

Amino Acid Transport by *Rhizobium leguminosarum*.



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Introduction.

Symbiotic nitrogen fixation involves the exchange of ammonium for dicarboxylates between the plant and bacteroid. We recently proposed that ammonium assimilation may be shut down in the bacteroid because of amino acid cycling¹. In addition it has long been known that glutamate supplied to isolated bacteroids stimulates secretion of aspartate and alanine^{2,3}. Conclusive evidence for the importance of an amino acid cycle for ammonium assimilation by the plant was demonstrated by mutation of the two principal broad range amino acid transporters; the general amino acid permease (Aap) and the branched chain amino acid permease (Bra)¹. Plants inoculated with strains mutated in either Aap or Bra retained a Fix⁺ phenotype whereas the double mutant, while retaining

nitrogenase activity, was effectively Fix⁻.

Our current model (Fig 1.) now predicts that malate and glutamate are supplied to the bacteroid, allowing ammonium assimilation shutdown, with ammonium and aspartate, returned to the plant.

However, the exact amino acids, that move via the Aap or Bra, remain unidentified. Their identification is difficult because the Aap and Bra are broad range amino acid transporters. Here we report that mutation of the Bra can lead to a narrowing of the solute specificity so that only alanine and leucine are solutes. In addition complementation using the *Pseudomonas* Bra also confirms the role of alanine transport alone being necessary for effective plant growth.

Fig 1. Model For Amino Acid Exchange During Symbiosis.

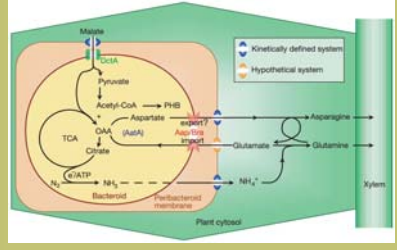


Fig 2. Branched Chain Amino Acid Permease (Bra):-

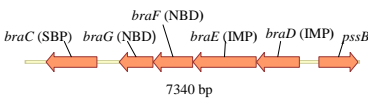


Fig. Bra3C:-

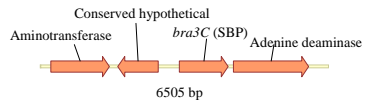


Fig 3. Symbiotic Effect of Double SBP Mutant:-

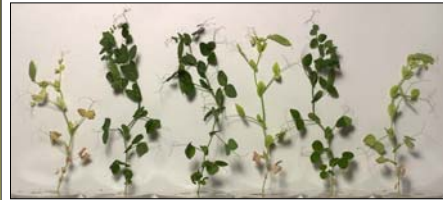


Fig 4. Amino Acid Transport by SBP Mutants:-

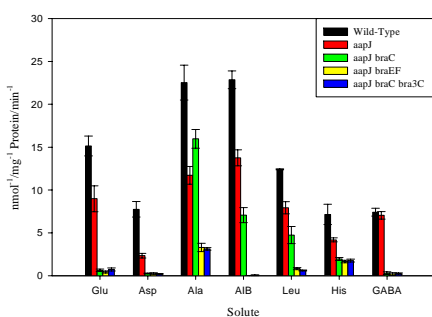
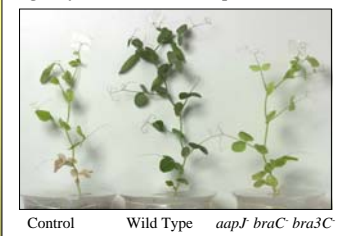


Fig 5. Symbiotic Effect of Triple SBP Mutant:-



Alanine Transport Via a Secondary Bra Solute Binding Protein (SBP) is enough for Fix⁺ Phenotype.

Mutation of the solute binding proteins of both Aap (AapJ) and Bra (BraC) was shown to result in a strain still capable of eliciting a Fix⁺ phenotype on pea plants (Fig 3.). The transport phenotype of this strain (Fig 4.) showed transport of only alanine and leucine at a significant level. This demonstrates that alanine export by the bacteroid is enough for ammonium assimilation by the plant to occur.

It was deduced that another solute binding protein was capable of interaction with the membrane components of the Bra. Sequence analysis of the *R.leguminosarum* genome revealed several BraC homologues but one orphan solute binding protein in particular showed identity to that of the Leu/Ile/Val solute binding proteins from other bacteria and so this was termed Bra3C (Fig 2.). Mutation of this Bra3C, to yield a triple solute binding protein mutant, resulted in a loss of the transport of alanine by free-living cells (Fig. 4) and resulted in a Fix⁻ phenotype on pea plants (Fig 5.).

Complementation of Fix⁺ in Aap Bra Double Mutant using *Pseudomonas* Bra.

The *Pseudomonas aeruginosa* Bra (pa_Bra) has been reported to be specific for aliphatic amino acids, such as alanine⁴. A strategy to complement an Aap and Bra double mutant with the more alanine specific pa_Bra was adopted. The transport phenotype of an *aap bra* strain complemented with the pa_Bra showed high transport rates of both alanine and leucine in free-living cells (Fig 6). The plant phenotype of this strain compared to wild type and its parent showed successful complementation for the Fix⁺ phenotype. The dry weights of the complemented *aap bra* strain showed a 25% reduction compared to that of the plants inoculated with wild type. Bacteroids recovered from plant nodules of the complemented strain showed 100% retention of the plasmid based pa_Bra.

Fig 6. Transport Phenotype of Adapted *Pseudomonas* Bra

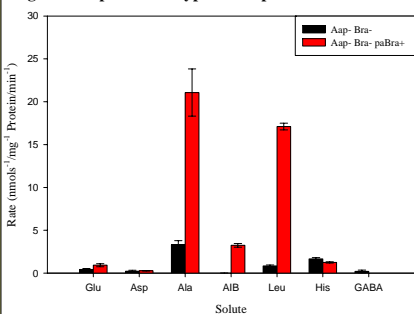
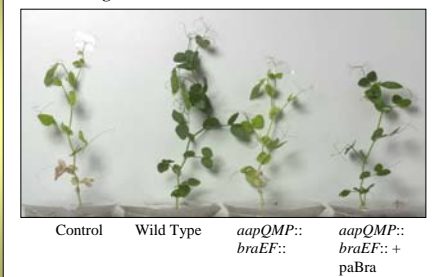


Fig 7. Complementation of Fix⁺ in Aap Bra Double Mutant using *Pseudomonas* Bra.



Conclusions and Future Work.

We have shown that alanine transport alone is sufficient for a Fix⁺ phenotype on pea plants. Since alanine secretion from isolated bacteroids is well documented this suggests that export of alanine via Aap/Bra enables the plant to assimilate nitrogen.

Whilst we have shown that alanine secretion alone is sufficient for normal plant assimilation of nitrogen, we do not know what amino acid is donated to the bacteroid, or even if an amino acid is donated?

The strategies employed above excluded transport of aspartate and glutamate via Aap/Bra so any transaminating donor would have to enter via another transport system. The alternative is that alanine is synthesised *de novo* from ammonium and a 2-keto acid. Double mutation of GOGAT and AldA is being carried out to assess the symbiotic effect of the total removal of bacteroid ammonium assimilation.

References:-

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