DELETION ANALYSIS OF THE *SINORHIZOBIUM MELILOTI* GENOME

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By

BRANISLAVA MILUNOVIC, B.Sc.,M.Sc.

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AUTHOR: Branislava Milunovic, B.Sc., M.Sc. (University of Western Ontario)

SUPERVISOR: Professor Turlough M. Finan

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PREFACE

As chapters 3, 4, and 5 were prepared as manuscripts, some of the work described was done by others. I have acted as the primary author in each case and with input from my supervisor and collegues in the laboratory. I wrote the material for manuscripts that will be submitted for publication. In Chapter 4 and 5 Tim Soh confirmed deletion structures by PCR as part of his summer project that I designed and principally supervised. In Chapter 4 George diCenzo built certain genetic constructs and tested these in plant assays as part of his summer project that I designed and principally supervised. I repeated experiments that yielded useful data and the data that appears in this thesis is my own.

ABSTRACT

The *Sinorhizobium meliloti* genome consists of 6204 predicted protein-coding regions of which approximately 2000 are proteins of unknown function (PUFs). To identify functions of *S. meliloti* PUFs, we employed the FRT/Flp recombination system to delete large gene clusters and then screened for phenotypes. Large-scale deletions have been mainly used to define minimal gene sets that contain only those genes that are essential and sufficient to sustain a functioning cell. To adapt FRT/Flp for use in *S. meliloti*, we used an already constructed pTH1522-derived integration gene library of the *S. meliloti* genome (pTH1522 carries a single FRT site). A second FRT site was inserted at defined locations in the genome through integration of a second plasmid (pTH1937) that also carries a single FRT site. Here we outline how this Flp/FRT system was used to delete defined regions and hence generate multiple gene knock-out mutants. This system was used to delete 32 and 56 defined regions from the 1340 Kb pSymA and 1678 Kb pSymB megaplasmid, respectively. The structures of the resulting megaplasmid deletion mutants were confirmed by PCR analysis. Carbohydrate and nitrogen utilization phenotypes were associated with the deletion of specific regions. Deleting large, regions of the genome helped us to identify phenotypes such as inability to grow on minimal media with fucose, maltotriose, maltitol, trehalose, palatinose, lactulose and galactosamine as sole carbon source. For several FRT-flanked regions, few or no recombinants were recovered which suggested the presence of essential genes. Through this strategy, two essential genes *tRNA*^{arg} and *engA* located on the pSymB and three

toxin/antitoxin-like systems, *sma0471*/*sma0473*, *sma2105* and *sma2230*/*sma2231* on pSymA megaplasmid were identified.

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ABBREVIATIONS USED

bp	base pairs
CFU	colony forming units
DMSO	dimethyl sulfoxide
dNTPs	deoxyribonucleotide triphosphate dATP, dCTP, dGTP and dTTP
DTT	dithiothreitol
EDTA	ethylenedinitrilotetraacetic acid
IPTG	isopropyl – ß – D - thiogalactopyranosid
LB	Luria broth
LBmc	Luria broth supplemented with 2.5 mM $MgSO_4$ and 2.5 mM $CaCl_2$
MCS	multiple cloning site
MOI	multiplicity of infection
MOPS	3-(N-morpholino) propanesulfonic acid
mRNA	messenger RNA
nt	nucleotides
OD	optical density
ORF	open reading frame
oriT	origin of transfer
PCR	polymerase chain reaction
PFU	plaque forming units
PSK	post segregational killing
rpm	revolutions per minute
TA	toxin antitoxin
tRNA	transfer RNA
X – gal	5 - Bromo – 4 – chloro – 3 – indolyl – β – D – galactopyranoside