Mutants of *Bacillus stearothermophilus* defective in the uptake and metabolism of acetate

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Mutants unable to utilize acetate as sole source of carbon and energy were isolated from ethyl-methanesulphonate-mutagenized cultures of a prototrophic *Bacillus stearothermophilus* strain. Three groups of mutants had identifiable defects. Thirteen of the mutants lacked one or both enzymes of the inducible anaplerotic glyoxylate shunt, and three mutants were auxotrophic for isoleucine and valine when grown on acetate, in a manner analogous to acetohydroxyacid synthase I mutants of enteric bacteria. One mutant was defective specifically in acetate uptake, providing direct evidence for an acetate transport system in *B. stearothermophilus*. The acetate uptake system was uncoupler-sensitive and had a relatively high K_m (in the range 300–350 μM). *Escherichia coli* was also shown to possess a saturable acetate uptake system, but its K_m was much lower (15 μM).

Introduction

The prototroph *Bacillus stearothermophilus* var. *nondiateticus* (Epstein & Grossowicz, 1969) utilizes a wide variety of compounds, including acetate, as single carbon and energy sources. Acetate is also the major product of overflow metabolism when glucose-grown chemostat cultures are phosphate, sulphur, magnesium or potassium limited. Under potassium limitation, 40% of the input glucose carbon can be converted to acetate (Pennock & Tempest, 1988).

In the work described in this paper, mutants in acetate metabolism were sought with the hope of redirecting the overflow metabolism; in particular, it was intended to isolate acetate kinase and phosphotransacetylase mutants, blocked in the interconversion of acetyl-CoA and acetate.

Fluoroacetate, a toxic analogue of acetate, has been used to select for mutants of *Escherichia coli* and *Salmonella typhimurium* unable to grow on acetate (Brown *et al.*, 1977; Guest, 1979; LeVine *et al.*, 1980). Fluoroacetate is converted intracellularly into fluorocitrate, which inhibits aconitase (Mager *et al.*, 1955,

Glusker, 1971). Mutants resistant to fluoroacetate have been identified as defective in acetate kinase (*ack*), phosphotransacetylase (*pta*) or both; some resistant mutants with normal levels of both these enzymes have also been described (Guest, 1979). Screens for acetate non-utilizers have produced similar genotypes to the above and, in addition, mutants defective in the glyoxylate cycle (Wilson & Maloy, 1987).

This paper describes the results of selection and screening procedures adopted to isolate *B. stearothermophilus* mutants with a functional TCA cycle but defects in the ability to utilize acetate as sole carbon source. One novel mutant type defective in acetate transport was identified, focussing our attention on potential acetate transport systems in bacteria.

Methods

Bacteria. The bacterial strains used are listed in Table 1.

Bacteriological media. *B. stearothermophilus* was routinely grown at 55 °C unless otherwise stated. Generally, the strains were subcultured on nutrient agar plates (Oxoid) and TSY medium (Liao *et al.*, 1986) was used as a rich broth for liquid cultures. Evans' minimal salts medium (Evans *et al.*, 1970) was modified as described by Pennock & Tempest (1988) and was solidified where appropriate with agar to 1.75% (w/v). Sole sources of carbon and energy were added to minimal salts to the following final concentrations: glucose, 5 g l⁻¹; sodium succinate, 60 mM; sodium acetate, sodium malate and sodium lactate, 40 mM.

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Fac^R, fluoroacetate resistant.

Table 1. *Bacterial strains*

Strain	Genotype	Source or reference
<i>B. stearotheophilus</i> var. <i>nondiastaticus</i>	Wild-type	Pennock & Tempest (1988)
AM1032	<i>icl-124</i>	This paper
AM1033	<i>icl-209</i>	
AM1034	<i>icl-210</i>	
AM1035	<i>icl-653</i>	
AM1049	<i>icl-567</i>	
AM1036	<i>mas-526</i>	
AM1037	<i>icl mas-331</i>	
AM1038	<i>icl mas-539</i>	
AM1039	<i>icl mas-585</i>	
AM1040	<i>icl mas-603</i>	
AM1041	<i>icl mas-606</i>	
AM1042	<i>icl mas-613</i>	
AM1043	<i>icl mas-518</i>	
AM1045	<i>ace-333; ilv-requiring</i>	
AM1046	<i>ace-494; ilv-requiring</i>	
AM1047	<i>ace-640; ilv-requiring</i>	
AM1048	<i>ace-271; uptake mutant</i>	
<i>E. coli</i>		Guest (1979)
JRG911	Wild-type	
JRG1061	<i>ack</i>	
JRG1078	<i>ack pta</i>	
JRG1089	<i>pta</i>	

Preparation of spores and mutagenesis. Spores were prepared by spreading 200 µl of a mid-exponential TSY culture of *B. stearotheophilus* onto lactate minimal plates and incubating at 55 °C for 24–36 h. Spores and any remaining vegetative cells were harvested by suspension in 10 ml of sterile distilled water, washed by repeated centrifugation and resuspension in 30 ml of sterile distilled water, and resuspended in sterile distilled water. The suspension was then heated at 96 °C for 10 min, cooled and stored at 4 °C.

Spores were mutagenized according to the method of Ito & Spizizen (1971). The spore preparation was diluted to 10⁸ c.f.u. ml⁻¹ in 0.1 M-sodium phosphate buffer, pH 7.0. Ethyl methanesulphonate (EMS) was added to a final concentration of 450 mM, and the suspension incubated for 1 h at 30 °C, washed three times in sodium phosphate buffer, and stored at 4 °C. Growing cells were recovered from the spores following 5 h incubation in TSY at 55 °C; the resulting cultures were either plated immediately or stored at –20 °C in the presence of 15% (v/v) glycerol.

Vegetative cells were also mutagenized: cells grown to mid-exponential phase at 55 °C in TSY were equilibrated at 42 °C, EMS was added to 750 mM and the cells incubated for 1 h at 42 °C before washing and plating.

Preparation of extracts and enzyme assays. *B. stearotheophilus* cells (25 ml) were grown in minimal media, with the sole carbon source indicated, to an OD₆₀₀ of 0.7. Cells grown on succinate and then induced in the presence of acetate were grown in succinate minimal medium to an OD₆₀₀ of 0.7, harvested, resuspended in prewarmed acetate minimal medium and incubated for a further hour. Cells (10 ml) were then harvested, washed in 50 mM-potassium phosphate buffer, pH 7.0, and resuspended in 1 ml of the same buffer. The cells were then sonicated and the extracts centrifuged at 12000 r.p.m. for 5 min at 4 °C; the supernatants were retained on ice and assayed immediately. Assays on extracts of *B. stearotheophilus* and *E. coli* cells were done at 48 °C and 37 °C respectively. Acetate kinase was measured according to the method of Nishimura & Griffith (1981), by coupling the formation of

ATP with hexokinase and glucose-6-phosphate dehydrogenase: the formation of NADPH was measured at 340 nm. Phosphotransacetylase was assayed according to the method of Wofford *et al.* (1986), measuring the appearance of the thioester bond of acetyl-CoA at 233 nm. Isocitrate lyase was assayed in the forward direction according to Reeves *et al.* (1971), by measuring the formation of glyoxylic acid phenylhydrazone at 324 nm. The malate synthase assay of Reeves *et al.* (1971) was modified to measure the formation of free CoA. 5,5'-Dithio(2-nitrobenzoic acid) was substituted for the 20 mM-ATP, and 20 mM-pyrophosphate buffer, pH 8.0, was used instead of 1 M-Tris/HCl, pH 8.0. The increase of absorbance was measured at 412 nm. Soluble protein was measured according to the method of Bradford (1976), using bovine serum albumin as a standard.

Acetate uptake studies. Cells (25 ml) were grown in glucose plus acetate minimal medium to an OD₆₀₀ of 0.7. They were transferred to prewarmed centrifuge tubes and pelleted briefly in a bench centrifuge at 37 °C. The pellets were then resuspended in prewarmed Evans' salts (55 °C), centrifuged at 37 °C, resuspended in 300 µl of minimal salts and maintained at 55 °C. To measure acetate uptake, 100 µl of the cell suspension was added to 2 ml of prewarmed Evans' salts and the cells were aerated at 50 °C for 7 min. [2-¹⁴C]Acetate [20 µl; 38 mM, 20 µCi ml⁻¹ (740 kBq ml⁻¹)] was added, and then 100 µl samples were removed at 10 s intervals and rapidly filtered through nitrocellulose discs, under pressure. The discs were washed with 10 ml of buffer (50 mM-potassium phosphate buffer, pH 7.0, 10 mM-sodium acetate) to wash off unincorporated radiolabelled acetate. These discs were then dissolved in scintillation fluid and the radioactivity measured in a Beckman LS1801 liquid scintillation counter. To determine cell protein, 25 µl of cells was added to 4 ml of water, 20 µg of lysozyme was added and the mixture incubated at 37 °C for 20 min. A 100 µl sample was removed for protein estimation.

Reagents. The bacteriological media were obtained from Oxoid and Difco. All chemicals used were of analytical reagent grade (Sigma or BDH) except ADP (Boehringer). The nitrocellulose discs (Millipore) had a pore size of 0.45 µm. Scintillation fluid was Cocktail T 'Scintran' from BDH. [2-¹⁴C]Acetate and L-[1,4,5-³H]leucine were obtained from Amersham.

Results

Mutagenesis of B. stearotheophilus

Incubation of *B. stearotheophilus* spores in 450 mM-EMS at 30 °C for 1 h resulted in about 50% kill, with 10% of the survivors being auxotrophs as estimated by replica plating on minimal medium. *B. stearotheophilus* spores are more susceptible to the lethal and mutagenic effects of EMS than are spores of *B. subtilis* 168 (Ito & Spizizen, 1971) or *B. cereus* NCIB 8122 (Necasek *et al.*, 1966). Incubation of *B. stearotheophilus* vegetative cells in 75 mM-EMS for 1 h at 42 °C gave approximately 99% kill, and about 6% of the survivors were auxotrophs. Prolonged incubation in EMS of either spores or vegetative cells ultimately results in the survival of only EMS-resistant mutants (data not shown).

Isolation of acetate-negative mutants

Two general approaches involving selection and screening, respectively, were adopted. Fluoroacetate-resistant

(Fac^R) mutants were selected on minimal lactate medium containing 125–175 mM-fluoroacetate (the minimum selective concentration was 100 mM, compared with the 10–50 mM range reported for *E. coli* selection by Brown *et al.*, 1977). Resistant colonies (1 per 10⁵ cells) were then purified on glucose minimal medium and transferred to plates containing acetate, lactate, succinate or glucose as sole carbon sources. TCA cycle mutants could be excluded by confirming the ability of strains to grow on glucose, lactate and succinate as carbon sources.

Mutants that failed to grow on acetate but that could grow on all the other carbon sources (Ace⁻) represented 5–10% of the possible Fac^R colonies tested. Mutants in the 100 and 300 series came from the same mutagenized spore preparation, and those in the 200 and 400 series were derived from a second, independently mutagenized, spore population.

In the second approach, colonies derived from mutagenized spores or cells were replica plated from TSY, minimal succinate or minimal lactate media to minimal acetate; potential Ace⁻ colonies were then purified on minimal glucose and rechecked on the various carbon sources to yield the 500 and 600 series mutants, respectively.

Glyoxylate pathway mutants

Table 2 shows the activities of isocitrate lyase and malate synthase in wild-type *B. stearothermophilus* grown on different carbon sources. The enzymes of the glyoxylate pathway are present at very low levels in succinate-grown cells, but are high in acetate-grown cells, as would be expected. The presence of both carbon sources led to intermediate expression levels, considerably lower than

in acetate-grown cells. Because Ace⁻ mutants could not be cultured in these conditions, mutant and wild-type cells grown in succinate medium were then induced in acetate medium for 1 h before assay. The wild-type showed at least as high an activity for acetate kinase and about one-third of the activity of malate synthase compared with the acetate-grown strain. Twenty-four mutants showing Ace⁻ phenotypes from all the isolation procedures were tested for isocitrate lyase and malate synthase activity. Thirteen of these mutants had decreased activities in one or both of the glyoxylate pathway enzymes (Table 2). The mutants of class 1 were defined as isocitrate lyase (*icl*) mutants; class 1b represents a leaky mutant with some residual activity. One mutant was defective only in malate synthase (*mas*) activity (class 2). The largest group, class 3, consisted of mutants defective in both activities (*icl mas*). One mutant retained some activity of both enzymes. Regulatory, polar or deletion mutations could be responsible for the phenotype of class 3 mutants; the relative infrequency of simple malate synthase mutants may suggest that mutations in this gene frequently have polar effects, and that the *icl* gene lies downstream of *mas* in an operon analogous to the situation in *E. coli* and *S. typhimurium*. On the basis of these results, over half the Ace⁻ mutants isolated were defective in the glyoxylate pathway.

Screen for acetate kinase or phosphotransacetylase mutants

Extracts of *B. stearothermophilus* cells grown on glucose and then for 1 h in acetate contain levels of acetate kinase [2 µmol min⁻¹ (mg protein)⁻¹] and phosphotransacetylase [6 µmol min⁻¹ (mg protein)⁻¹] comparable to

Table 2. Glyoxylate pathway enzyme activities in *B. stearothermophilus*

Results are means of at least two experiments.

		Enzyme specific activity		Genotype/phenotype
		Isocitrate lyase	Malate synthase	
Carbon source		nmol min ⁻¹	(mg protein) ⁻¹	
Succinate + acetate		0.16	23	} Wild-type
Succinate		0.026	8	
Acetate		0.42	210	
Succinate then acetate		0.71	62	
Class	Mutant allele	Percentage of wild-type*		
1a	124, 209, 210, 653	<0.5	75–100	<i>icl</i>
1b	567	6.4	95	<i>icl</i>
2	526	100	<2.0	<i>mas</i>
3a	331, 539, 585, 603, 606, 613	<1.4	<2.0	<i>icl mas</i>
3b	518	12	<9.0	<i>icl mas</i>

* All mutants were grown on succinate then acetate as above.

those of *E. coli* JRG911 grown under similar conditions [$3 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$ and $8 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$, respectively]. To confirm the validity of the assay procedure, *E. coli* acetate kinase (*ack*) and phosphotransacetylase (*pta*) mutants JRG1061, 1078 and 1089 (Guest, 1979) were analysed; the estimated levels of phosphotransacetylase in *pta* mutants was $<2\%$ that of wild-type; residual activities of acetate kinase in *ack* mutants, however, were 30% of wild-type.

Thirty-eight *B. stearotherophilus* mutants derived from the various isolation procedures were assayed; most of those chosen for study could grow on both succinate and lactate, although several that did not were also screened in case the phenotypes of *ack* or *pta* mutants might differ from the predicted simple Ace^- . All thirty-eight mutants tested had wild-type activities of both enzymes.

Isoleucine- and valine-requiring mutants

Several acetate-negative mutants of *E. coli* (Dailey & Cronan, 1986) and *S. typhimurium* (Dailey *et al.*, 1987; Wilson & Maloy, 1987) will grow on acetate if the medium is supplemented with isoleucine and valine. These mutants lack the isoenzyme acetohydroxyacid synthase I. This enzyme catalyses the first step in the biosynthetic pathways of isoleucine and valine, and this particular isoenzyme is essential for the pathway to function in acetate-grown (but not glucose-grown) cells.

Forty-three Ace^- mutants of *B. stearotherophilus* were tested; three (AM1045, AM1046, AM1047) were found to be Ace^+ if isoleucine and valine were added to the growth medium at $50 \mu\text{g ml}^{-1}$. These are presumed to be analogous to the mutants of similar phenotype in the enteric bacteria.

Acetate uptake studies

Acetate uptake was measured over as short a time period as possible (40 s, Fig. 1) in an attempt to distinguish acetate uptake from assimilation. *B. stearotherophilus* vegetative cells proved to be prone to lysis if cultures are held at room temperature or 37°C , and also lyse during prolonged storage at 50°C without a carbon source; cells were kept above 42°C whilst washing, and cells were assayed immediately after preparation. Even under optimized conditions for harvesting and washing cells, each cell suspension could only be assayed once – significant lysis occurred in stored, unaerated cells during the 15 min required to complete an uptake experiment. The variation in activity of different batches of cells was generally within a twofold range.

Fig. 1. Acetate uptake by *B. stearotherophilus*. Cells were incubated with $[2-^{14}\text{C}]$ acetate, harvested at 10 s intervals onto membrane filters and washed. ○, AM1116 (wild-type) cells; ●, AM1116 cells in the presence of $0.5 \mu\text{M}$ -CCCP; ■, AM1048 (mutant) cells.

Results of a typical uptake experiment are shown in Fig. 1. The rate of uptake is linear over the 40 s period of sampling; experiments were routinely done with $38 \mu\text{M}$ -acetate; under these conditions the rate of acetate uptake in glucose-grown cells was $0.72 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$ whereas that in cells grown on a mixture of acetate and glucose was $8.9 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$, suggesting acetate-dependent induction. The addition of $0.5 \mu\text{M}$ -CCCP as a respiratory uncoupler led to inhibition, suggesting that the uptake process is active, or at least cannot be maintained in the absence of a proton gradient. Glyoxylate pathway mutants were also tested and these took up $[2-^{14}\text{C}]$ acetate at the same rate as the wild type.

Eight Ace^- mutants which were neither glyoxylate-cycle-defective nor isoleucine- and valine-requiring mutants were tested for their ability to take up acetate. Of these, mutant AM1048 was reproducibly defective in uptake (Fig. 1). To ensure that this apparent decrease in uptake was not due to a more rapid cell death in this strain, we measured uptake of $\text{L-[1,4,5-}^3\text{H]leucine}$ and of $[2-^{14}\text{C}]$ acetate simultaneously in a dual labelling experiment. Leucine was taken up by the mutant cells at about 50% of the wild-type rate; this is within the range that could be due to cell batch differences. The same cell suspension failed, as previously, to take up significant concentrations of acetate.

Acetate uptake in wild-type *B. stearotherophilus* was measured at increasing concentrations of acetate, to determine whether the system was saturable (Fig. 2). This did appear to be the case, although the variability between cell suspensions means that detailed kinetic analysis is impossible. A V_{max} of about 60 nmol min^{-1}

Fig. 2. Specific acetate uptake by *B. stearothermophilus* cells at various concentrations of acetate. Rates of acetate uptake were calculated from data as shown in Fig. 1; the graph includes data from experiments done on two separate occasions (●, ■). The K_m , calculated as the concentration of acetate at which half the maximum velocity of acetate uptake was obtained, is approximately 300–350 μM -acetate, and the estimated value of $V_{\max}$ is 60 $\text{nmol min}^{-1} (\text{mg protein})^{-1}$.

Fig. 3. Specific acetate uptake by succinate-grown *E. coli* cells at various concentrations of acetate. Inset: Eadie-Hofstee plot of the data in the main figure, giving estimates of $K_m = 15 \mu\text{M}$ and $V_{\max} = 50 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$.

$(\text{mg protein})^{-1}$ was estimated; the apparent K_m at half-maximum velocity was about 300–350 μM -acetate.

Following the discovery of a possible *B. stearothermophilus* acetate transport system, we tested whether *E. coli* possesses a similar system. Fig. 3 shows that acetate uptake is saturable in succinate-grown *E. coli* cells. The kinetics appear more classically hyperbolic; the K_m for acetate uptake at 37 °C in *E. coli* was about 15 μM and $V_{\max}$ was 50 $\text{nmol min}^{-1} (\text{mg protein})^{-1}$. Acetate kinase and phosphotransacetylase mutants of *E. coli* transported acetate at the same rate as the wild-type (results not shown), confirming that the uptake system was independent of these enzymes.

Discussion

The spectrum of *B. stearothermophilus* mutants with defects in acetate metabolism obtained in this study differs from those obtained previously in *E. coli* and *S. typhimurium*, as no acetate kinase or phosphotransacetylase mutants were obtained. Several explanations for this are possible. There may be more than one enzyme with these activities, or an alternative biochemical pathway may exist to convert acetate to acetyl-CoA. Alternatively, lesions in *pta* or *ack* genes may affect growth on glucose or other substrates as these enzymes may be required to recycle CoA in conditions of carbon excess, when acetate is excreted. Brown *et al.* (1977) have shown that *pta* mutants of *E. coli* excrete virtually no acetate into the growth medium, but these strains can grow on glucose. Van Dyk & LaRossa (1987) have shown that *pta* and *ack* gene products are also essential in α -ketobutyrate metabolism in *S. typhimurium*, converting propionyl-CoA to propionyl phosphate and propionyl phosphate to propionate, respectively. A sulphometuron-methyl-induced build-up of α -ketobutyrate during growth of *S. typhimurium* in glucose minimal medium is lethal (LaRossa *et al.*, 1987; Van Dyk & LaRossa, 1987), but there is no reason to suppose that such a build-up would occur in *B. stearothermophilus* cells grown on glucose, succinate or lactate.

The glyoxylate pathway mutants of *B. stearothermophilus* are analogous to those found in *E. coli* and *S. typhimurium*. It is likely, therefore, that the isocitrate lyase and malate synthase (*icl* and *mas*) genes form an operon in *B. stearothermophilus* which is transcribed from *mas* to *icl* analogous to those of *E. coli* (Cortay *et al.*, 1988) and *S. typhimurium* (Wilson & Maloy, 1987).

For a significant proportion (about 35%) of Ace^- mutants, it was not possible to identify any enzymological defect. There must be additional components essential to acetate-specific metabolism, but as exemplified by the isoleucine/valine-requiring mutants, not all may be directly associated with the catabolic pathway. Guest (1979) reported the isolation of acetate-negative fluoroacetate-resistant mutants which were wild-type for both acetate kinase and phosphotransferase; these mutants, designated *facB*, mapped close to *ack* and *pta*, suggesting a relationship with acetate metabolism.

Our results suggest that an active acetate uptake system is present in *B. stearothermophilus*. Brown *et al.* (1977) showed that an inducible acetyl-CoA synthetase, acetate kinase and phosphotransacetylase are involved in the long-term assimilation of acetate in *E. coli*, but there have been no reports of measurement of acetate transport.

It has been suggested that weak acids such as acetate permeate the cytoplasmic membrane by passive diffu-

sion in their undissociated form (Kell *et al.*, 1981). Boenigk *et al.* (1989), however, reported a carrier-mediated acetate transport system in *Acetobacterium woodii*, which excretes copious amounts of acetate. The apparent K_m of this transport system was 850 μM . The actual mechanism of transport was not established, but it could involve either facilitated diffusion of the acid or a proton/acetate symport.

The *B. stearotheophilus* acetate transport system appears to have a relatively low affinity for acetate, but it is acetate-inducible and uncoupler-sensitive, although some caution is necessary in interpreting the effect of uncouplers on transport systems in intact cells, as they may affect intracellular pH, resulting in indirect effects. The transport systems of *B. stearotheophilus* and *A. woodii* are both low affinity systems whose concentration dependence shows sigmoidal kinetics. In contrast, the acetate transport system detected in *E. coli* is of much higher affinity; the maximum uptake rates in *E. coli* and *B. stearotheophilus* are much higher than that reported for *A. woodii*.

The importance of the uptake system in the catabolism of acetate in *B. stearotheophilus* is suggested by the Ace^- phenotype of AM1048. It was not possible to test whether fatty acids, metabolized via acetyl-CoA, could act as a carbon source, as the wild-type parent proved unable to grow on oleate. Both wild-type and mutant could, however, grow on minimal medium containing ethanol, another two-carbon compound that is likely to be metabolized via acetyl-CoA. The mutant formed somewhat smaller colonies on ethanol than did the wild-type: this could be an indirect result of the acetate transport defect, or might reflect a second residual mutation in the strain. Despite this complication, the ability to grow on ethanol suggests that the strain can still metabolize internal acetate or acetyl-CoA.

The evidence presented argues strongly that both *B. stearotheophilus* and *E. coli* possess specific transport systems for acetate, although a full analysis of either uptake system is beyond the scope of this paper.

The behaviour of mutant AM1048, which failed to take up acetate despite having normal levels of acetate kinase and phosphotransacetylase, argues that in *B. stearotheophilus* the observed uptake of radiolabelled acetate is not the result of simple diffusion followed by rapid metabolism, although it would have been helpful to be able to undertake transport studies in a metabolically blocked strain. The *E. coli* uptake system was of much higher affinity; here no transport mutant was available, but metabolically blocked strains showed the same uptake behaviour as the wild type. The low K_m of the uptake system, at 15 μM , was also very different from the published K_m values of the enzymes that might be responsible for acetate assimilation – acetate kinase and

acetyl-CoA synthase have K_m values of 7 mM and 200 μM , respectively (Fox & Roseman, 1986; Brown *et al.*, 1977). The two systems may have different roles in the two organisms; as already discussed, bacilli excrete acetate during growth on rich media or glucose, and a relatively high K_m would be appropriate for a system designed to effect excretion of excess intracellular acetate, whereas the low K_m of the *E. coli* system might suggest an efficient uptake system to scavenge extracellular acetate.

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