Introduction

When studying bacteria, it is important to know how each species grows and responds to certain conditions that can be found in their natural environment. These conditions may include nutrient starvation, oxygen limitation, hyperosmolality, acidic or basic conditions and metal toxicity. At Rhizobium can exist in two states, as a free-living saprophyte in the soil and in a symbiotic relationship with leguminous plants. The latter involves specific molecular signal exchange between the legume and the free-living bacteria, ending with Rhizobium forming root nodules on the plant. How Rhizobium respond to environmental stress may effect the way it nodulates with legumes and is important to characterize.

Results

Various different stresses were tested, each one being commonly found in the natural soil environment of Rhizobium. They induced hypersensitivity (100 mM sucrose & 100 mM NaCl), acidity (pH 5.75), metal toxicity (100 µM CuCl2, 100 µM ZnCl2, 100 µM MnCl2), oxygen limitation, hyperosmolarity, acid or basic conditions.

General stress responses which are normally controlled by a single, or a few master regulators, provide cross-protection against a wide variety of environmental cues, regardless of the initial stimulant. This response is effective in allowing the cell to survive, but it may not be enough to let the cell grow under the stress conditions.

Under prolonged stress conditions cells employ specific stress responses, which utilize highly integrated networks of genetic and physiological adaptation mechanisms. Usually, there is also a clear relationship between cellular response systems and global regulators, adding another level of control to the cell’s emergency stress response and long-term reactions.

How is Stress Detected?

This project utilizes a library (LB) in order to study stress response in R. leguminosarum 3841. The library was created by randomly cutting up 3841’s genomic DNA into 2-3 kb pieces, cloning them into a specifically created plasmid (pOT1) and inserting the plasmid into 3841, ready for screening. Green Fluorescent Protein (GFP) is the reporter for pOT1 and was used to determine the differential fluorescence induction (DFI) strategy of screening the library.

Screening

DIF allows large populations of bacteria to be screened and the level of fluorescence can be accurately monitored to judge their level of expression (fig. 2). GFP has numerous advantages over other reporters, including being able to detect its presence in single cells and requires no fixation.


Current / Future Work

Further characterization of the stress response of each clone is being performed. This will involve testing each clone on different types of stress, such as microaerobic conditions and oxidative shock, which will help to establish the specificity of each stress response. It may also provide an insight into the crossovers of stress response mechanisms that can occur within Rhizobium.

In order to determine the regulation of each identified insert, the plasmids were individually conjugated into a mutant 3841 library. This library contains approximately a million mutants created using random transposon insertion. When screened under identical stress conditions, the colonies should fluoresce as before. Mutants that do not do so are due to the “knock-out” of a regulatory gene in the chromosome that is in cis, that is being isolated and analysed. This should provide a valuable insight into the regulatory mechanisms involved in the adaptation to stress in R. leguminosarum.

The complete genome sequence of R. leguminosarum (currently underway) will contribute to this project considerably. This project may also provide a function to previously unknown or hypothetical proteins.

References


Table 1. A Selection of Clones. Table shows the unique plasmid and strain number given to each clone, the most appropriate hit found with BLAST, a hit (DD) and similarity (Sim) percentage. Obviously the hits may not be exactly what the inserts are, but they do provide a clue to their identity. Also shown is a score (+, ++, ++++) for how much each clone produced under hyperosmotic (Osm) and acidic stress (pH).

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Strain</th>
<th>Hits</th>
<th>Osm%</th>
<th>pH</th>
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<tbody>
<tr>
<td>pRU1504</td>
<td>RU1507</td>
<td>ATP-binding component of the transport system for glycine betaine and proline</td>
<td>61</td>
<td>60</td>
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<tr>
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<td>RU1506</td>
<td>Hypothetical transmembrane protein</td>
<td>67</td>
<td>65</td>
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<tr>
<td>pRU1507</td>
<td>RU1508</td>
<td>Hypothetical protein</td>
<td>66</td>
<td>72</td>
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<tr>
<td>pRU1509</td>
<td>RU1510</td>
<td>Putative ATP-dependent DNA ligase protein</td>
<td>83</td>
<td>88</td>
</tr>
</tbody>
</table>

Fig. 2. (left) The pOT1 Plasmid. Unique restriction sites are shown in red, others are shown in black. The DNA from Rhizobium was inserted at the full site, destroying the site in the presence. Also shown are the open reading frames for gfp, mobilisation, replication and gusA resistance.

Adaptation in Rhizobium to Environmental Stress

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