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Evolutionary and molecular origins of a phenotypic switch in *Pseudomonas fluorescens* SBW25

A thesis submitted in partial fulfilment of the requirements for the degree of Ph.D. in Evolutionary Genetics at Massey University, Auckland, New Zealand.

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Survival in the face of unpredictable environments is a challenge faced by all organisms. One solution is the evolution of mechanisms that cause stochastic switching between phenotypic states. Despite the wide range of switching strategies found in nature, their evolutionary origins and adaptive significance remain poorly understood. Recently in the Rainey laboratory, a long-term evolution experiment performed with populations of the bacterium *Pseudomonas fluorescens* SBW25 saw the *de novo* evolution of a phenotypic switching strategy. This provided an unprecedented opportunity to gain insight into the evolution and maintenance of switching strategies.

The derived ‘switcher’ genotype was detected through colony level phenotypic dimorphism. Further microscopic examination revealed the cellular basis of phenotypic switching as the bistable (ON/OFF) expression of a capsule. Transposon mutagenesis demonstrated that the structural basis of the capsule was a colanic acid-like polymer encoded by the Pflu3656-wzb locus. Subsequently, whole genome re-sequencing enabled elucidation of the series of mutational events underlying the evolution of capsule bistability: nine mutations were identified in the switcher. Present in both forms of the switcher, the final mutation – a point mutation in a central metabolic pathway – was shown to be the sole mechanistic cause of capsule switching; it ‘set the stage’ for a series of molecular events directly responsible for bistability.

Two models were proposed to explain capsule switching at the molecular level: the genetic amplification-reduction model, and the epigenetic feedback model. Collective results of biochemical and genetic assays proved consistent with the epigenetic model, whereby a decrease in flux through the pyrimidine biosynthetic pathway activates an already-present feedback loop. Subsequent analysis of a second switcher (evolved independently of and in parallel with the first) revealed a radically different genetic route leading to phenotypically and mechanistically similar capsule switching.
In addition to providing the first empirical insight into the evolutionary bases of switching strategies, the work presented in this thesis demonstrates the power of natural selection – operating on even the simplest of organisms – to forge adaptive solutions to evolutionary challenges; in a single evolutionary step, selection took advantage of inherent intracellular stochasticity to generate an extraordinarily flexible phenotype.
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<th>Meaning</th>
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<tbody>
<tr>
<td>ACP</td>
<td>Acetylated cellulosic polymer</td>
</tr>
<tr>
<td>AP-PCR</td>
<td>Arbitrary primed-polymerase chain reaction</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic local alignment search tool</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>c-di-GMP</td>
<td>Cyclic-dimeric-guanosine monophosphate</td>
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<tr>
<td>CP</td>
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<td>CPSase</td>
<td>Carbamoyl phosphate synthetase</td>
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<td>dNTP</td>
<td>dinucleotide triphosphate</td>
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<td>DUF</td>
<td>Domain of unknown function</td>
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<tr>
<td>g</td>
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<tr>
<td>GDP/GTP</td>
<td>Guanosine di-/tri- phosphate</td>
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<td>GDP-L-fucose</td>
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<td>Gm</td>
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<tr>
<td>HK</td>
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<td>Km</td>
<td>Kanamycin</td>
</tr>
<tr>
<td>LB</td>
<td>Lysogeny broth</td>
</tr>
<tr>
<td>M-W-W test</td>
<td>Mann-Whitney-Wilcoxon test</td>
</tr>
<tr>
<td>NF</td>
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<tr>
<td>OD</td>
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<tr>
<td>PDE</td>
<td>Phosphodiesterase</td>
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<tr>
<td>REE</td>
<td>Reverse evolution experiment</td>
</tr>
<tr>
<td>RR(r)</td>
<td>Response regulator (receiver)</td>
</tr>
<tr>
<td>SM</td>
<td>Smooth morph</td>
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<td>Tc</td>
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<td>TCSTP</td>
<td>Two-component signal transduction pathway</td>
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<td>X-gal</td>
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