Methylamine and Ammonium Transport Systems in *Rhizobium leguminosarum* MNF3841

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As the sole source of nitrogen, methylamine supported the growth of a range of species of *Rhizobium*. The methylamine assimilatory system was inducible in *R. leguminosarum* MNF3841, whereas the capacity to utilize NH_4^+ as a nitrogen source was constitutive. An uptake system for [^{14}C]methylamine (methylamine permease) was induced by growth of MNF3841 on methylamine or ethylamine. The uptake was sensitive to 2,4-dinitrophenol, azide and carbonyl cyanide *m*-chlorophenylhydrazone. The methylamine permease had a K_m of 0.035 mM, a $V_{\max}$ of 2.2 nmol min $^{-1}$ (mg protein) $^{-1}$ and a K_i for ammonium of 1.5 mM. Most of the [^{14}C]methylamine accumulated by cells was rapidly incorporated into TCA-insoluble materials. An NH_4^+ -sensitive methylamine-accumulating system distinct from the methylamine permease was demonstrated in ammonia-limited cells grown in continuous culture. This system, the ammonium permease, had a K_m of 0.11 mM (for methylamine), a K_i for NH_4^+ of 0.007 mM and a $V_{\max}$ of 2.5 nmol min $^{-1}$ (mg protein) $^{-1}$. Methylamine was accumulated by chemostat-grown, N-limited cells and could exchange with unlabelled methylamine. Treatment with carbonyl cyanide *m*-chlorophenylhydrazone caused efflux of the accumulated methylamine, whereas high concentrations of NH_4^+ did not. Thus *R. leguminosarum* possesses a specific methylamine permease which is quite distinct from the ammonium permease.

INTRODUCTION

The mechanism of ammonia transport is of obvious importance to understanding the physiology of nitrogen fixation by *Rhizobium*, since both isolated bacteroids (Bergersen & Turner, 1967) and cells induced for nitrogenase in the laboratory (O'Gara & Shanmugam, 1976; Tubb, 1976; Bergersen & Turner, 1978) liberate into the medium as $^{15}\text{NH}_3$ most of the $^{15}\text{N}_2$ they fix.

Studies of ammonia transport mechanisms in bacteria have been approached in two major ways: the measurement of intracellular and extracellular ammonia concentrations (e.g. Dilworth & Glenn, 1982), and the use of [^{14}C]methylamine as an analogue for ammonium (Stevenson & Silver, 1977).

In *Azotobacter vinelandii*, measurements of intra- and extracellular ammonia indicated that a 100-fold higher concentration existed inside the cells (Kleiner, 1975), but since cell suspensions were apparently toluenized prior to the measurements of intracellular ammonia, these data must be treated with caution. Similar concentration differences have been reported for *Clostridium pasteurianum* and *Klebsiella pneumoniae* (Kleiner & Fitzke, 1979; Kleiner, 1982). By contrast, in *Rhizobium leguminosarum* grown on either histidine or NH_4^+ , no evidence was found for a gradient in ammonia concentration across the cell membrane (Dilworth & Glenn, 1982).

While uptake of methylamine by eukaryotic cells appears to parallel uptake of NH_4^+ (Kleiner, 1981), the situation in prokaryotes is not as definitive. Since methylamine uptake on an ammonium carrier would be expected to be less efficient than ammonium uptake, the inhibition constant (K_i) for ammonium competition with methylamine would be expected to be lower than

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Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

the Michaelis constant (K_m) for methylamine uptake (Kleiner, 1981). Data which conform to this prediction have been reported for *A. vinelandii* (Barnes & Zimniak, 1981), *C. pasteurianum* (Kleiner & Fitzke, 1981), *K. pneumoniae* (Kleiner, 1982), *Azospirillum* species (Hartmann & Kleiner, 1982) and *Rhodospirillum rubrum* (Alef & Kleiner, 1982). While the ideal situation for measuring methylamine uptake would require that no further metabolism of methylamine occurs, it is clear that for *A. vinelandii* (Barnes & Zimniak, 1981), *C. pasteurianum* (Kleiner & Fitzke, 1981) and *K. pneumoniae* (Kleiner, 1982) methylamine is extensively metabolized to a less polar compound, probably *N*⁵-methylglutamine (γ -glutamylmethylamide). However, methylamine does not serve as a nitrogen source for the growth of any of these bacteria.

In bacteria capable of using methylamine as a source of nitrogen for growth the situation appears to be different. In *Pseudomonas* species MA, growth with methylamine as a sole source of carbon and nitrogen results in the synthesis of a transport system of considerable capacity but high K_m (1 to 3 mM), which is insensitive to ammonium inhibition (Bellion *et al.*, 1980). When the same strain is grown on succinate with methylamine as the sole nitrogen source, a second transport system is induced which has a low K_m (0.016 mM) and relatively low capacity, and which shows weak competitive inhibition ($K_i = 4.8$ mM) by NH_4^+ (Bellion & Weyland, 1982).

In bacteroids of *R. leguminosarum* loaded anaerobically with radioactive methylamine, Laane *et al.* (1980) showed that oxygen was necessary for methylamine export, and inferred that this applied to NH_4^+ movement also. Dilworth & Glenn (1982) found that [¹⁴C]methylamine uptake into *R. leguminosarum* WU235 was an active process that was only slightly inhibited by a considerable molar excess of NH_4^+ , and suggested that uptake of methylamine was not necessarily a good index of ammonium uptake in this strain. In *Rhizobium meliloti* also, ammonium did not significantly inhibit methylamine uptake (Osburne, 1982), and efflux could not be demonstrated in response to ammonium additions (Wiegel & Kleiner, 1982). By contrast, experiments with cowpea *Rhizobium* 32H1 showed that NH_4^+ did inhibit methylamine uptake at very low concentrations, and that counterflow of methylammonium and ammonium ions could occur (Gober & Kashket, 1983). Extensive conversion of methylamine to a compound like *N*⁵-methylglutamine also occurred, but whether this strain could grow on methylamine was not apparent. Methylamine transport was not induced in aerobic cultures using glutamate as the nitrogen source, but was induced in the same medium under the microaerophilic growth conditions needed to induce nitrogenase (Gober & Kashket, 1983). It is unclear how cowpea *Rhizobium* strains grow on low concentrations of NH_4^+ under aerobic conditions (Bergersen & Turner, 1976) if a vital ammonium transport system cannot be synthesized.

It thus appears that where an organism is able to use methylamine as a nitrogen source for growth, methylamine uptake is likely to be via a specific inducible transport system that is unconnected with NH_4^+ transport.

In this paper we present evidence for the existence in *R. leguminosarum* MNF3841 of two permease systems which will transport methylamine. The first is induced only by growth on methylamine (or ethylamine) and we regard it as a methylamine permease. The second is derepressed only under nitrogen limitation (ammonia or nitrate), is very sensitive to inhibition by ammonium ions and appears to be an NH_4^+ permease.

METHODS

Organisms. The rhizobia used in this study are shown in Table 1.

Media. Strains were tested for growth on a solid medium containing the mineral salts used by Brown & Dilworth (1975) with phosphate at 0.3 mM, mannitol (10 mM) and nitrogen sources at 10 mM. *Rhizobium leguminosarum* MNF3841 was grown in batch culture in liquid medium of the same composition supplemented with 40 mM-HEPES (pH 7.2).

Nodulation and preparation of bacteroids. Pea plants (*Pisum sativum* L. cv. Greenfeast) were nodulated by *R. leguminosarum* MNF3841 in pots and bacteroids isolated as described by Glenn *et al.*, (1980): they were used immediately after isolation.

Continuous culture. The medium for continuous culture was similar to that for batch cultures, but contained 20 mM-HEPES, pH 7.2. The carbon source was fructose or sucrose (10 mM). Cells were cultured under NH_4^+ -limitation (0.5 mM- NH_4^+ ; $D = 0.04$ or 0.12 h^{-1}), methylamine limitation (0.5 mM-methylamine; $D = 0.04 \text{ h}^{-1}$),

Table 1. *Rhizobium* strains used

Fast-growing rhizobia	Source
<i>R. leguminosarum</i> WU235	Institute of Agriculture, University of Western Australia, Australia
<i>R. leguminosarum</i> MNF3841	Str ^r derivative of strain 300 of Johnston & Beringer (1975)
<i>R. trifolii</i> TA1	Dr D. L. Chatel, W. Australian Department of Agriculture, Australia
Cowpea <i>Rhizobium</i> NGR234	Dr M. J. Trinick, CSIRO, Canberra, Australia
Slow-growing rhizobia	Source
Cowpea <i>Rhizobium</i> 32H1	Dr J. Burton, Nitragin Co., USA
Cowpea <i>Rhizobium</i> CB756	Dr R. A. Date, CSIRO, Brisbane, Australia
<i>Rhizobium japonicum</i> WU40	Institute of Agriculture, University of Western Australia, Australia
<i>Rhizobium lupini</i> WU8	

phosphate limitation (0.05 mM; $D = 0.12 \text{ h}^{-1}$) or under O_2 limitation ($D = 0.092 \text{ h}^{-1}$). The chemostats had working volumes of 75 ml and were operated at 28 °C.

Uptake experiments. Cells were prepared for uptake measurements as described by Dilworth & Glenn (1982) except that they were centrifuged and washed rather than Millipore-filtered. Competitors or inhibitors were added 60 s prior to the addition of 0.1 mM-[^{14}C]methylamine, and samples were taken at intervals (up to 10 min), filtered and washed with 10 ml minimal salts solution; radioactivity on the filter was measured by liquid scintillation counting (Hudman & Glenn, 1980). [^{14}C]Methylamine hydrochloride (specific activity 43.3 mCi mmol $^{-1}$; 1.6 GBq mmol $^{-1}$) was from New England Nuclear.

Incorporation experiments. Cells of MNF3841 were grown at 28 °C in mannitol-methylamine to a cell density of 0.11 mg dry wt ml $^{-1}$, centrifuged, washed twice and resuspended at a cell density of approximately 0.175 mg dry wt ml $^{-1}$ in minimal salts-mannitol containing 0.4 mM-[^{14}C]methylamine (0.2 μCi). Samples (0.5 ml) were taken at intervals into cold 5% (w/v) TCA containing 0.05% methylamine, stored for 30 min, filtered and washed twice with the same solution.

Growth experiments. Cells of strain MNF3841 were grown on minimal salts medium containing mannitol (10 mM) plus either methylamine or NH_4^+ or nitrate (10 mM) to a cell density of approximately 0.31 mg dry wt ml $^{-1}$, centrifuged, washed with minimal salts solution, and resuspended in a similar medium containing the appropriate nitrogen source at 10 mM.

Analytical methods. Ammonia was measured by the phenol-hypochlorite method of Fawcett & Scott (1960) and methylamine by a modification of the ninhydrin method of Spies (1957), with a heating time of 20 min at 100 °C for colour development. Bacterial protein was measured by the Lowry method with bovine serum albumin as the standard.

RESULTS

Growth of rhizobial strains on methylamine. All fast-growing strains of *Rhizobium* (*R. leguminosarum* WU235 and MNF3841, *Rhizobium trifolii* TA1 and cowpea *Rhizobium* NGR 234) grew on solid media with methylamine as the sole source of nitrogen. Slow-growing strains (*Rhizobium lupini* WU8, *Rhizobium japonicum* WU40 and cowpea *Rhizobium* CB756 and 32H1) also grew, *R. lupini* being the slowest.

Growth of MNF3841 on methylamine as a nitrogen source. Cells grown in mannitol-methylamine to a cell density of approximately 0.23 mg dry wt ml $^{-1}$ and resuspended in the same medium grew immediately with a mean generation time of 10.3 h. Cells grown and resuspended in mannitol- NH_4^+ grew immediately with a generation time of 3.5 h. Methylamine-grown cells transferred to mannitol- NH_4^+ also grew immediately with a low generation time (3.5 h).

Cells grown in mannitol- NH_4^+ and transferred to mannitol-methylamine showed a limited increase in turbidity for 2 h after which growth ceased. After 5 h the cells began to grow with a mean generation time of 9.9 h, a rate virtually identical to that observed previously for cells growing on methylamine as a nitrogen source.

Cells of strain MNF3841 grown on a mannitol-minimal salts medium containing nitrate (10 mM) as the nitrogen source grew with a mean generation time of 4.5 h when resuspended in a similar medium. Nitrate-grown cells resuspended in mannitol- NH_4^+ media grew immediately with a generation time of 3.5 h, providing additional evidence that cells grown on nitrogen sources other than NH_4^+ are able to grow immediately when transferred to it.

Substrate consumption rates. During growth experiments similar to those described, samples of supernatant were taken to measure the utilization rates for NH_4^+ and methylamine. Ammonium- or methylamine-grown cells of MNF3841 consumed NH_4^+ at 2.1 and $1.8 \mu\text{mol h}^{-1} (\text{mg dry wt})^{-1}$, respectively, and there was no change in the rate of utilization with time. Methylamine-grown cells when resuspended in fresh methylamine medium utilized methylamine at $0.5 \mu\text{mol h}^{-1} (\text{mg dry wt})^{-1}$. When ammonium-grown cells were resuspended in methylamine-containing medium they did not utilize any methylamine for 5 h and thereafter consumed it at a rate of $0.6 \mu\text{mol h}^{-1} (\text{mg dry wt})^{-1}$.

These growth data provided clear evidence that the methylamine utilization system in *R. leguminosarum* MNF3841 was inducible and that it was clearly different from the NH_4^+ -consuming system.

The methylamine uptake system. Cells of *R. leguminosarum* MNF3841 were grown on a variety of nitrogen sources, and their rate of [^{14}C]methylamine uptake was examined after they had been centrifuged and washed. Cells grown on methylamine and ethylamine as the sole nitrogen source were able to take up [^{14}C]methylamine [1.5 and $1.09 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$, respectively] but cells grown on nitrate, glutamate or NH_4^+ had only low levels of uptake [0.01 , 0.03 and $0.08 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$, respectively]. These data were consistent with the induction of the methylamine permease before methylamine could be utilized as a nitrogen source of rhizobial growth.

The addition of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP; 0.025 mM) or 2,4-dinitrophenol (0.5 mM) or azide (1 mM) inhibited uptake of [^{14}C]methylamine by more than 95% . The process thus appeared to be active and dependent on an energized membrane, though these conclusions are limited by the possible effects of further metabolism of methylamine.

Kinetic data. The apparent K_m for the methylamine permease was determined on five occasions over the concentration range 0.01 to 0.15 mM and the following values were obtained: Lineweaver-Burk plot, 0.035 mM (SD, 0.010); Hans-Woolf, 0.050 mM (SD, 0.012); and non-linear regression (Duggleby, 1981), 0.03 mM . The V_{max} was $2.2 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$.

The effect of ammonium and amines on methylamine transport. The capacity of cells grown on methylamine as sole N source to accumulate [^{14}C]methylamine in the presence of NH_4^+ and amines was investigated. The accumulation of methylamine by adapted cells of MNF3841 was found to be relatively insensitive to NH_4^+ . Ammonium was a poor competitive inhibitor with a K_i of 1.5 mM . Ethylamine was a more potent inhibitor of methylamine transport with a K_i of 0.5 mM . Propylamine was also a poor inhibitor of methylamine transport with a K_i of 2.6 mM . Glycine appeared to have no significant effect on methylamine uptake even when present in a 100-fold excess. In the presence of 1 mM-NH_4^+ the apparent K_m for methylamine uptake was 0.118 mM .

Methylamine transport in NH_4^+ -grown cells. Ammonium-grown cells of MNF3841 have a low, but measurable, rate of [^{14}C]methylamine transport [$0.08 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$]. To determine if this represented a basal level of the methylamine permease, or if transport involved another, separate uptake system, the K_i values for NH_4^+ , ethylamine and propylamine were determined. The K_i values were 0.55 mM for ethylamine, 1.2 mM for NH_4^+ and 2.6 mM for propylamine, values which are essentially the same as those obtained for methylamine-grown cells. It appeared therefore that the low rate of methylamine transport observed in NH_4^+ -grown cells was due to a basal level of the methylamine permease, not an NH_4^+ permease.

Efflux experiments with the methylamine permease. Cells grown in batch culture on mannitol-methylamine were loaded with [^{14}C]methylamine and then treated with methylamine (25 mM) or NH_4^+ (25 mM) or CCCP (0.02 mM) or azide (1 mM). None of these treatments resulted in a loss of radioactivity from the cells.

Incorporation into TCA-insoluble material was therefore measured as a fraction of the total accumulated label as described in Methods. Samples (0.1 ml) were also filtered to measure the rate of accumulation of label. About 85% of the radioactivity was incorporated into TCA-insoluble material, thus explaining the lack of efflux described earlier.

Chemostat culture studies. Cells from chemostat cultures of MNF3841 grown under a variety of limitations were examined for their capacity to transport [^{14}C]methylamine. Methylamine-limited cells transported methylamine via a system which was relatively insensitive to NH_4^+ (Table 2). The K_i (for NH_4^+) for this system was 1.25 mM and the K_m 0.050 mM (Hans-Woolf), indicative of the methylamine permease described for batch cultures. Phosphate-limited cells and oxygen-limited cells showed only a basal activity of the methylamine permease (Table 2).

Cells growing on 0.5 mM- NH_4^+ were ammonia-limited; the effluent NH_4^+ concentration was 0.016 mM. Increasing the NH_4^+ concentration to 1 mM resulted in a doubling of the cell density from 0.19 to 0.38 mg dry wt ml $^{-1}$. Such cells showed a [^{14}C]methylamine uptake activity which was clearly sensitive to NH_4^+ inhibition (Table 2). Cells from nitrate-limited chemostats also showed NH_4^+ -sensitive [^{14}C]methylamine uptake.

Kinetics of the ammonium permease. [^{14}C]methylamine uptake in NH_4^+ -limited cells of MNF3841 was investigated in more detail – particularly the effect of substrate concentration and the effect of NH_4^+ . The K_m values quoted are the means of four separate determinations and the SDS were less than 0.015 mM. The K_m determined over the range 0.010 to 0.150 mM was 0.117 mM (Lineweaver-Burk) or 0.11 mM (Hans-Woolf) with a $V_{\max}$ of 2.5 nmol min $^{-1}$ (mg protein) $^{-1}$. Over the range 0.005–0.15 mM, NH_4^+ was a powerful competitive inhibitor (K_i 0.007 mM; Fig. 1). When the kinetic characteristics of this system were compared with those for the methylamine permease, it was clear that the apparent K_m values for methylamine varied only two- to threefold, while the NH_4^+ sensitivity of the two systems was vastly different. Because the NH_4^+ -sensitive system found in cells growing under inorganic N limitation has the characteristics predicted for NH_4^+ transport we are terming it a rhizobial ammonium permease.

Table 2. Rates of [^{14}C]methylamine uptake in the presence and absence of 1 mM- NH_4^+ by *R. leguminosarum* MNF3841 grown under various limitations

Limitation	Dilution rate (h $^{-1}$)	Control rate [nmol min $^{-1}$ (mg protein) $^{-1}$]	Rate in presence of 1 mM- NH_4^+ [nmol min $^{-1}$ (mg protein) $^{-1}$]
Methylamine	0.04	1.87	1.13
Nitrate	0.04	0.73	0.052
Ammonia	0.04	0.76	0.078
	0.12	1.03	0.060
Oxygen	0.09	0.02	—
Phosphate	0.12	0.06	—

Fig. 1. Dixon plot showing the effect of NH_4^+ on the uptake of [^{14}C]methylamine by NH_4^+ -limited chemostat cells of *R. leguminosarum* MNF3841. Methylamine concentration was 0.02 mM (○) or 0.1 mM (●).

Fig. 2. The effect of methylamine, CCCP or NH_4^+ on the retention of accumulated $[^{14}\text{C}]$ methylamine by NH_4^+ -limited chemostat-grown cells of *R. leguminosarum* MNF3841. Cells were allowed to take up $[^{14}\text{C}]$ methylamine for 5 min, after which time (a) 25 mM-methylamine, (b) 0.02 mM-CCCP, or (c) 25 mM- NH_4Cl was added to the cells.

Characteristics of the rhizobial ammonium permease. Cells from ammonia-limited chemostats were unable to accumulate $[^{14}\text{C}]$ methylamine when treated with CCCP (0.02 mM), azide (1 mM) or 2,4-dinitrophenol (1 mM). Like the methylamine permease this system was probably active and dependent on an energized membrane.

Efflux of radioactive $[^{14}\text{C}]$ methylamine from NH_4^+ -limited cells. The efflux of $[^{14}\text{C}]$ methylamine from NH_4^+ -limited cells was followed after they were allowed to accumulate label for 5 min and then treated with 25 mM (final concentration) methylamine. Samples taken over the next 25 min showed clear evidence for efflux of label when there was a large external concentration of methylamine (Fig. 2a). The half-time for the exchange was about 25 min. This was clearly different from the methylamine-grown cells which rapidly incorporated most of the radioactivity into TCA-precipitable material. When a similar experiment was carried out on cells accumulating $[^{14}\text{C}]$ methylamine by treating them with CCCP (0.02 mM), a clear loss of label from the cells occurred (Fig. 2b), a result again quite distinct from that with methylamine-grown cells.

It might be considered that NH_4^+ itself should induce an efflux of $[^{14}\text{C}]$ methylamine from cells of *R. leguminosarum* MNF3841. However, there was no NH_4^+ -induced efflux of intracellular $[^{14}\text{C}]$ methylamine (Fig. 2c). This result is in accord with our previous work (Dilworth & Glenn, 1982), which showed that relatively high concentrations of ammonia (at least greater than 2.5 mM) rapidly equilibrate across the rhizobial membrane. Under these conditions, there will be a high concentration (25 mM) of ammonia inside the cells within 1.5 min which will effectively prevent label from leaving.

The data presented provide clear evidence that in laboratory-grown cultures of *R. leguminosarum* there are two separate and distinct permeases capable of transporting methylamine. One of these is the true methylamine permease induced by growth on methylamine; the other has the characteristics of an NH_4^+ permease and is produced only when the cells are grown under inorganic nitrogen limitation.

Methylamine transport in isolated bacteroids. Pea bacteroids accumulated $[^{14}\text{C}]$ methylamine only very slowly [$0.024 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$]. This uptake was relatively insensitive to inhibition by 1 mM- NH_4^+ [$0.017 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$]. Control experiments showed that these bacteroids were able to accumulate $[^{14}\text{C}]$ succinate, but unable to take up fructose, a pattern described previously for this strain (Glenn *et al.*, 1980; Glenn *et al.*, 1984). The rates and ammonia insensitivity of the accumulation of $[^{14}\text{C}]$ methylamine by bacteroids suggest that this material was accumulated by a basal level of the methylamine permease and not via the ammonium permease.

DISCUSSION

The results presented suggest that *R. leguminosarum* MNF3841 resembles *Pseudomonas* species MA (Bellion *et al.*, 1980; Bellion & Weyland, 1982) in its ability to synthesize a methylamine permease with methylamine as a nitrogen source for growth. Thus, the apparent K_m values for methylamine and the degree of inhibition by NH_4^+ are low, and the permeases are induced by growth on alkylamines rather than by NH_4^+ . Induction of the transport system apparently parallels induction of the necessary enzymes for methylamine metabolism, and leads to extensive incorporation of methylamine carbon into cells of MNF3841.

Cells of MNF3841 grown on high (10 mM) concentrations of nitrogen sources other than methylamine also transport methylamine to a limited extent, and for ammonia-grown cells the system responsible has the same properties as that induced by methylamine. Accordingly, under most conditions used for growth of *Rhizobium* in the laboratory, measurement of methylamine transport is likely to represent repressed levels of a methylamine permease relatively insensitive to inhibition by NH_4^+ . Such appears to be the case for *R. meliloti* (Osborne, 1982). If the other enzymes of methylamine utilization are induced to roughly the same extent as the permease, efflux of radioactive methylamine would not be expected, a result noted by Wiegel & Kleiner (1982) for *R. meliloti*.

Since the low level of [^{14}C]methylamine uptake by pea bacteroids has the characteristics of the methylamine permease rather than the ammonium permease, it is probable that the bacteroid environment is non-limiting with respect to nitrogen. It is also probable that active methylamine export by bacteroids (Laane *et al.*, 1980) will involve repressed levels of the methylamine permease and be unreliable as an index of ammonia export.

Growth of MNF3841 in a chemostat on limiting ammonia or nitrate results in a methylamine uptake activity with entirely different kinetic parameters. This system is inhibited by low concentrations of NH_4^+ , as are the methylamine transport activities of *A. vinelandii* (Barnes & Zimniak, 1981), *C. pasteurianum* (Kleiner & Fitzke, 1981), *K. pneumoniae* (Kleiner, 1982), *Azospirillum* spp. (Hartmann & Kleiner, 1982) and *R. rubrum* (Alef & Kleiner, 1982), none of which is able to use methylamine as a source of nitrogen for growth. Unlike *R. leguminosarum*, these other organisms appear to be able to synthesize an NH_4^+ permease under conditions where nitrogen is not limiting. In *R. leguminosarum*, however, nitrogen-excess conditions appear to lead to repressed levels of a methylamine permease rather than an NH_4^+ permease.

The situation in the cowpea *Rhizobium* (*Bradyrhizobium*) 32H1 is somewhat confusing. Methylamine transport in this strain is mediated by a system with a low K_m for methylamine and is inhibited by low concentrations of NH_4^+ (Gober & Kashket, 1983). Methylamine taken up can efflux, though not necessarily as methylamine, and the general picture would seem to be one of an organism unable to use methylamine for growth. However, we find that this strain grows on plates with methylamine as the sole source of nitrogen, albeit slowly. Another unusual finding is that only under microaerophilic conditions does strain 32H1 produce the methylamine-transporting (NH_4^+ permease) system (Gober & Kashket, 1983), yet it can grow under aerobic conditions with NH_4^+ as the nitrogen source (Bergersen & Turner, 1976). The apparent inconsistency may be resolved if 32H1 also produces the NH_4^+ permease system at low nitrogen concentrations, but relies on a diffusive mechanism for ammonia uptake at higher concentrations, as suggested for *R. leguminosarum* by Dilworth & Glenn (1982).

Further study of ammonium transport in fast-growing rhizobia such as *R. leguminosarum* will need to use ammonia- or nitrate-limited chemostats to separate the ammonium permease from the methylamine permease, as well as mutants completely lacking the methylamine permease system to allow the ammonium permease to be studied directly.

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REFERENCES

- ALEF, K. & KLEINER, D. (1982). Evidence for an ammonium transport system in the N_2 -fixing phototrophic bacterium *Rhodospirillum rubrum*. *Archives of Microbiology* **132**, 79–81.
- BARNES, E. M. & ZIMNIAK, P. (1981). Transport of ammonium and methylammonium ions by *Azotobacter vinelandii*. *Journal of Bacteriology* **146**, 512–516.

- BELLION, E. & WEYLAND, L. (1982). Methylamine uptake in *Pseudomonas* species MA: utilization of methylamine as the sole nitrogen source. *Journal of Bacteriology* **149**, 395–398.
- BELLION, E., ALI KHAN, M. Y. & ROMANO, M. J. (1980). Transport of methylamine by *Pseudomonas* sp. MA. *Journal of Bacteriology* **142**, 786–790.
- BERGERSEN, F. J. & TURNER, G. L. (1967). Nitrogen fixation by the bacteroid fraction of breis of soybean root nodules. *Biochimica et biophysica acta* **141**, 507–515.
- BERGERSEN, F. J. & TURNER, G. L. (1976). The role of O_2 limitation in control of nitrogenase in continuous cultures of *Rhizobium* sp. *Biochemical and Biophysical Research Communications* **73**, 524–531.
- BERGERSEN, F. J. & TURNER, G. L. (1978). Activity of nitrogenase and glutamine synthetase in relation to availability of oxygen in continuous cultures of cowpea *Rhizobium* sp. supplied with excess ammonium. *Biochimica et biophysica acta* **538**, 406–416.
- BROWN, C. M. & DILWORTH, M. J. (1975). Ammonia assimilation by *Rhizobium* cultures and bacteroids. *Journal of General Microbiology* **86**, 39–48.
- DILWORTH, M. J. & GLENN, A. R. (1982). Movements of ammonia in *Rhizobium leguminosarum*. *Journal of General Microbiology* **128**, 29–37.
- DUGGLEBY, R. G. (1981). A nonlinear regression programme for small computers. *Analytical Biochemistry* **110**, 9–18.
- FAWCETT, J. K. & SCOTT, J. E. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology* **13**, 156–159.
- GLENN, A. R., POOLE, P. S. & HUDMAN, J. F. (1980). Succinate uptake by free-living and bacteroid forms of *Rhizobium leguminosarum*. *Journal of General Microbiology* **119**, 267–271.
- GLENN, A. R., ARWAS, R., MCKAY, I. A. & DILWORTH, M. J. (1984). Fructose metabolism in wild-type, fructokinase-negative and revertant strains of *Rhizobium leguminosarum*. *Journal of General Microbiology* **130**, 231–237.
- GOBER, J. W. & KASHKET, E. R. (1983). Methylammonium uptake by *Rhizobium* sp. 32H1. *Journal of Bacteriology* **153**, 1196–1201.
- HARTMANN, A. & KLEINER, D. (1982). Ammonium (methylammonium) transport by *Azospirillum* spp. *FEMS Microbiology Letters* **15**, 65–67.
- HUDMAN, J. F. & GLENN, A. R. (1980). Glucose uptake by free-living and bacteroid forms of *Rhizobium leguminosarum*. *Archives of Microbiology* **128**, 72–77.
- JOHNSTON, A. W. B. & BERINGER, J. E. (1975). Identification of the *Rhizobium* strains in pea root nodules using genetic markers. *Journal of General Microbiology* **87**, 343–350.
- KLEINER, D. (1975). Ammonium uptake by nitrogen fixing bacteria. I. *Azotobacter vinelandii*. *Archives of Microbiology* **104**, 163–169.
- KLEINER, D. (1981). The transport of NH_3 and NH_4^+ across biological membranes. *Biochimica et biophysica acta* **639**, 41–52.
- KLEINER, D. (1982). Ammonium (methylammonium) transport by *Klebsiella pneumoniae*. *Biochimica et biophysica acta* **688**, 702–708.
- KLEINER, D. & FITZKE, E. (1979). Evidence for ammonia translocation by *Clostridium pasteurianum*. *Biochemical and Biophysical Research Communications* **86**, 211–217.
- KLEINER, D. & FITZKE, E. (1981). Some properties of a new electrogenic transport system: the ammonium (methylammonium) carrier from *Clostridium pasteurianum*. *Biochimica et biophysica acta* **641**, 138–147.
- LAANE, C., KRONE, W., KONINGS, W. & VEEGER, C. (1980). Short-term effect of ammonium chloride on nitrogen fixation by *Azotobacter vinelandii* and by bacteroids of *Rhizobium leguminosarum*. *European Journal of Biochemistry* **103**, 39–46.
- O'GARA, F. & SHANMUGAM, K. T. (1976). Regulation of nitrogen fixation by rhizobia. *Biochimica et biophysica acta* **437**, 313–321.
- OSBURNE, M. A. (1982). *Rhizobium meliloti* mutants altered in ammonium utilization. *Journal of Bacteriology* **151**, 1633–1636.
- SPIES, J. R. (1957). Colorimetric procedures for amino acids. *Methods in Enzymology* **3**, 467–477.
- STEVENSON, R. & SILVER, S. (1977). Methylammonium uptake by *Escherichia coli*: evidence for a bacterial NH_4^+ transport system. *Biochemical and Biophysical Research Communications* **75**, 1133–1139.
- TUBB, R. S. (1976). Regulation of nitrogen fixation in *Rhizobium* spp. *Applied and Environmental Microbiology* **32**, 483–488.
- WIEGEL, J. & KLEINER, D. (1982). Survey of ammonium (methyl-ammonium) transport by aerobic N_2 -fixing bacteria – the special case of *Rhizobium*. *FEMS Microbiology Letters* **15**, 61–63.