Characterisation of a second ABC transporter of *Rhizobium leguminosarum* with broad specificity for amino acids

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Introduction

The importance of amino acid metabolism by *Rhizobium leguminosarum* has been highlighted by the recent report that alanine is a major product of nitrogen fixation by this bacterium[1]. N_2-fixation by *R. leguminosarum* takes place in symbiosis with leguminous plants. It is not known how the alanine and ammonium produced by N_2-fixation are secreted from the bacteroid and made available to the plant. Also, in order to establish a *Rhizobium*-legume symbiosis, the bacteria must thrive in the soil environment, competing with many other organisms for nutrients. Transporters of key nutrients, such as, amino acids may give a competitive advantage to *Rhizobium* spp., allowing them to better colonize roots.

The general amino acid permease (Aap) of *R. leguminosarum*[2] is a member of the solute binding protein dependent (SBP) ABC transporter subfamily, but is unusual in that it is involved in the uptake of a broad range of amino acids. During studies on the Aap, a second high affinity transporter of amino acids was identified. The properties of this permease are presented here. Although homologous to the LIV-I transporters of *E. coli* and *Pseudomonas aeruginosa*, the solute specificity is broader than reported for these homologues.

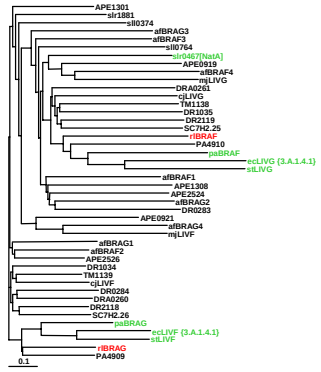

Figure 1. Phylogenetic tree of members of the HAAT family of ABC transporters. Amino acid sequences of the ATP binding proteins of known members of the HAAT family were aligned using ClustalW. The sequences are identified by the designated protein name or protein number assigned by the sequencing projects. The *R. leguminosarum* permease reported here is highlighted in red. Other characterised transporters are shown in green.

Sequence analysis of rlBra

An operon encoding a second ABC transporter of amino acids in *R. leguminosarum* is located in a region upstream of the *pssB* gene, which is involved in the production of exopolysaccharide. Sequencing of this region revealed five ORF products with homology to SBP ABC transporters (the sequence data has been submitted to the EMBL database under accession number AJ272047). These comprised one periplasmic binding protein (**BraC**), two nucleotide-binding proteins (**BraFG**) and two integral membrane proteins (**BraDE**). BLAST searches identified extensive similarity to the branched chain amino acid transporters (LIV-I) of *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*, which transport leucine, isoleucine, valine, alanine and threonine. For example, the *P. aeruginosa* BraCDEFG proteins share 41, 50, 44, 43 and 56 % identity respectively with the *R. leguminosarum* homologues described here. Therefore, this new operon represents the *R. leguminosarum* equivalent of these transporters. The hydrophobic amino acid uptake transporter (HAAT) family (TC number 3.A.1.4) of ABC transporters is expanding as bacterial genomes are completed (see figure 1). However, very few of these transporters have been characterised. The data presented here should caution against assuming the solute specificity from sequence homology.

Table 1. Growth of *R. leguminosarum* strains

	Growth of strains following 7 days incubation at 28°C on AMA containing 10 mM Glutamate
A34 (*aap*$^+$ *bra*$^+$)	+++
RU1356 (*aap*$^-$ *bra*$^+$)	-
RU1131 (*aap*$^+$ *braE*$^-$)	+++
RU1470 (*aap*$^+$ *braC*$^-$)	+++
RU1357 (*aap*$^-$ *braE*$^-$)	-
RU1472 (*aap*$^-$ *braC*$^-$)	-
RU1357 pIJ1427 (*braDEFGC* cosmid)	+++
RU1357 pBIO206 (*braE::TnPhoA* cosmid)	-

Characterisation of rlBra

Data from a number of experiments indicates that rlBra has a broader solute specificity than reported for previously characterised members of the HAAT family.

1. *aap*- strains of *R. leguminosarum* are unable to grow on glutamate as a sole carbon/nitrogen source. Over-expression of rlBra on a cosmid complements this growth defect. However, a chromosomal copy of rlBra is not sufficient for growth (Table 1).

2. Mutation of *bra* by transposon insertion into either *braE* or *braC* decreases the uptake of a range of amino acids, including polar amino acids (glutamate and histidine). Mutation of both *bra* and *aap* decreases the uptake rate of most amino acids tested (i.e. with the exception of arginine) to almost undetectable levels (figure 2).

3. Overexpression of *bra* or *aap* on cosmids in a *aap*$^-$ *bra*$^-$ strain leads to a large increase in uptake rate of all amino acids tested, including arginine. A control cosmid containing a *TnPhoA* insertion in *braE* shows no elevation of transport rate (figure 3).

4. In an *aap*$^-$ strain, uptake of glutamate is inhibited by all amino acids tested. Such competitive inhibition indicates that these amino acids are transported by the same permease (rlBra). However, glutamate and arginine do not inhibit the uptake of leucine (figure 4). This is the result of the lower affinity of rlBra for glutamate than for leucine (Table 2). The same is probably true for arginine but the kinetics are confused by the presence of an uncharacterised high affinity transporter of this solute.

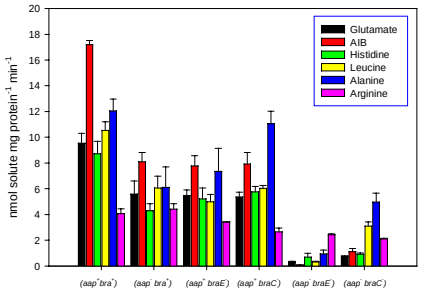

Figure 2. Solute uptake by *R. leguminosarum* strains. Solute uptake was assayed with 25 µM 0.125 µCI solute by the rapid filtration method. The genotype, rather than the strain name, is used for simplicity.

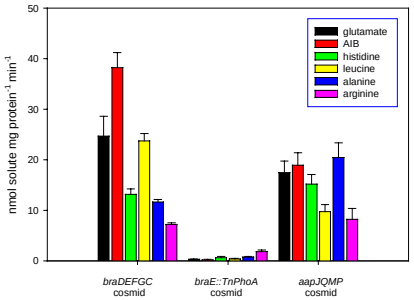

Figure 3. Solute uptake by a *aap*$^-$ *bra*$^-$ *R. leguminosarum* strain containing cosmids. Solute uptake was assayed with 25 µM 0.125 µCI solute by the rapid filtration method. The strains used were RU1357 (*ΔaapJQMP braE::TnphoA*) containing either pIJ1427 (*braDEFGC* cosmid), pBIO206 (*braE::TnPhoA* cosmid) or pRU3024 (*aapJQMP* cosmid).

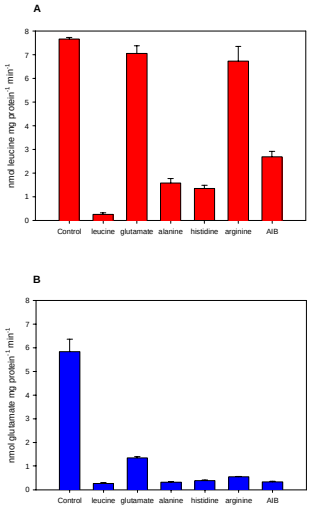

Figure 4. Inhibition of ^{14}C-leucine and ^{14}C-glutamate uptake by other amino acids.
Uptake of 25 µM (0.125 µCi) ^{14}C-leucine (A) and ^{14}C-glutamate (B) uptake was assayed by the rapid filtration method. Competing solutes were added to a final concentration of 0.5 mM.

Table 2. Kinetics of solute uptake by rlBra.

Solute	K_m	V_{max} (nmol mg protein^{-1} min^{-1})
Leucine	205 ± 82 nM	7.15 ± 1.05
Alanine	173 ± 37 nM	3.51 ± 0.26
α-amino isobutyric acid (AIB)	97 ± 56 nM	5.42 ± 1.16
Glutamate	56 ± 11 µM	17.09 ± 1.58
Histidine	78 ± 29 nM	2.78 ± 0.26

References

1. Allaway, D. *et al.* (2000) *Molecular Microbiology* **36** 508-515

2. Walshaw, D.L. & Poole, P.S. (1996) *Molecular Microbiology* **21** 1239-1252