

# Adaptation in *Rhizobium* to Environmental Stress

Marc Fox, Claire Vernazza & Philip Poole

The University of Reading

School of Animal and Microbial Sciences, PO Box 228, Reading, RG6 6AJ.

e-mail: m.a.fox@reading.ac.uk



## 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, hypersmolarity, acid or basic conditions and metal toxicity. *Rhizobia* 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 *Rhizobia* forming root nodules on the plant. How *Rhizobia* responds to environmental stress may affect the way it nodulates with legumes and so is important to characterise.

Two types of stress responses operate in microorganisms: general stress response and specific stress response.

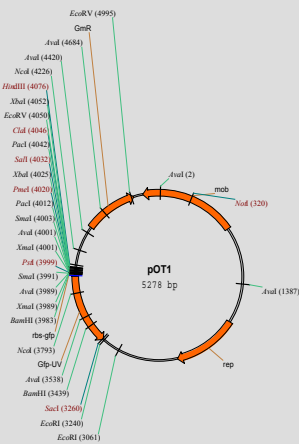
General stress responses, which are normally controlled by a single, or a few master regulators, provide cross-protection against a wide variety of environmental cues, regardless of the initial stimulant. This response is effective in allowing the cell to survive, but it may not be enough to let the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

Under prolonged stress conditions cells employ specific stress responses, which utilise highly integrated networks of genetic and physiological adaptation mechanisms. Usually, there is also a complex 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 utilises a library (LB3) in order to study stress response in *R. leguminosarum* 3841. The library was created by randomly cutting up 3841's genomic DNA into 2-3kb pieces, cloning them into a specifically created plasmid (pOT1) and inserting the plasmids into 3841, ready for screening. Green Fluorescence Protein (GFP) is the reporter for pOT1 and uses the differential fluorescence induction (DFI) strategy of identifying genes expressed by bacteria. LB3 was further selected so that it contained only clones that expressed no reporter under unstressed conditions. Therefore, any stress activated genes present in the plasmids would produce GFP, allowing them to be easily identified and isolated.

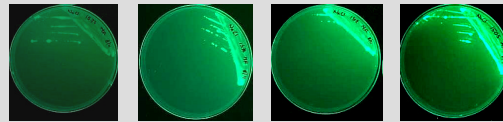
**Fig. 1, (right) The pOT1 Plasmid.** Unique restriction sites are shown in red, others are shown in black. The DNA from *Rhizobia* was inserted at the *SalI* site, destroying the site in the process. Also shown are the open reading frames for *gfpUV*, mobilisation, replication and gentamycin resistance.



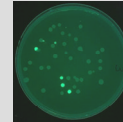
## Screening

DFI 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 it requires no co-factors.

**Fig. 2, (below) GFP Expression.** Expression of GFP from colonies, containing pOT1 plasmids with different promoters, screened under a UV transilluminator at approximately 390nm. Expression ranges from none to low to medium to high.



Around 10,000 clones from LB3 were screened on minimal media with a stress. GFP producing colonies were identified and isolated. Isolated colonies were then confirmed as being only stress induced. Liquid cultures were also made so fluorescence readings could be recorded.



**Fig. 3, (left) Screened Plate.** An example of a plate examined under UV. As can easily be seen, four colonies have high GFP expression, two have some, the rest none. This indicates six clones each containing an insert that responds to stress.

## Results

Various different stresses were tested, each one being commonly found in the natural soil environment of *Rhizobia*. They induced hypersmolarity (100mM sucrose & 100mM NaCl), acidity (pH 5.75), metal toxicity (100µM ZnCl<sub>2</sub>, 100µM AlCl<sub>3</sub> & 30µM CuCl<sub>2</sub>), nutrient starvation (no magnesium), root exudate and microaerobic conditions.

To date thirty clones have been isolated as containing inserts that activate under hypersmolarity and/or acidic stress, each producing GFP to varying degrees. These clones have been sequenced and compared by BLAST to search for homologues (table 1).

Plasmid	Strain	Hits	ID%	Sim%	Osm	pH
pRU843	RU1507	ATP-binding component of the transport system for glycine betaine and proline	61	68	+++	-
pRU844	RU1508	Hypothetical transmembrane protein	47	65	++	+++
pRU846	RU1510	Hypothetical protein	66	72	+++	++
pRU850	RU1514	Putative ATP-dependant DNA ligase protein	83	89	+	+++
pRU853	RU1517	FixG	93	94	+	+
pRU856	RU1520	Carboxypeptidase-related protein	39	53	++	+
pRU859	RU1506	ExsI, a putative transcriptional regulator protein	36	58	+	-
pRU860	RU1505	ProV, a glycine betaine/L-proline transport ATP-binding protein	83	89	+++	+
pRU865	RU1527	Two component sensor kinase/response regulator hybrid	55	69	++	+
pRU867	RU1529	Hypothetical outer membrane protein, homologous to NodT	55	66	++	+++
pRU868	RU1530	RmrA, an efflux transport protein	45	58	-	+
pRU869	RU1531	A two component sensor/response regulator hybrid	57	70	-	+
pRU870	RU1532	ABC transporter, substrate binding protein	79	89	++	+

**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, its identity (ID) 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 GFP each produced under hypersmolarity (Osm) and acidic stress (pH).

## Current / Future Work

Further characterisation 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 crossover of stress response mechanisms that can occur within *Rhizobia*.

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 mutagenesis. When screened under identical stress conditions, the colonies should fluoresce as before. Mutants that do not are due to the "knock-out" of a regulatory gene in the chromosome in that mutant. These colonies are 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 enormously. This project may also provide a function to previously unknown or hypothetical proteins.

## References

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