

Adaptation in *Rhizobium* to Environmental Stress



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Introduction

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 *Rhizobia* eliciting the formation of root nodules on the plant. In the soil and rhizosphere *Rhizobium* may be exposed to conditions that include nutrient starvation, oxygen limitation, hyperosmolarity, acid or basic conditions and metal toxicity. How *Rhizobia* responds to environmental stress may affect nodulation and nitrogen fixation process. This makes stress response an important factor to characterise.

Screening

This project utilises a library (LB3) in order to study stress response in *R. leguminosarum* 3841. The library was created by restriction digesting 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 present in pOT1. Clones from LB3 were screened on various kinds of minimal media, each designed to induce stressful conditions commonly found in the natural soil environment. GFP producing colonies were then selected (Fig. 1). Stress induced fluorescence was then confirmed and quantitated in liquid culture.



Fig. 1. (above) Stress induction. A plate under osmotic stress examined under UV light. As can be seen, four colonies have high GFP expression, two have some, the rest none.

Results

Thirty clones have been isolated with inserts that activate under stress (predominantly hyperosmosis and acidic stress). A selection of these clone results are displayed in the below table.

Plasmid	Hits	ID%	Sim%	Osm	pH
pRU843	Glycine Betaine/L-Proline transport protein ProV	66	79	+++	-
pRU846	Hypothetical protein	93	96	+++	++
pRU848	Fatty aldehyde dehydrogenase	59	71	++	+
pRU855	Carboxypeptidase-related protein	36	51	++	+
pRU857	MFS transporter	61	73	++	+
pRU861	Two-component response regulator	57	70	+++	++
pRU862	Hypothetical protein	47	59	+++	++
pRU863	ABC transporter, substrate-binding protein	73	84	+++	-
pRU867	Hypothetical outer membrane protein, homologous to NodT	55	66	++	+++
pRU871	RmrA, an efflux transport protein	45	58	-	+
pRU872	Putative transport transmembrane protein	62	73	+++	+++

Table 1. The unique plasmid number given to each clone, the most appropriate hit found using the BLAST software with its identity (ID) and similarity (Sim) percentage. Also shown is a score for how much GFP each produced under hyperosmosis (Osm) and acidic stress (pH).

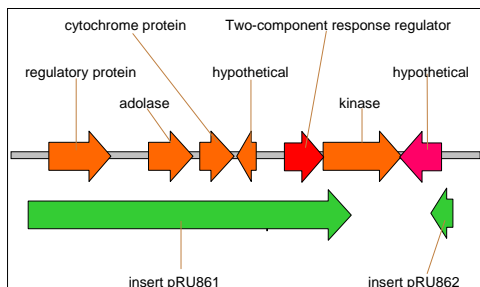


Fig. 2 A genomic region of 3841 containing a two-component response regulator and a hypothetical gene of interest.

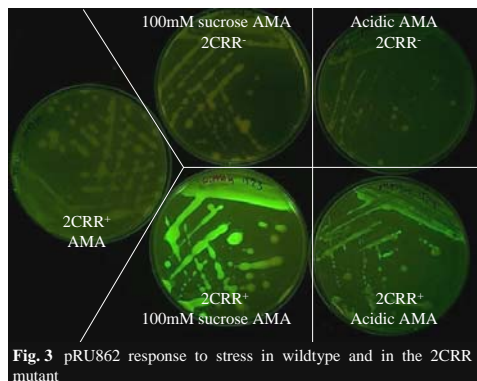


Fig. 3 pRU862 response to stress in wildtype and in the 2CRR mutant

Mutants

Two genes that were targeted were a two-component response regulator (2CRR) and a hypothetical protein (Hypo) which were identified in pRU861 and pRU862 (Fig. 2). When the 2CRR was knocked out, pRU862 was no longer activated under stress conditions (Fig. 3), indicating that it regulated the hypothetical protein.

However, the two genes did not prove to be vital for growth under high osmotic conditions (Table 2) and did not affect the nodulation or fixation of pea plants (Figs. 4a & b).

Future Work

This strategy, along with others employed by the lab, can be used to identify regulator of stress response in 3841. The role, if any, this stress response plays in nodulation and/or nitrogen fixation can then be further investigated.

	AMS		0.3M suc AMS		0.5M suc AMS	
Wildtype	3.81	100%	4.21	110.5%	9.04	275.6%
2CRR	3.95	100%	4.22	106.8%	7.32	203.3%
Hypo	4.60	100%	4.13	89.8%	10.36	271.9%

Table 2. Mean generation times (MGT) of wildtype and the two mutants (hours) under stress and not. Blue figure shows increase in MGT compared to non-stressed conditions.



Fig. 4a



Fig. 4b

Fig. 4. Pea plant phenotypes of uninoculated plants and those inoculated with wildtype, 2CRR⁻ (a) or Hypo⁻ (b) mutants.

References

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