Rhizobium leguminosarum has a second general amino acid permease with unusually broad substrate specificity and high similarity to branched chain amino acid transporters (Bra/LIV) of the ABC family

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Introduction

The completed genome sequences of α-proteobacteria, including *S. meliloti*, *M. loti* and *A. tumifaciens* have revealed a multiplicity of genes encoding putative transport proteins, especially ABC transporters involved in uptake of small molecules. One of the best characterised ABC transporters of rhizobia is the general amino acid permease (Aap) of *R. leguminosarum* (ref. 5). This permease belongs to the polar amino acid transporter subfamily (PAAT), one of the two main subfamilies of ABC transporters of amino acids (Ref. 2). Mutation of *aap* leads to a decrease in the uptake rates of a number of amino acids (Fig. 1). However, a significant rate of uptake remains and can be attributed to a second general amino acid permease of *R. leguminosarum* (rlBra; Ref. 3). This permease has high sequence identity to members of the hydrophobic amino acid transporter (HAAT) subfamily (Fig. 2), the second main subfamily of ABC transporters of amino acids. This subfamily includes the branched chain amino acid (Bra) permease of *Pseudomonas aeruginosa* and LIV-I transporter of *E. coli*. It has become apparent that this subfamily also includes transporters of urea and short chain amides. Like Aap, rlBra is unusual as it transports a broad range of amino acids. Indeed, rlBra has the broadest solute specificity of any characterised bacterial amino acid transporter.

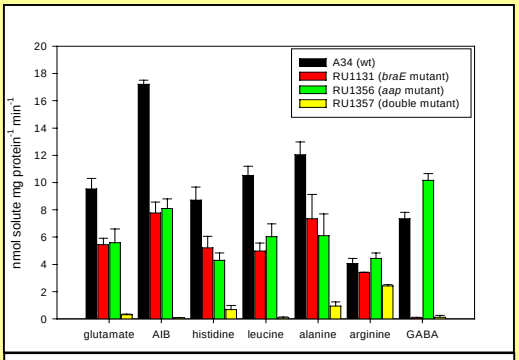

Figure 1. Solute uptake by *R. leguminosarum* strains. Solute uptake was assayed with 25 μM 0.125 μCi (^{14}C or ^{3}H) solute by the rapid filtration method. AIB = α-aminoisobutyric acid.

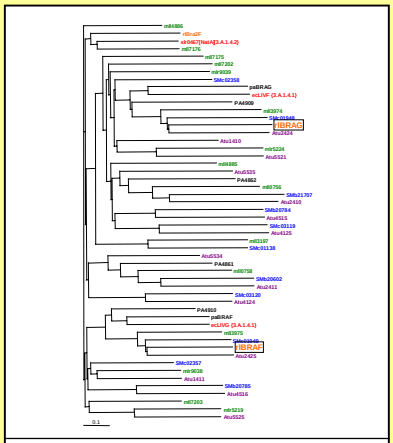

Figure 2. Phylogenetic tree of members of the HAAT family of ABC transporters. Amino acid sequences of the ATP binding proteins of selected members of the HAAT family were aligned using ClustalW. The sequences are identified by the designated protein name or protein number assigned by the sequencing projects. The *R. leguminosarum* permease reported here is highlighted by boxed text. Characterised transporters are shown in red, *S. meliloti* in blue, *M. loti* in green, *A. tumefaciens* in purple and *P. aeruginosa* in black.

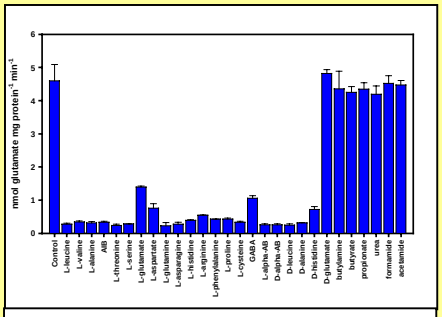

Figure 3. Inhibition of ^{14}C-glutamate uptake in RU1356 (aap$^-$ bra$^+$) by other solutes. Uptake of 25 μM (0.125 μCi) ^{14}C-glutamate was assayed by the rapid filtration method. Competing solutes were added to a final concentration of 0.5 mM. AIB = α-aminoisobutyric acid GABA = γ-aminobutyric acid.

Characteristics of rlBra

- The *bra* operon encodes one periplasmic binding protein (**BraC**), two nucleotide-binding proteins (**BraFG**) and two integral membrane proteins (**BraDE**).
- RlBra transports a broad range of solutes, including acidic and basic polar amino acids (L-glutamate, L-arginine and L-histidine), in addition to neutral amino acids (L-alanine and L-leucine) (Fig.1 & 3). However, rlBra has a much lower affinity for glutamate than for other tested solutes (see Table 1).
- Amino and carboxyl groups are required for transport by rlBra as butylamine, butyrate and propionate do not inhibit glutamate uptake.
- Solutes transported by rlBra do not have to be α-amino acids. Consistent with this, rlBra is the first ABC transporter to be shown to transport γ-aminobutyric acid (GABA) (Fig.1 & 3).
- Transport does not appear to be stereo-specific as D-amino acids cause significant inhibition of L-glutamate uptake (Fig. 3).
- Unlike other solutes tested, L-alanine uptake is not dependent on the solute binding protein rlBraC (data not shown). Therefore, a second, unidentified solute binding protein may interact with the rlBraDEFG membrane complex during L-alanine uptake.
- As with Aap, solute transport by rlBra is bidirectional, but the 10^3-10^4 higher affinity for uptake compared with efflux supports a substantial concentration gradient and provides a net uptake of solutes (Ref 1).

Table 1. Kinetics of solute uptake by the Aap and Bra of *R. leguminosarum*

	Bra		Aap	
	K_m	V_{max} (nmol solute mg protein^{-1} min^{-1})	K_m	V_{max} (nmol solute mg protein^{-1} min^{-1})
L-leucine	205 ± 82 nM	7.15 ± 1.05	515 ± 144 nM	3.17 ± 0.24
L-alanine	173 ± 37 nM	3.51 ± 0.26	509 ± 206 nM	6.22 ± 0.70
AIB	97 ± 56 nM	5.42 ± 1.16	807 ± 383 nM	3.94 ± 0.59
L-histidine	78 ± 29 nM	2.78 ± 0.26	200 ± 110 nM	4.17 ± 0.48
L-glutamate	56 ± 11 μM	17.09 ± 1.58	200 ± 66 nM	4.81 ± 0.52
GABA*	288 ± 121 nM	6.17 ± 0.82	na	na

Cells were grown on glucose/ammonia minimal medium. Kinetics were derived from the mean rates determined for at least two independent cultures. The standard errors were determined from the non-linear regression curve.
* GABA kinetics were determined in the wild type strain (A34; *aap*$^+$ *bra*$^+$), as the Aap has no affect on uptake of this solute.
na = not applicable as Aap does not transport GABA.

Amino acid transport is required for productive nitrogen fixation

Proteomic analysis of peribacteroids confirms Aap and rlBra are both expressed in bacteroids and leak into the peribacteroid space during sample preparation (ref. 4). Although plants inoculated with single *aap* or *bra* mutants are indiscernible from plants inoculated with the wild-type strain, plants inoculated with *aap bra* double mutants are yellow in colour and appear nitrogen starved (Fig. 4). However, the roots of these plants contain nodules with active nitrogenase (as discerned by acetylene reduction). Therefore, not only are *aap* and *bra* expressed in bacteroids, at least one of these ABC transporters must be active for productive nitrogen fixation.

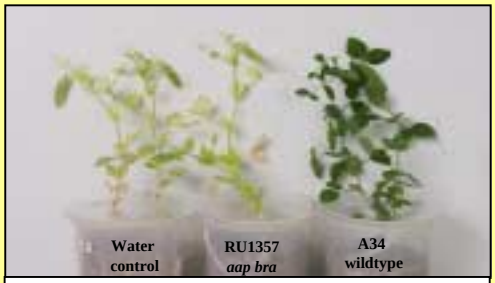

Figure 4. Effect of blocking amino acid uptake in bacteroids on plant symbiotic performance.

References

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