DELETION ANALYSIS OF THE *SINORHIZOBIUM MELILOTI* GENOME
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By

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TITLE: Deletion Analysis of the Sinorhizobium meliloti genome

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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 colleagues 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.
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 – β – D - thiogalactopyranosid
LB  Luria broth
LBmc  Luria broth supplemented with 2.5 mM MgSO₄ and 2.5 mM CaCl₂
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