extension	72	1 min for each 1 kb	
Terminal extension	72	5	1

Analysis:

6. Aliquot 2 µl of PCR product into 5 µl of 1× loading dye, and load into a 0.8-1% agarose gel containing 0.1 ng/ml ethidium bromide.
7. Run the agarose gel at 100 V for 60 min.
9. Visualise PCR products using a gel documentation system.

E. 5. Survival Assay in Acidic pH

Reference: (Waterman & Small, 1996)

Note: This method was used to test acid resistance of environmental *E. coli* in (Chiang *et al.*, 2011).

1. Inoculate a single bacterial colony into 10 ml of 1× LB in a sterile 50 ml Erlenmeyer flask, and incubate the culture overnight at 37°C with shaking (200 rpm).
2. Subculture to ~10^5 cells into fresh 1× LB adjusted to pH 2.5 with hydrochloric acid.
3. Incubate culture at 37°C with shaking (200rpm) for 2 h.
4. Serially plate culture onto LB plates, and incubate overnight at 37°C.
5. Determine percent survival: (final CFU/ml)/(initial CFU/ml) × 100.

E. 6. Survival Assay in Osmotic Stress

Reference: (Dong *et al.*, 2009)

Note: This method was used to test osmotic stress resistance of environmental *E. coli* in (Chiang *et al.*, 2011).

1. Inoculate a single bacterial colony into 5 ml of 1× LB in a sterile test tube, and incubate the culture overnight at 37^{o}C on the rotating wheel.
2. Transfer overnight culture to a sterile 15 ml polypropylene tube, and centrifuge culture 4,000 × *g* for 10 min. Discard supernatant.
3. Resuspend pellet in an equal volume of sterile 0.9% NaCl, and centrifuge culture 4,000 × *g* for 10 min. Discard supernatant.
4. Resuspend pellet in an equal volume of sterile 0.9% NaCl.
5. Dilute culture to ~10^7 cells into fresh LB containing 15 mM H_2O_2 in a sterile 50 ml Erlenmeyer flask, and incubate 37^{o}C with shaking (200 rpm).
6. Take samples of culture over time. Serial plate each sample immediately, and plate onto LB plates.
10. Determine percent survival: (final CFU/ml)/(initial CFU/ml) × 100.

E. 7. Protein Extraction by Sonication

1. Inoculate a single bacterial colony into 5 ml of 1× LB in a sterile test tube containing the appropriate antibiotic, and incubate the culture overnight at 37°C on the rotating wheel.
2. Transfer the overnight culture to a sterile 15 ml polypropylene tube (if samples are needed for exponential or stationary phase, subculture overnight culture into 1 × LB, and at an OD_{600} of ~0.3, sample 30 ml, and at an OD_{600} of ~1.5, sample 10 ml). Centrifuge culture 4,000 × *g* for 10 min. Discard supernatant.
3. Add half volume of 50 mM potassium phosphate buffer, pH 7.
4. Centrifuge culture 4,000 × *g* for 10 min. Discard supernatant.
5. Add half volume of 50 mM potassium phosphate buffer, pH 7.
6. Centrifuge culture 4,000 × *g* for 10 min. Discard supernatant.
7. Resuspend pellet in 1 ml of 50 mM potassium phosphate buffer, pH 7, and transfer cells to ice-cold sterile 15 ml polystyrene conical tube (OD_{600} ~ 15).
8. Sonicate sample.
9. Transfer sonicated sample to sterile 1.5 ml microcentrifuge tube, and centrifuge 10,000 × *g* for 15 min at 4°C.
10. Transfer supernatant to new microcentrifuge tube.

E. 8. Protein Quantification by Bradford Assay

Reference: (Bradford, 1976)

1. To determine the protein concentration of one unknown, mix 19.2 ml Bio-Rad Protein Assay Dye Reagent Concentrate (Catalog #500-0006) with 4.8 ml sterile ddH$_2$O in a sterile 50 ml polypropylene tube (i.e., 80% Bio-Rad dye).
2. Aliquot 1 ml of mix to each of 21 disposable glass test tubes (keep extra 3 ml in case unknown needs retesting. See step #7).
3. Add the following amounts of 1 mg/ml bovine serum albumin, vortexing for 5 s after each addition (perform in triplicate - 18 test tubes used):

Tube	1 mg/ml bovine serum albumin (μl)
1	0
2	1
3	3
4	5
5	7
6	9

4. Add 5 μl of unknown to each of the three remaining test tubes, vortexing for 5 s after each addition.
5. Allow the test tubes to sit for 5 min at room temperature (no longer than 30 min).
6. Measure OD$_{595}$ of each tube.
7. Create a standard curve for the known protein concentrations, and determine the unknown concentration by using the line equation for the standard curve. If the unknown concentration is too high to plot within the standard curve, use the extra 80% Bio-Rad dye to test a smaller quantity or dilution of the unknown.

E. 9. Native PAGE Analysis of Catalase Activity

Reference: (Gregory & Fridovich, 1974)

Note: This method was used to assay catalase activity of environmental *E. coli* in (Chiang *et al.*, 2011).

1. Prepare reagents for a polyacrylamide gel as detailed below.

   30% Acrylamide/0.8% Bis:

Acrylamide	90 g
N,N`-methylene-bis-acrylamide	2.4 g
ddH$_2$O	to 300 ml

   Filter solution through a 0.45 μM filter into a dark sterile bottle, and store at 4^{o}C.

   1.5 M Tris-Cl, pH 8.8:

Tris base	91 g
ddH$_2$O	300 ml
(*Adjust to pH 8.8 with 1 N HCl*)	
ddH$_2$O	to 500 ml

   Store solution at 4^{o}C.

   10× Running Buffer:

Tris base	30 g
Glycine	144 g
ddH$_2$O	to 1 L

   Store solution at room temperature.

   3× Native Sample Dye:

Glycerol	30 ml
1 M Tris-Cl, pH 6.8	18.8 ml
1% bromophenol blue	1.5 ml
ddH$_2$O	to 100 ml

   Store solution at room temperature.

2. Wipe spacer plates and short plates with 70% ethanol (prepare for two gels).
3. Secure plates in a casting frame, and assemble in a casting stand.

4. Mix reagents as detailed below, swirling after each addition. Keep cool and avoid bubbles when mixing. Makes two gels (one to stain for catalase and one to serve as a protein loading control):

Reagent	Volume (ml)
ddH_2O	7
1.5 M Tris-Cl, pH 8.8	3.75
30% Acry/0.8% Bis	4.75
10% APS	0.15
TEMED (N,N,N`,N`-Tetramethylethylenediamine)	0.015

5. Pipette mixture between gel casting glass, and insert comb. Let gels solidify (~30 min).
6. While gels solidify, prepare protein for loading. Make 50-100 µl of 0.5 or 1 µg/µl protein with 1× native sample dye.
7. After gels solidify, remove comb, and assemble gels into gel unit. Add required amount of 1× running buffer.
8. Load 5 µg of protein into wells for each gel. Include a protein ladder, e.g., Fermentas PageRuler Prestained Protein ladder.
9. Run gels at 120 V for 1.5 h.
10. Remove gels from gel apparatus. Stain one gel for protein and one gel for catalase.
11. For protein gel, soak gel in 0.1% Coomassie Blue solution (0.5 g Coomassie Brilliant Blue - 200 ml methanol - 50 ml acetic acid - 250 ml ddH_2O → filter solution using filter paper) for 1 h. Destain with destain solution (450 ml methanol - 100 ml acetic acid - 450 ml ddH_2O) for 24 h, replacing the destain solution 2-3×. Take photos.
12. For catalase gel, soak gel in horseradish peroxidase solution (2.5 mg horseradish peroxidase dissolved in 50 ml of 50 mM potassium phosphate buffer, pH 7) for 15 min. Decant solution, and rinse gel in running water for 1 min.
13. Soak catalase gel in 5 mM H_2O_2 (45 µl of 30% H_2O_2 in 50 ml of 50 mM potassium phosphate buffer, pH 7) for 10 min. Decant solution, and rinse gel in running water for 1 min.
14. Soak catalase gel in 0.5 mg/ml diaminobenzidine (25 mg diaminobenzidine dissolved in 50 ml of 50 mM potassium phosphate buffer, pH 7) for 30 min to 24 h. Take photos.

E. 10. Western Blot

Reference: This method was modified by T. Dong and S. M. Chiang from (Lam & Mutharia, 1994).

Note: This method was used to detect RpoS and AppA in environmental *E. coli* in (Chiang *et al.*, 2011).

1. Prepare reagents for a polyacrylamide stacking gel as detailed below.

   30% Acrylamide/0.8% Bis:

Acrylamide	90 g
N,N`-methylene-bis-acrylamide	2.4 g
ddH$_2$O	to 300 ml

   Filter solution through a 0.45 μM filter into a dark sterile bottle, and store at 4^{o}C.

   1.5 M Tris-Cl, pH 8.8 or pH 6.8:

Tris base	91 g
ddH$_2$O	300 ml
(*Adjust to pH 8.8 or 6.8 with 1 N HCl*)	
ddH$_2$O	to 500 ml

   Store solution at 4^{o}C.

   10× Running Buffer with SDS:

Tris base	30 g
Glycine	144 g
SDS	10 g
ddH$_2$O	to 1 L

   Store solution at room temperature.

   10× TBS-T:

NaCl	43.5 g
1 M Tris-Cl, pH 8	50 ml
Tween 20	2.5 ml
ddH$_2$O	to 500 ml

   Store solution at room temperature.

10× Transfer Buffer:

Tris base	6.05 g
Glycine	28.8 g
Methanol	400 ml
ddH_2O	to 2L

Store solution at room temperature, and place 1× transfer buffer on ice or at 4^oC for 30 min before use.

2× SDS/Loading Buffer:

1 M Tris-Cl, pH 6.8	12.5 ml
Glycerol	8.7 ml
β-mercaptoethanol	2.5 ml
10% SDS	10 ml
1% Bromophenol blue	1 ml
ddH_2O	15.3 ml

Store solution at room temperature or at 4^oC for long term storage.

Sample collection:

2. Inoculate a single bacterial colony into 5 ml of 1× LB in a sterile test tube containing the appropriate antibiotic, and incubate the culture overnight at 37^oC on the rotating wheel.
3. Subculture cells 1:10,000* into 50 ml of 1× LB without antibiotics (1:5,000 for *rpoS* mutants to compensate for a longer *rpoS* mutant lag phase).
    *Eight generations of growth are needed prior to sampling to ensure that there is no protein carryover from the overnight culture.
4. Take samples at the desired OD_{600}. For exponential phase (OD_{600} ~0.3), take 1 ml of sample; for stationary phase (OD_{600} ~1.5), take 0.2 ml of sample; and for overnight culture, take 0.1 ml of sample (record OD_{600}). If samples will not be processed immediately, add 150 μg/ml chloramphenicol final concentration to stop protein synthesis.
5. Centrifuge samples at 11,000 × *g* for 1 min. Discard supernatant using a pipette (supernatant is high in salt and will produce wide bands in the polyacrylamide gel).
6. Resuspend samples in 1× SDS/loading buffer so that the calculated OD_{600} of the samples are ~1, e.g., to the pellet from 1 ml of exponential phase cells and to the pellet from 0.2 ml of stationary phase, add 0.3 ml of 1× SDS/loading buffer.
7. Place samples in boiling water for 5 min.
8. Centrifuge samples at 10,000 × *g* for 1 min.

SDS-PAGE:

9. To prepare polyacrylamide stacking gel, wipe spacer plates and short plates with 70% ethanol (prepare for two gels).

10. Secure plates in a casting frame, and assemble in a casting stand.
11. Mix reagents as detailed below for separating gel, swirling after each addition. Keep cool and avoid bubbles when mixing. Makes two gels (one for Western blot and one to serve as a protein loading control):

Reagent	Volume (ml)
ddH_2O	4
1.5 M Tris-Cl, pH 8.8	2.5
10% SDS	0.1
30% Acry/0.8% Bis	4.4
10% APS	0.1
TEMED (N,N,N`,N`-Tetramethylethylenediamine)	0.01

12. Pipette mixture between gel casting glass up to ~5.5 cm from the bottom, and add water on top. Let gels solidify (15-30 min).
13. After gels solidify, pour out water using a Kim-wipe to help drain.
14. Mix reagents as detailed below for stacking gel, swirling after each addition. Keep cool and avoid bubbles when mixing:

Reagent	Volume (ml)
ddH_2O	3.6
1.5 M Tris-Cl, pH 6.8	0.63
10% SDS	0.05
30% Acry/0.8% Bis	0.66
10% APS	0.05
TEMED (N,N,N`,N`-Tetramethylethylenediamine)	0.005

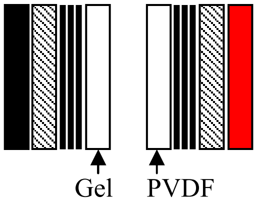
15. Pipette mixture between gel casting glass, and insert comb. Let gels solidify (~30 min).
16. After gels solidify, remove comb, and assemble gels into gel unit. Add required amount of 1× running buffer with SDS.
17. Load 10 µl of protein into wells for each gel. Include a protein ladder, e.g., Fermentas PageRuler Prestained Protein ladder.
18. Run gels at 50 V for 0.5 h or until protein has reached the separating gel.
19. Increase the voltage to 100 V for 1-1.5 h or until protein has reached the bottom of the separating gel.

Western blot:

20. Remove gels from gel apparatus. Stain one gel for protein, and use the other gel for Western blot.
21. For protein gel, soak gel in 0.1% Coomassie Blue solution (0.5 g Coomassie Brilliant Blue - 200 ml methanol - 50 ml acetic acid - 250 ml ddH_2O → filter

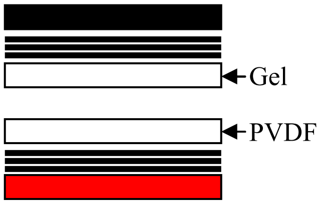
solution using filter paper) for 1 h. Destain with destain solution (450 ml methanol - 100 ml acetic acid - 450 ml ddH_2O) for 24 h, replacing the destain solution 2-3×. Take photos.

22. For Western blot, transfer protein from the polyacrylamide gel to a PVDF membrane by (a) wet transfer or (b) semi-dry transfer.
    a. West transfer: Soak 7 × 9 cm PVDF membrane in methanol for 1-5 min. Transfer membrane to ice-cold 1× transfer buffer. Also soak the sandwich cassette, six 7 × 9 cm filter papers, and the separating layer of the polyacrylamide gel in ice-cold 1× transfer buffer. Assemble sandwich as shown below, minimizing air bubbles between layers, and fill the gel unit with ice-cold 1× transfer buffer. Run transfer at 90 V for 1 h or at 25 V overnight at 4°C.



Wet transfer: Black (cathode) side → sandwich mesh → 3 filter papers → separating layer of polyacrylamide gel → PVDF membrane → 3 filter papers → sandwich mesh → red (anode) side

    b. Semi-dry transfer: Soak 7 × 9 cm PVDF membrane in methanol for 1-5 min. Transfer membrane to ice-cold 1× transfer buffer. Also soak six 7 × 9 cm filter papers and the separating layer of the polyacrylamide gel in ice-cold 1× transfer buffer. Assemble sandwich as shown below, minimizing air bubbles between layers using a roller. Run transfer at 20 V for 30 min.



Semi-dry transfer (bottom up): Base (anode) → 3 filter papers → PVDF membrane → separating layer of polyacrylamide gel → 3 filter papers → Lid (cathode)

23. Take membranes out from chambers, and incubate in 5% milk (made in 1× TBS-T) with shaking for 1 h at room temperature or overnight at 4°C.
24. Cut edges of membrane to fit within a 100 mm Petri dish. Add 10 ml of 1:10,000 dilution of primary antibody.
25. Incubate membrane overnight at 4°C with gentle shaking.
26. Wash membrane 3× with 1× TBS-T for 10 min each on shaker at room temperature.
27. Incubate membrane for 1 h with 10 ml of 1:3,000 dilution of secondary antibody with shaking at room temperature.
28. Wash membrane 3× with 1× TBS-T for 10 min each on shaker at room temperature.
29. Mix 5 ml of reagent 1 and 5 ml of reagent 2 of ECL staining solution (Amersham GE Healthcare, Inc.) in Petri dish.

30. Using tweezers, gently allow excess TBS-T to drain off membrane, and place membrane in Amersham detection solution for 1 min with shaking.
31. Seal membrane in Saran wrap, eliminating leaks, and in dark room, expose Amersham Hyperfilm ECL to the membrane for 10 s to 1 min. Develop film.

E. 11. Motility Assay

Reference: This method was used to detect motility of environmental *E. coli* in (Chiang *et al.*, 2011).

1. Aliquot 200 µl of 0.15% LB agar to a sterile 96-well microtitre plate. Let solidify.
2. Stab single bacterial colonies into agar.
3. Incubate plate at 37°C overnight without shaking, and observe.

E. 12. Genomic DNA Extraction from Bacterial Cultures

Reference: This procedure was modified by M. Kirchhof (2002) and S. M. Chiang from (Miller, 1992).

1. Inoculate a single bacterial colony into 10 ml of 1× LB in a sterile 50 ml Erlenmeyer flask containing the appropriate antibiotic, and incubate the culture overnight at 37^{o}C with shaking (200 rpm).
2. Aliquot 1.5 ml of overnight culture into a microcentrifuge tube, and centrifuge at 13,000 × *g* for 1 min. Discard supernatant.
3. Resuspend pellet in 467 µl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). Add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K, and gently invert microcentrifuge tube 6-8× to mix. Incubate at 37^{o}C for 1 h.
4. After incubation, add 1 ml phenol-chloroform mix (50% phenol, pH 8 - 48% chloroform - 2% isoamyl alcohol) to microcentrifuge tube. Invert tube 6-8× to mix, and centrifuge at 13,000 × *g* for 10 min.
5. Transfer aqueous phase to a new microcentrifuge tube, carefully avoiding the organic phase. Add 1 ml phenol-chloroform mix to microcentrifuge tube. Invert tube 6-8× to mix, and centrifuge at 13,000 × *g* for 10 min.
6. Transfer aqueous phase to a new microcentrifuge tube, carefully avoiding the organic phase. Add 1 ml chloroform to microcentrifuge tube. Invert tube 6-8× to mix, and centrifuge at 13,000 × *g* for 10 min.
7. Transfer aqueous phase to a new microcentrifuge tube, carefully avoiding the organic phase. Add 1 ml of 100% ethanol and 50 µl of 3 M sodium acetate (pH 5.2) to microcentrifuge tube. Invert tube 6-8× to mix, and place tube on ice for 30 min.
8. Centrifuge microcentrifuge tube at 13,000 × *g* for 10 min. Remove supernatant using a pipettor.
9. Resuspend pellet in 1 ml of 70% ethanol. Centrifuge microcentrifuge tube at 13,000 × *g* for 10 min. Remove supernatant using a pipettor.
10. Air dry pellet by placing microcentrifuge tube at 37^{o}C for 30 min.
11. Resuspend genomic DNA in 50-100 µl of ddH_2O or TE buffer.
12. Measure OD_{260} and OD_{280} to determine DNA concentration and purity.
    - ➢ $[DNA] = 50\ \mu g/\mu l \times \text{dilution} \times OD_{260}$
    - ➢ OD_{260}/OD_{280} of pure DNA = 1.8-2.0

E. 13. RNA Isolation from 24 h and 48 h Bacterial Cultures using Microcentrifuge Tubes

Reference: This procedure was modified by S. M. Chiang from a large scale RNA extraction procedure (M. Trudeau, 2004).

Method questions:

- What is the mechanism by which DEPC (diethyl pyrocarbonate) functions to remove RNase? Why is it important to de-activate DEPC?
  DEPC reacts with histidine and disables RNase, but it can interact with RNA as well. Autoclaving inactivates DEPC through hydrolysis (forming ethanol and CO_2*).*
- Why do we use acidic phenol in RNA extraction?
  DNA tends to stay in acidic phenol, while RNA comes out into the aqueous phase.
- What is the purpose of keeping the RNA on ice after adding hot phenol?
  The samples cool on ice to allow for complete dissociation of nucleoproteins.
- What is the purpose of the chloroform washes?
  Chloroform denatures lipids and further helps to separate nucleic acids from the organic phase (isoamyl alcohol reduces foaming).

1. Prepare RNase-free reagents and supplies as detailed below.

   DEPC ddH$_2$O:

Diethyl pyrocarbonate (DEPC)	0.5 ml
ddH2O	499.5 ml

   Shake mixture vigorously. Incubate at 37°C for 1 h or room temperature for overnight. Autoclave.

   RNase-free 1.5 ml microcentrifuge tubes and RNase-free tips:
   Wipe storage container or tip box with RNase Zap, and rack tips and tubes from unopened, RNase-free bags using gloves wiped with RNase Zap. Autoclave.

Sample collection:

2. Inoculate 10 ml of 1× LB with a single bacterial colony, and incubate at 37°C with shaking (200 rpm).
3. At 24 h and 48 h post-inoculation, aliquot 1.5 ml of culture into sterile 1.5 ml microcentrifuge tubes.
4. Centrifuge tubes at 11,000 × *g* for 1 min, and discard supernatant.

5. Flash freeze bacterial pellets in liquid nitrogen, and store at –80°C.

RNA isolation:

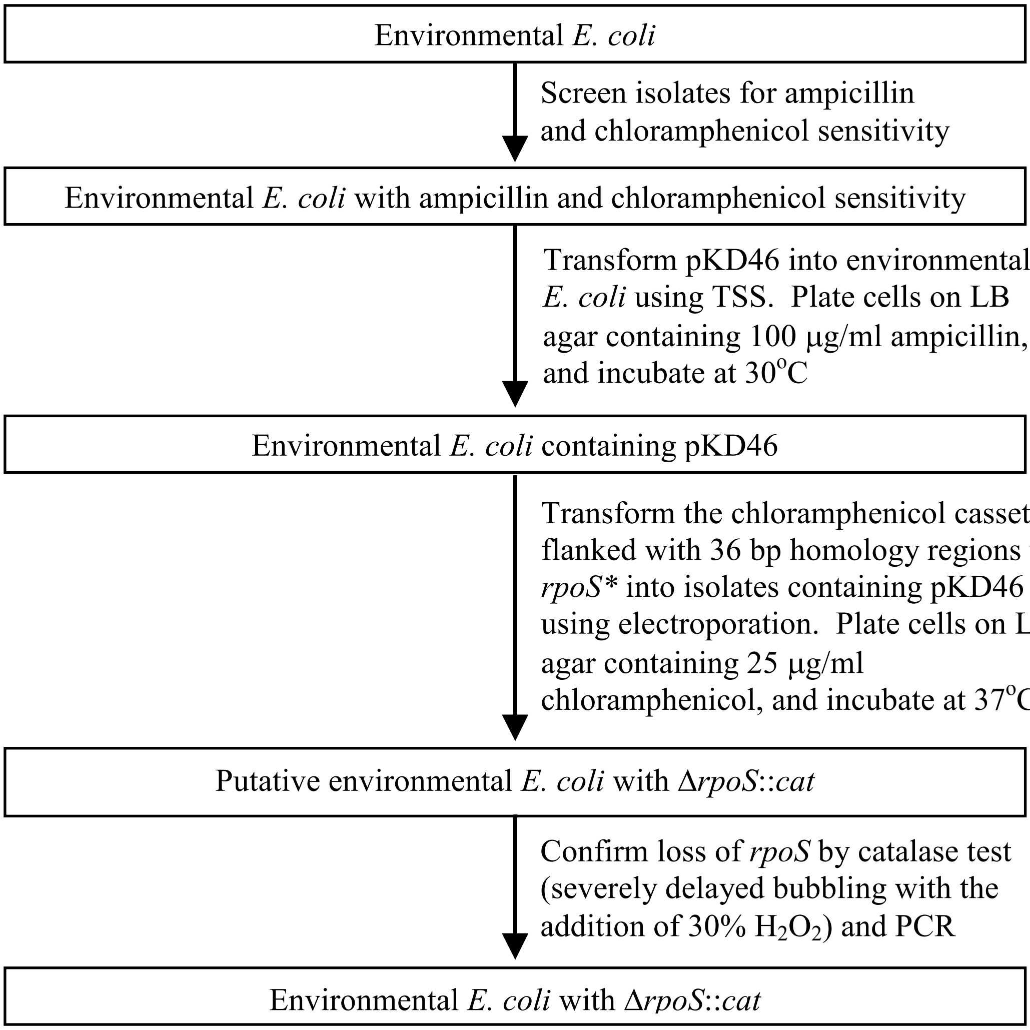
Note: Perform all steps quickly and on ice when possible. Typically, inversion, not vortexing, is used for mixing so to prevent shearing of genomic DNA.

6. Preparation: -let bacterial pellets thaw on ice.
   -preheat acidic phenol at 65°C (make sure to loosen bottle cap).
7. Vortex bacterial pellets to resuspend in residual supernatant.
8. Add 0.5 ml boiling 2% SDS - 16 mM EDTA (heated in microwave). Thoroughly mix the bacterial pellets with the SDS/EDTA by repeated inversion for 30 s.
9. Add 1 ml of 65°C acidic phenol, pH 4.3. Mix thoroughly by inversion.
10. Place tubes in 65°C bath for 3 min, inverting every 30 s to mix.
11. Place tubes on ice for 10 min, inverting occasionally.
12. Centrifuge tubes at 13,000 × *g* for 10 min.
13. Transfer aqueous phase to a new RNase-free 1.5 ml microcentrifuge tubes. Add DEPC ddH$_2$O to make up lost volume of the aqueous phase (to 0.5 ml).
14. Repeat steps 8-12 two more times or until no white matter is visible at the interface.
15. Add 1 ml of room temperature phenol-chloroform mix (50% phenol - 24% chloroform - 1% isoamyl alcohol). Mix gently by inversion.
16. Centrifuge tubes at 13,000 × *g* for 5 min, and transfer aqueous phase to a new RNase-free 1.5 ml microcentrifuge tube. Add DEPC ddH$_2$O to make up lost volume of the aqueous phase (to 0.5 ml).
17. Add 1 ml of room temperature chloroform-isoamyl alcohol mix (96% chloroform - 4% isoamyl alcohol). Mix gently by inversion.
18. Centrifuge tubes at 13,000 × *g* for 5 min, and transfer aqueous phase to new RNase-free 1.5 ml microcentrifuge tube.
19. Add 0.1 volumes (40 μl) of 3 M sodium acetate (pH 5.2, RNase-free), and invert several times to mix.
20. Add 1 ml of absolute ethanol, and invert several times to mix.
21. Store tubes at –20°C for 60 min or overnight.
22. Centrifuge tubes at 13,000 × *g* for 30 min at 4°C to pellet RNA. Discard supernatant.
23. Wash pellet with 0.5 ml of 70% RNase-free ethanol.
24. Centrifuge tubes at 13,000 × *g* for 15 min at 4°C. Discard supernatant.
25. Dry RNA pellet for 15 min at room temperature.
26. Add 20 μl of DEPC ddH$_2$O to resuspend RNA pellet.
27. Measure OD$_{260}$ and OD$_{280}$ to determine RNA concentration and purity.
    - ➢ [RNA] = 40 μg/μl × dilution × OD$_{260}$
    - ➢ OD$_{260}$/OD$_{280}$ of pure RNA = 2.0
28. Check RNA integrity by running 2-5 μg of RNA on a 1% agarose gel (ribosomal subunit bands: 23S, 16S, 5S should be visible).

E. 14. Flowchart to Construct *rpoS* Mutants (Δ*rpoS*::*cat*) of Environmental *E. coli*

Reference: This method was modified by S. M. Chiang from (Datsenko & Wanner, 2000).

Note: This method was modified to work on environmental *E. coli* isolates with only moderate success. Of ~50 isolates screened for antibiotic sensitivity in the first step, only four Δ*rpoS*::*cat* derivatives of environmental *E. coli* isolates were successfully constructed. Remember to include *E. coli* K-12 MG1655 in the procedure as a positive control. For electroporation, prepare cells as in Appendix E. 18.



Environmental *E. coli*

Screen isolates for ampicillin and chloramphenicol sensitivity

Environmental *E. coli* with ampicillin and chloramphenicol sensitivity

Transform pKD46 into environmental *E. coli* using TSS. Plate cells on LB agar containing 100 μg/ml ampicillin, and incubate at 30°C

Environmental *E. coli* containing pKD46

Transform the chloramphenicol cassette flanked with 36 bp homology regions to *rpoS** into isolates containing pKD46 using electroporation. Plate cells on LB agar containing 25 μg/ml chloramphenicol, and incubate at 37°C

Putative environmental *E. coli* with Δ*rpoS*::*cat*

Confirm loss of *rpoS* by catalase test (severely delayed bubbling with the addition of 30% H_2O_2) and PCR

Environmental *E. coli* with Δ*rpoS*::*cat*

*To amplify the *cat* gene with 36 bp homology regions to *rpoS*, use pKD3 plasmid as template and the following primers (small letters → *rpoS* homology regions):
Fw: 5’- atgttccgtcaagggatcacgggtaggagccaccttGTGTAGGCTGGAGCTGCTTC -3’
Rv: 5’- cctcgcttgagactggcctttctgacagatgcttacCATATGAATATCCTCCTTAG -3’

E. 15. Preparation of Chemically Competent Cells (TSS)

Reference: (Chung *et al.*, 1989)

1. Inoculate a single bacterial colony into 10 ml of 1× LB in a sterile 50 ml Erlenmeyer flask containing the appropriate antibiotic, and incubate the culture overnight at 37^{o}C with shaking (200 rpm).
2. Subculture 1:50 into 50 ml of 1× LB in a sterile 250 ml Erlenmeyer flask, and incubate cells at 37^{o}C with shaking (200 rpm).
3. At an OD_{600} of 0.4, transfer 0.5 ml of culture to a sterile 1.5 ml microcentrifuge tube, and dilute 1:1 with 2× TSS (20% PEG 3350 - 10% DMSO - 50 mM $MgCl_2$ in 1× LB, adjusted to pH 6.5). Keep on ice.
4. Cells are now ready for transformation, or flash-freeze cells in dry ice-ethanol bath prior to storing at -80^{o}C. Thaw cells on ice if used later.

E. 16. Transformation using Chemically Competent Cells (TSS)

Reference: (Chung *et al.*, 1989)

1. Add 1-5 µl of DNA (0.1 - 0.5 µg DNA) to 0.1 ml of TSS cells in a sterile 1.5 ml microcentrifuge tube on ice. Mix by tapping tube. Remember to include a positive control to test the competency of the cells (e.g., pUC19) and a negative control to test for contaminants (no DNA added).
2. Keep microcentrifuge tube on ice for 30 min.
3. Add 0.9 ml of 1× LB with 20 mM glucose, and grow cells at 37^{o}C (30^{o}C for strains carrying temperature sensitive plasmids, e.g., pKD46) with shaking (200 rpm) for at least 1.5 h or for approximately two generations of growth.
4. Plate 50 µl of cells onto plates containing the selective antibiotic. Incubate plates at 37^{o}C (30^{o}C for strains carrying temperature sensitive plasmids).
5. Leave the remainder transformed cells on bench overnight. Plate remainder of cells the following day if no transformants were detected in the first plating.

E. 17. Preparation of Electrocompetent Cells for *E. coli* K-12

Reference: (Dower *et al.*, 1988)

1. Inoculate a single bacterial colony into 10 ml of 1× LB in a sterile 50 ml Erlenmeyer flask containing the appropriate antibiotic, and incubate the culture overnight at 37°C with shaking (200 rpm).
2. Subculture 1:50 into 50 ml of 1× LB in a sterile 250 ml Erlenmeyer flask, and incubate cells at 37°C with shaking (200 rpm).
3. At an OD_{600} of 0.4, place culture on ice for 30 min.
4. Centrifuge cells at 4,000 × *g* at 4°C for 15 min. Discard supernatant.
5. Resuspend cells with an equal volume of 10% ice-cold glycerol.
6. Centrifuge cells at 4,000 × *g* at 4°C for 15 min. Discard supernatant.
7. Resuspend cells with an equal volume of 10% ice-cold glycerol.
8. Centrifuge cells at 4,000 × *g* at 4°C for 15 min. Discard supernatant.
9. Resuspend cells in 3 ml of 10% ice-cold glycerol.
10. Centrifuge cells at 4,000 × *g* at 4°C for 15 min. Discard supernatant.
11. Resuspend cells in 1 ml of 10% ice-cold glycerol.
12. Aliquot cells into 1.5 ml sterile microcentrifuge tubes.
13. Cells are ready for electroporation, or flash-freeze cells in liquid nitrogen or dry ice-ethanol bath prior to storing at -80°C. Thaw cells on ice if used later.

E. 18. Preparation of Electrocompetent Cells for Environmental *E. coli*

Reference: (Murphy & Campellone, 2003)

1. Inoculate a single bacterial colony into 10 ml of 1× LB in a sterile 50 ml Erlenmeyer flask containing the appropriate antibiotic, and incubate the culture overnight at 37°C (or 30°C for strains carrying a temperature sensitive plasmid, e.g., pKD46) with shaking (200 rpm).
2. Subculture 1:100 into 50 ml of 1× LB in a sterile 250 ml Erlenmeyer flask, and incubate cells at 37°C (or 30°C for strains carrying a temperature sensitive plasmid) with shaking (200 rpm). If strain contains pKD46 for gene deletion procedure, add 20 mM arabinose final concentration to induce plasmid expression.
3. At an OD_{600} of 0.5, place culture in 42°C incubator for 15 min, swirling flask every 3 min.
4. Place flask in ice bath for 10 min, swirling every 2 min.
5. Aliquot 2 × 20 ml of culture into two sterile 50 ml polypropylene tubes. Centrifuge tubes at 4,000 × *g* for 10 min, and pour off supernatant.
6. Resuspend pellet in 1 ml of ice-cold 20% glycerol - 1 mM MOPS (unbuffered), and transfer suspension to a sterile 1.5 ml-microcentrifuge tube.
7. Centrifuge microcentrifuge tube 11,000 × g for 1 min. Pour off supernatant.
8. Resuspend pellet in 1 ml of ice-cold 20% glycerol - 1 mM MOPS (unbuffered).
9. Centrifuge microcentrifuge tube 11,000 × g for 1 min. Pour off supernatant.
10. Resuspend pellet in 1 ml of ice-cold 20% glycerol - 1 mM MOPS (unbuffered).
11. Centrifuge microcentrifuge tube 11,000 × g for 1 min. Pour off supernatant.
12. Resuspend pellet in 90 µl of ice-cold 20% glycerol - 1 mM MOPS (unbuffered).
13. Cells are ready for electroporation, or flash-freeze cells in liquid nitrogen or dry ice-ethanol bath prior to storing at -80°C. Thaw cells on ice if used later.

E. 19. Transformation by Electroporation

Reference: (Murphy & Campellone, 2003) and (Taketo, 1988)

1. Prechill 0.2 cm electroporation cuvette (Bio-Rad *E. coli* Pulser cuvette, 0.2 cm electrode gap) on ice.
2. Add 50 µl of electrocompetent cells and 1-5 µl of DNA (0.1 - 0.5 µg DNA) to the electroporation cuvette, and tap gently on the tabletop to mix. Remember to include a positive control to test the competency of the cells (e.g., pUC19) and a negative control to test for contaminants (no DNA added).
3. Wipe the cuvette sides with Kim-wipes to dry, and place in electroporator.
4. Pulse cuvette 2.25 kV (or use *E. coli* preset "Ec2" on Bio-Rad MicroPulser, 2.5 kV). If using Bio-Rad Gene Pulser II, set machine to 2.25 kV, 25 µF, and 200 Ω, and hold down two red pulser buttons simultaneously until a beep is heard.
5. Immediately take out cuvette, and add 0.6 ml of 1× LB.
6. Pour contents of cuvette into 2.4 ml of 1× LB in sterile test tubes, and incubate at 37^{o}C (30^{o}C for strains carrying temperature sensitive plasmids) on rotating wheel for 1.5 h or for approximately two generations of growth.
7. Transfer 1.5 ml of cells to sterile 1.5 ml microcentrifuge tubes, and centrifuge cells at 11,000 × *g* for 1 min. Discard supernatant.
8. Add remainder 1.5 ml of cells to pellet, and centrifuge cells at 11,000 × *g* for 1 min. Discard supernatant, except for ~100 µl.
9. Resuspend pellet in residual supernatant, and plate 50 µl of cells onto plates containing the selective antibiotic. Incubate plates at 37^{o}C (30^{o}C for strains carrying temperature sensitive plasmids).
10. Leave the remainder electroporated cells on bench overnight. Plate remainder of cells the following day if no transformants were detected in the first plating.

E. 20. Biofilm in 96-Well Microtitre Plate

Reference: (Merritt *et al.*, 2005)

1. Prepare bacterial cultures by either (a) or (b):
    a) **For fewer strains (< 20)**, inoculate 5 ml of 1× LB with single bacterial colonies. Incubate overnight at 37^{o}C with shaking (200 rpm). Subculture 1:100 into fresh 1× LB, and aliquot 100 µl into sterile, untreated microtitre plate wells (Corning 96-well flat bottom polystyrene plates). Ensure that some wells have uninoculated media to serve as blanks.
    b) **For a large screen (> 20 strains)**, inoculate 100 µl 1× LB in microtitre plate wells with single colonies. Incubate overnight at 37^{o}C. Using a 48-prong inoculating manifold, replica plate cultures into fresh 100 µl of 1× LB in sterile, untreated microtitre plate wells (Corning 96-well flat bottom polystyrene plates). Ensure that some wells have uninoculated media to serve as blanks.
2. Incubate cells at room temperature (~23^{o}C) for 48 h without agitation to allow formation of biofilm.
3. After incubation, empty out planktonic bacteria and media into waste tub by brisk shaking.
4. Rinse out plates by immersing plate 3× in water and shaking out water each time into waste tub.
5. Add 125 µl of 0.1% crystal violet to each well, and let sit for 10 min at room temperature.
6. Shake out and rinse plates 3× with water or until no dye comes from the wells.
7. Invert plates onto paper towels, and air-dry the wells.
8. Add 200 µl of acetone - ethanol (20% acetone - 80% ethanol) to each of the wells, and let sit for 10-15 min at room temperature.
9. Pipette contents of wells briefly to mix, and aliquot 125 µl of solution to new microtitre plates.
10. Obtain readings at OD_{600} using a microtitre plate reader.

E. 21. References for Standard Operating Procedures

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