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Isolation and Characterization of *ack* and *pta* Mutations in *Azotobacter vinelandii* Affecting Acetate-Glucose Diauxie†

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Azotobacter vinelandii **mutants defective for acetate utilization that were resistant to fluoroacetate (FA) were isolated. FA-resistant mutant AM6 failed to transport [^{14}C]acetate and lacked enzymatic activity for both acetate kinase and phosphotransacetylase. Growth of wild-type *A. vinelandii* was sensitive to 10 mM glycine; however, all FA-resistant strains were resistant to glycine toxicity. Isolated mutants that were spontaneously resistant to glycine were also resistant to FA and lacked both acetate kinase and phosphotransacetylase activity. The glycine-resistant mutant AM3, unlike mutant AM6, was capable of growth on acetate. The mutant strain AM6 was unable to growth under acetate-glucose diauxie conditions. Glucose utilization in this mutant, unlike that in wild-type *A. vinelandii*, was permanently arrested in the presence of acetate. Revertants of strain AM6 were selected on plates with acetate or acetate-glucose. Two classes of revertants were isolated. Class I revertant mutants AM31 and AM35 were positive for both acetate kinase and phosphotransacetylase activities. These revertants were also sensitive to both FA and glycine. Class II revertant strains AM32 and AM34 still lacked acetate kinase and phophotransacetylase activity. Both of these revertants remained resistant to FA and glycine.**

Researchers in our laboratory have been investigating acetate-glucose diauxie in *Azotobacter vinelandii*, a nonsymbiotic nitrogen-fixing bacterium (S. George, C. Costenbader and T. Melton, manuscript in preparation). To investigate the molecular nature of acetate-glucose diauxie in *A. vinelandii*, we isolated mutants defective for diauxie, glucose, or acetate utilization. In this study we describe the isolation and characterization of mutants that are resistant to fluoroacetate (FA) and that exhibit hypersensitive repression of glucose utilization via acetate. In both *Salmonella typhimurium* and *Escherichia coli* two classes of FA-resistant mutants have been isolated (3, 9, 12). One class consists of *ack* mutants which are defective in acetate kinase (ATP:acetate phosphotransferase; EC 2.7.2.1) activity. The other major class of mutants selected as FA-resistant are *pta* mutants which are defective in phosphotransacetylase (acetyl-coenzyme A [CoA]:orthophosphate acetyltransferase; EC 2.3.1.8) activity. The genetic loci *ack* and *pta* have been identified in both *E. coli* and *S. typhimurium* and mapped near *purF*, which is at 49 min of their respective linkage maps (1, 26). Other FA-resistant *E. coli* mutants consist of *facA* mutants, which lack both *ack* and *pta* activities, and *facB* mutants, which retain both of these enzyme activities (9). These genetic lesions also map near the *purF* site.

Diauxic growth has been observed in a wide variety of bacteria. The classic example is the growth of *E. coli* in medium containing lactose and glucose (18). Under these conditions glucose is the first carbon source that is metabolized, and the organism does not metabolize lactose until glucose is exhausted from the growth medium. Glucose is known to repress the expression of genes needed to code for proteins necessary for lactose utilization (19). Pastan and Perlman (20) found that the addition of cAMP to growth media containing both glucose and lactose relieved the repressive effects of glucose on the expression of the lactose operon. Ucker and Signer (32) have reported a succinate-lactose diauxie in *Rhizobium meliloti*, with succinate being the preferred carbon source in this case. These investigators found that cAMP was not involved in the regulation of this diauxie phenomenon. *Pseudomonas oxalaticus* has been shown to exhibit diauxie when grown on oxalate in the presence of either acetate or formate (5). Oxalate utilization was inhibited while acetate or formate was preferentially metabolized. Oxalate transport was not inhibited, and it was postulated that an intermediate, oxalyl-CoA, was the target for formate and acetate inhibition. In *Pseudomonas fluorescens*, organic acids such as citrate or succinate have been found to repress glucose transport and the expression of enzymes needed for glucose catabolism (15). Diauxie has also been reported in *Arthrobacter crystallopoietes*, in which succinate exhibits repression of glucose transport and catabolism (10). Even though much work has been done at the molecular level to understand the molecular basis of diauxie in *E. coli*, very little is known about the molecular nature of diauxie in other organisms.

Results of this study suggest that there is an alternate pathway for the utilization of acetate in *A. vinelandii* independent of the acetate kinase and phosphotransacetylase system. Results of these mutational studies further support the fact that glucose utilization in *A. vinelandii* is regulated via acetate during diauxie.

MATERIALS AND METHODS

Chemicals. Sodium monofluoroacetic acid (FA) was obtained from Sigma Chemical Company, St. Louis, Mo. The ^{14}C-uniformly labeled substrates used in transport studies were [1,2-^{14}C]acetic acid (56.5 mCi/mmol) from New England Nuclear Corp., Boston, Mass., and D-[^{14}C]glucose (263 mCi/mmol) from Amersham Corp., Arlington Heights, Ill.

Bacterial strains and culture conditions. *A. vinelandii* OP was used as the wild-type strain for these studies. Cells were

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routinely grown at 28°C on a rotary shaker at 250 rpm in Burks nitrogen-free media (BM) (30). Acetate-glucose cultures were pregrown in BM-sucrose liquid media containing 30 mM ammonium acetate at 28°C with aeration. The bacterial cells were centrifuged and washed twice with Burks buffer (BB; pH 7.0), which contained 0.2 g of KH_2PO_4 and 0.8 g of K_2HPO_4 per liter of deionized water. Washed cells were inoculated into Klett flasks containing 20 ml of BM–25 mM glucose and 45 mM ammonium acetate. Growth was monitored turbidimetrically with a Klett-Summerson photoelectric colorimeter (no. 66 red filter).

Isolation of spontaneous mutants and revertants. Mutants defective for acetate utilization were selected as FA-resistant colonies growing on a BM-sucrose plate containing 10 mM FA. Plates were allowed to incubate for 4 to 7 days at 28°C. Single colonies were isolated and characterized further. Since FA-resistant cells fail to grow on acetate, revertants of these FA-resistant cells were selected on plates containing BM–50 mM potassium acetate and 30 mM NH_4Cl as a nitrogen source or on acetate-glucose plates containing BM–25 mM glucose and 45 mM ammonium acetate.

Transport studies. Cells used in transport studies were grown in 500 ml of the appropriate medium, harvested by centrifugation, washed twice with BB, and suspended in 5 ml of BB for every 20 Klett units of cell turbidity. The transport assay mixture contained 1 ml of BB and 2 ml of the cell suspension, which contained approximately 10^9 cells per ml in a 10-ml glass vial. The assay mixture was continuously mixed with a magnetic microspin bar. Aeration was supplied by a 15-cm Pasteur pipette attached to an air pump. Transport was initiated on the addition of 200 μl of 1 mM labeled substrate stock solution. Uptake was terminated by removing a 200-μl sample and placing it into 10 ml of BB. The sample was vortexed and immediately filtered through a Gelman membrane filter (pore size, 0.45 μm) mounted on a Millipore sampling manifold attached to a Gast vacuum pump. Filters were placed into mini scintillation vials, and 5 ml of scintillation fluid {Triton X-100–toluene scintillation fluid containing 666 ml of scintillized toluene, 333 ml of Triton X-100, 5.5 g of PPO [2,5-diphenyloxazole] and 0.15 g of dimethyl POPOP [1,4-bis(5-phenyloxazolyl)benzene]} was added. Samples were counted in a Packard liquid scintillation spectrometer. Cell dry weight was determined by drying 1 ml of cell suspension at 70°C. Uptake was expressed as nanomoles of labeled substrate taken up per milligram of cell (dry weight).

Preparation of cell extracts. Cultures were grown for 18 h in 500 ml of BM-sucrose media containing 30 mM ammonium acetate. Cultures were harvested by centrifugation and washed with BB containing 1 mM 2-mercaptoethanol and suspended in a final volume of 5 ml of BB containing 1 mM 2-mercaptoethanol. Crude extracts for enzyme assays were prepared by disrupting cells twice in a French press at 16,000 lb/in^2 and by centrifuging the broken cells at 31,000 × g for 20 min. The protein concentration of crude extracts was determined by using the Bio-Rad protein assay.

Enzyme assays. Acetate kinase activity was determined by a modification of the method of Lipmann and Tuttle (14). The reaction mixture contained 1 ml of hydroxylamine solution (28% hydroxylamine hydrochloride in an equal volume of 14% sodium hydroxide), 1 ml acetate buffer (pH 7.4), 0.1 ml of 0.1 M ATP (pH 7.0), and 0.9 ml of cell extract diluted with BB as necessary. The reaction mixture was incubated for 10 min at 30°C, and the reaction was stopped by the addition of 1 ml each of a 25% HCl solution and 12% trichloroacetic acid. The protein precipitate was removed by centrifugation. Color was developed by adding 1 ml of 5% $FeCl_3$, and the sample was read immediately in the Klett-Summerson colorimeter with filter no. 54. A reading of 69 Klett units above a reagent blank corresponds to 1.0 μmol of acetohydroxamic acid. One unit of enzyme is defined as the amount which produces 1 μmol of hydroxamic acid per min under the reaction conditions given above.

Phosphotransacetylase activity was assayed by the procedure of Stadtman (28). The reaction mixture consisted of 0.1 ml each of 0.1 M Tris (pH 8.0), 0.06 M dilithium acetyl phosphate, 0.16 mM Coenzyme A, and 0.1 M cysteine. A 0.6-ml sample of the appropriately diluted crude cell extract was added to start the reaction. After 10 min of incubation at 30°C, 0.1 ml of 0.5 M potassium arsenate (pH 8.0) was added to the reaction mixture, which was incubated for an additional 15 min. The residual acetyl phosphate was determined by the procedure with hydroxamic acid of Lipmann and Tuttle (14). One unit of phosphotransacetylase is defined as the amount of enzyme required to catalyze the arsenolysis of 1 μmol of acetyl phosphate per min.

Oxygen uptake. Oxygen uptake was measured with a YSI model 53 biological oxygen monitor (Yellow Springs Instruments Co., Inc.). Cells were pregrown in 20 ml of the appropriate medium, washed with 20 ml of BB twice, and suspended in BM-glucose or BM-acetate-glucose media to 15 Klett units and kept on ice until they were used in the assay. Cells were equilibrated to room temperature, and 3 ml was placed in the sample chamber in which the temperature was set at 28°C. The chamber was oxygenated with a magnetic stirrer for 3 min, after which the oxygen probe was inserted in the chamber. The rate was calculated as microatoms of oxygen per minute per milligram (dry weight).

Isolation of revertants of mutant AM6. Two approaches were employed to select revertants. The first method was to select directly for mutant AM6 revertants that were capable of growth on acetate media. This involved plating mutant AM6 on plates with acetate and incubating at 28°C until colonies appeared. These single-colony clones were isolated and characterized further. Mutants AM34 and AM35 were selected by this technique. The second method used to isolate revertants involved inoculating strain AM6 onto plates with acetate-glucose. The wild-type strain can grow on these plates, whereas the FA-resistant strain AM6 can not. Therefore, mutant AM6 was spread onto the plates with acetate-glucose which were incubated at 28°C until revertant colonies appeared. Mutants AM31 and AM32 are representative of revertants selected by this approach.

RESULTS

***A. vinelandii* FA-resistant mutants and revertants.** To study the genetic and regulator processes underlying the acetate-glucose diauxie growth of *A. vinelandii*, we isolated mutations which affected growth of this organism on acetate, acetate-glucose media, or both. Since acetate repressed glucose utilization during diauxie, we first attempted to isolate mutants defective for the transport or metabolism of acetate. To achieve this, we used FA to isolate spontaneous mutants that failed to utilize acetate. One such mutant, AM6, was isolated and was resistant to 10 mM FA. This mutant did not grow on acetate and was also defective for acetate-glucose diauxie (Fig. 1). However, strain AM6 grew normally on the other substrates tested (i.e., glucose, maltose, fructose, sucrose, sorbitol, melibiose, galactose, xylitol, arabitol, mannitol, gluconate, pyruvate, inositol, oxaloacetate, benzoic acid). Even though AM6 did not grow with acetate as the sole carbon source, the addition of 45 mM

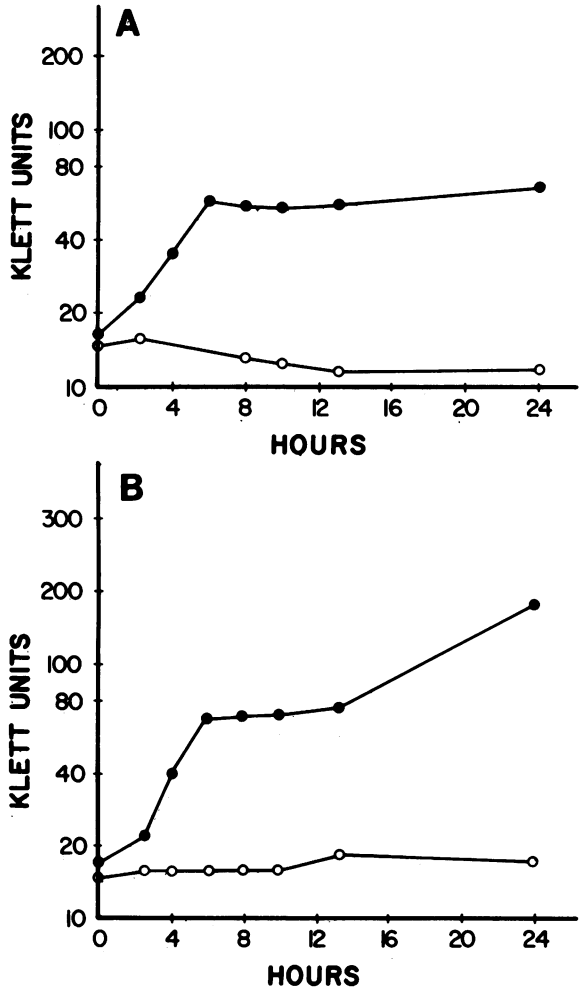

FIG. 1. Growth of *A. vinelandii* wild type and strain AM6 on acetate and under diauxie conditions. Strains were pregrown in BM–2% sucrose with 30 mM ammonium acetate at 28°C with aeration. Cells were washed with BB and used to inoculate a flask containing BM-45 mM ammonium acetate (A) or acetate-glucose media (B). Symbols: ●, growth of wild-type strain; ○, growth of strain AM6.

acetate inhibited growth of this mutant on glucose (Fig. 2). This inhibition could be reversed, however, if these cells were washed in BB and suspended in fresh glucose media. Acetate did not inhibit the growth of AM6 on the other substrates tested. The growth rate of mutant AM6 on glucose was identical to that of the wild-type strain.

Growth of wild-type *A. vinelandii* was sensitive to 20 mM glycine when it was grown on media containing glucose or sucrose as the sole carbon source. Unlike the wild type, mutant AM6 was resistant to glycine. All FA-resistant strains isolated so far in the laboratory are also glycine resistant, and mutants that are resistant to glycine are in turn resistant to FA. Growth of *A. vinelandii* is also sensitive to 10 mM threonine and cysteine. However, FA-resistant strains are still sensitive to these amino acids, and cells resistant to these amino acids remain sensitive to FA and glycine.

Transport of acetate and glucose in mutant AM6. To determine the reason that mutant AM6 failed to grow on acetate, we first examined the transport of radioactive ace-

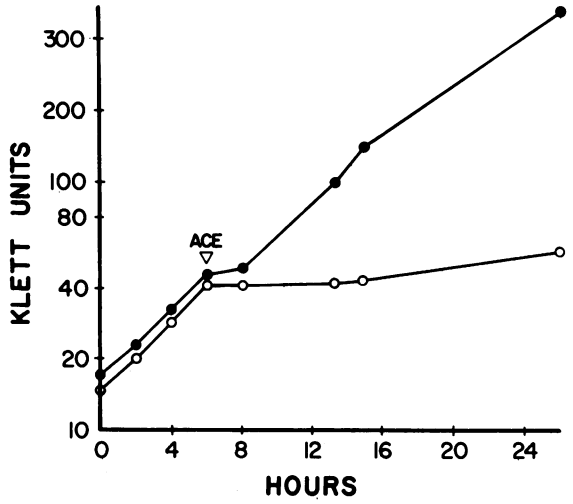

FIG. 2. Growth inhibition of strain AM6 on glucose by acetate. Strain AM6 (○) and the wild-type strain (●) were pregrown as described in the legend to Fig. 1. Cells were inoculated in BM–25 mM glucose media with 30 mM ammonium chloride and grown at 28°C with aeration for 6 h, at which time potassium acetate (ACE) was added to a final concentration of 45 mM.

tate. Transport of acetate was significantly decreased in mutant AM6 compared with that in the wild type (Fig. 3). It should be noted that even though there was a significant decrease in acetate transport in this mutant, it still had the capacity to transport acetate to some extent, as indicated by the inhibition of glucose utilization by this substrate. Transport of acetate in mutant AM3, a spontaneous glycine-resistant isolate, was also examined. Even though all glycine-resistant strains were FA resistant, mutant AM3 transported acetate to approximately the same extent as the wild type (Fig. 3). Therefore, it appears that in glycine-resistant cells, acetate transport is normal. This is also demonstrated by the fact that glycine-resistant cells are still capable of acetate-glucose diauxie growth. Therefore, there are at least two systems for acetate transport in *A. verlandii*: one that is sensitive to FA and another that is insensitive to

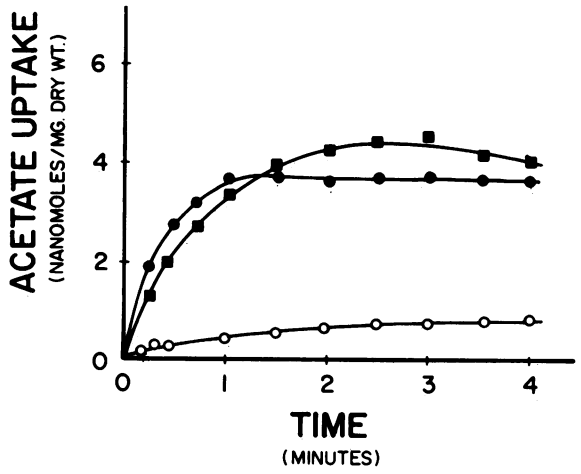

FIG. 3. Time course of [^{14}C]acetate uptake in strains of *A. vinelandii*. Cells were prepared for transport as described in the text. The uptake of acetate (63 μM) was measured at 28°C, using the *A. vinelandii* wild type (●), strain AM6 (○), and strain AM3 (■).

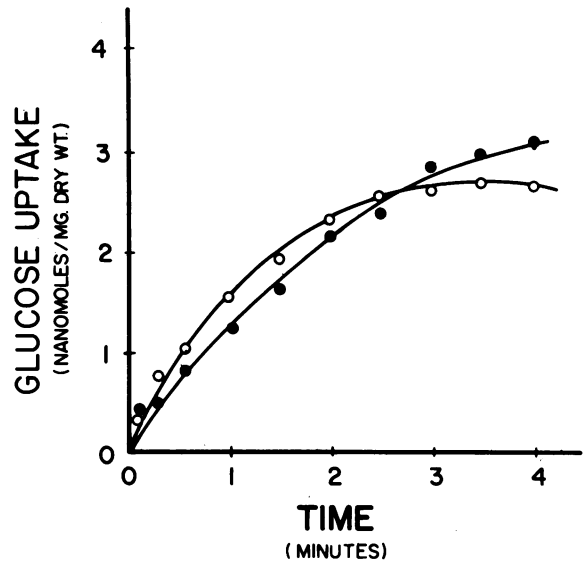

FIG. 4. Transport of glucose by the wild type (●) and strain AM6 (○) during the glucose phase of diauxie. Strains pregrown in 100 ml of BM–2% sucrose with 30 mM ammonium acetate at 28°C overnight were harvested and washed twice in BB. Cells were suspended in 500 ml of acetate-glucose media at 25 Klett units and grown for 14 h. At this time cells were harvested, washed twice in BB, and suspended in 5 ml of BB per 20 Klett units of cell suspension. The transport reaction mixture contained 1 ml of BB, 2 ml of cell suspension, 45 mM ammonium acetate, and 63 μM [^{14}C]glucose. Transport measurement was carried out as described in the text.

FA. Although the mutant AM6 does not grow under diauxie conditions, it is still capable of transporting glucose in the presence of acetate (Fig. 4). Apparently, inhibition of glucose utilization by acetate is not at the level of transport for this substrate.

Acetate kinase and phosphotransacetylase activity in FA-resistant mutants. Because the FA-resistant mutant AM6 failed to grow on acetate, acetate kinase and phosphotransacetylase activities were measured in this mutant and in the wild-type parent (Fig. 5). The activity for both of these enzymes was significantly decreased in AM6 as compared with that in the wild type. This mutant resembles the *facA* mutants isolated in *E. coli* (9). We also measured the activities of these enzymes in the glycine-resistant mutant AM3. This mutant also lacks activity for both of these enzymes (Fig. 4). However, unlike the FA-resistant AM6 mutant, mutant AM3 is capable of acetate transport.

Oxygen uptake studies. The data in Table 1 show the oxygen uptake rates for the wild type and the FA-resistant mutant AM6 utilizing glucose as the sole carbon source or under diauxie conditions. The rates of oxygen uptake on glucose were comparable in the two mutants, which was expected, because they both grew well on glucose as a substrate. Under diauxie conditions, cells were harvested 14 h into the growth period, which corresponds to the time after the diauxie lag. At this time, acetate was exhausted from the acetate-glucose medium and the wild type oxidized glucose. Even though the FA-resistant mutant AM6 is capable of growing on glucose as a sole carbon source, it cannot oxidize this substrate under diauxie conditions. However, if these cells are harvested, washed, and suspended in fresh glucose media, they are able to continue to grow.

Characterization of acetate and diauxie revertants of mutant AM6. The data presented above show that the FA-resistant mutant AM6 is defective for acetate transport, acetate kinase, and phosphotransacetylase activities. Since a mutant was selected which was spontaneously resistant to FA, it is reasonable to expect that the loss of transport, acetate kinase, and phosphotransacetylase activities are due to a single genetic mutation. To investigate this further, we set about to isolate revertants of mutant AM6 using two approaches. Revertants of mutant AM6 were selected as clones capable of growth either on acetate plates or on acetate-glucose plates. Table 2 shows the phenotype of representative revertants of mutant AM6. Mutants AM30, AM31, AM32, and AM33 were selected as revertants on acetate-glucose plates. All of these mutants grew on media containing glucose, acetate, or sucrose as the sole carbon source. Revertant strains AM30, AM32, and AM33 grew on media containing FA or glycine, indicating they were still resistant to these compounds. However, revertant strain AM31 was sensitive to both FA and glycine. Unlike the parental FA-resistant strain AM6, these revertants grew on acetate-glucose plates. Strains AM34 and AM35 were rever-

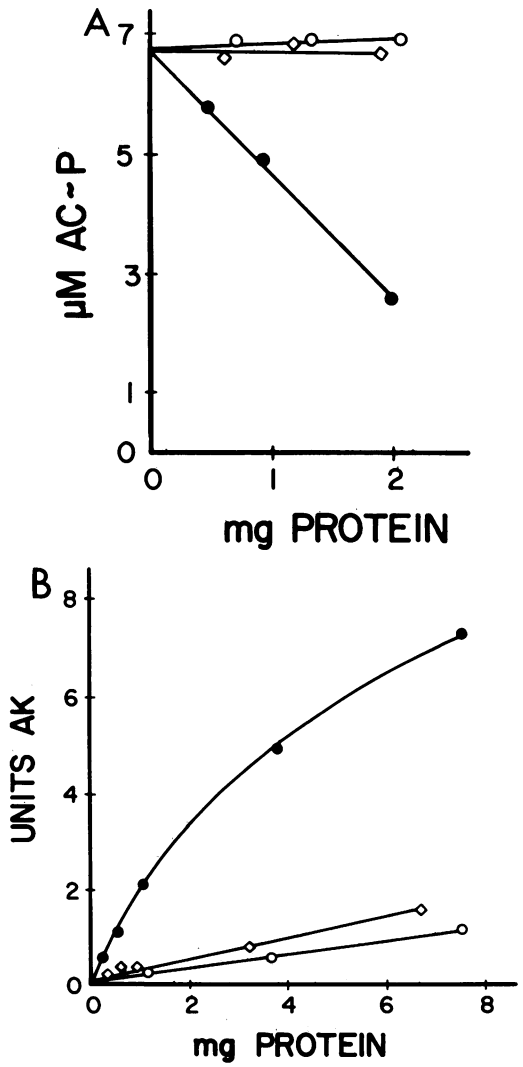

FIG. 5. Expression of phosphotransacetylase and acetate kinase in strains of *A. vinelandii*. Phosphotransacetylase (Ac-P) (A) and acetate kinase (AK) (B) were assayed in the wild-type strain (●), strain AM6 (○), and strain AM3 (◇), as described in the text.

TABLE 1. Oxygen uptake[a] by *A. vinelandii* strains during glucose growth

Strain	Growth conditions[b]	Rate of oxidation (μatom of O/min per mg [dry wt])
Wild type	Glucose	3.64
AM6	Glucose	3.71
Wild type	Diauxie	3.64
AM6	Diauxie	0.07

[a] Oxygen uptake was determined polarographically at 28°C.

[b] The bacterial cultures were pregrown in 20 ml of BM–2% sucrose medium with 30 mM ammonium acetate at 28°C overnight. Cells were harvested, washed twice in BB, and suspended into 10 ml of either BM-glucose or acetate-glucose media and allowed to grow for 14 h at 28°C. These cells were harvested, washed in BB, and suspended to 15 Klett units in either glucose or acetate-glucose media.

tants selected on acetate plates. Even though these mutants were selected by the same technique, strain AM34 remained resistant to FA and glycine (Table 2), while revertant AM35 was sensitive to these compounds. Therefore, two classes of revertants, each capable of growing on acetate, were obtained. However, one class retained its resistance to both glycine and FA and, therefore, resembles the glycine-resistant strains.

Table 3 shows the activity of acetate kinase and phosphotransacetylase in the wild type, mutant AM6, and representative revertants. Mutant AM6 lacked both acetate kinase and phosphotransacetylase activities. The glycine-resistant mutant AM3 was also defective for both of these enzymes. Spontaneously selected revertants of mutant AM6 fell into two distinct classes. Class I revertants such as strains AM31 and AM35 regained both acetate kinase and phosphotransacetylase activities. Class II revertants (AM32 and AM34) lacked activity for both of these enzymes and yet grew on acetate (Table 2). The designation of revertant classes is independent of the selection procedure used to revert the parental strain AM6 since each class included revertants isolated either with acetate or with acetate-glucose plates. Revertant strains AM31 and AM35, which were sensitive to both FA and glycine (Table 2), regained high levels of acetate kinase and phosphotransacetylase activities (Table 3).

TABLE 2. Phenotype of mutant AM6 and revertants

Carbon source[a]	Growth of the following strains[b]							
	WT	AM6	AM30	AM31	AM32	AM33	AM34	AM35
Sucrose	+	+	+	+	+	+	+	+
Acetate	+	−	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Acetate-glucose	+	−	+	+	+	+	+	+
Sucrose-FA	−	+	+	−	+	+	+	−
Glucose-glycine	−	+	+	−	+	+	+	−

[a] The bacterial cultures were pregrown in 20 ml of BM–2% sucrose medium at 28°C overnight. Cells were harvested, washed twice in BB, and suspended into 20 ml of BB. Cells were streaked into the following plates: BM–2% sucrose; BM–50 mM potassium acetate with 30 mM NH_4Cl; BM–2% glucose; BM–25 mM glucose with 45 mM ammonium acetate; BM–2% sucrose–10 mM FA; BM–2% glucose–20 mM glycine. After 2 days of incubation at 28°C, plates were scored for growth (+) or no growth (−).

[b] Strains tested were wild-type (WT), the FA-resistant mutant AM6, revertants of AM6 selected on acetate-glucose plates (AM30, AM31, AM32), and revertants of AM6 selected on acetate plates (AM34, AM35).

TABLE 3. Specific activity of acetate kinase and phosphotransacetylase in *A. vinelandii* strain AM6 and revertants

Strain[a]	Selection Procedure[b]	Sp act (μmol/min per mg of protein) of the following enzymes	
		Acetate kinase	Phosphotransaretylase
Wild type	None	1.80	2.10
AM6	FA^r	0.03	0.00
AM3	Gly^r	0.06	0.02
AM31	DP	1.20	1.80
AM32	DP	0.05	0.05
AM34	Ac	0.03	0.00
AM35	Ac	1.52	1.20

[a] Strains were grown as described in text.

[b] Selection procedure used to isolate strains: resistance to 10 mM FA (FA^r); resistance to 10 mM glycine (Gly^r); growth on acetate-glucose (diauxie) plates (DP), and growth on acetate plates (Ac).

DISCUSSION

In this report we described acetate utilization mutants of *A. vinelandii* that were resistant to FA or glycine. Mutations affecting acetate utilization in *E. coli* and *S. typhimurium* were resistant to FA (3, 9, 12). In *E. coli* the FA-resistant strains fall into four classes: class I, *ack* mutants that lack acetate kinase activity; class II, *pta* mutants that lack phosphotransacetylase activity; class III, *facA* mutants that lack both acetate kinase and phosphotransacetylase; class IV, *facB* mutants that are resistant to FA but positive for acetate kinase and phosphotransacetylase activities (9). The spontaneous FA-resistant mutants of *A. vinelandii* isolated in this study were defective for both acetate kinase and phosphotransacetylase activities. Guest (9) has reported that the largest class of FA-resistant mutants in *E. coli* are deficient in both acetate kinase and phosphotransacetylase activities. Interestingly, these mutants are also incapable of growth by glucose fermentation. Mutant AM6, which is resistant to FA, lacked activity for these two enzymes and cannot grow on acetate as the sole carbon source (Fig. 1). The defect in this mutant is due to a single mutational event since spontaneous revertants can be selected which regain both acetate kinase and phosphotransacetylase activities (Table 3). Results of preliminary transformational mapping indicate that this mutation is linked to the *rif* (rifampin) marker on the *A. vinelandii* chromosome (data not shown). It is unlikely that the mutation in strain AM6 is a deletion since this strain can be reverted for both acetate kinase and phosphotransacetylase activities simultaneously.

Another characteristic of FA-resistant mutants isolated during this study was their resistance to 10 mM glycine. The wild-type *A. vinelandii* was sensitive to glycine. Vela and Rosenthal (34), when studying the effect of peptone on *A. vinelandii* morphology, found the pleomorphism-inducing principle in peptone to be glycine. Giant cells, filamentous forms, and budding and branching cells were observed in cultures containing peptone or glycine. High concentrations of glycine are known to induce bacteriolysis or morphological alterations in a number of bacteria (8, 16, 21, 25). In *E. coli*, glycine can induce the formation of protoplasts (36). Park (J. T. Park, Biochem. J. **70**:2p, 1958) has shown that glycine induces the accumulation of UDP acetylamino sugars in *Staphylococcus aureus*. Strominger and Birge (31) concluded that UDP-acetylmuramic acid is the major com-

pound that accumulates and suggested that the inhibition of cell wall synthesis by high concentrations of glycine is probably due to the inhibition of the reaction in which L-alanine is added to UDP-acetylmuramic acid. Amino acid inhibition of growth has been reported for a number of bacterial species (6, 7, 11, 13, 17, 22, 23, 24, 29, 33, 35). Revertant strains AM31 and AM35 become sensitive to FA concomitant with sensitivity to glycine. These results indicate that a common element is responsible for the expression of sensitivity or resistance to these two compounds. This is further substantiated by the fact that strains that are resistant to 10 mM glycine are also resistant to FA (e.g., strain AM3). We also found that acetate can protect *A. vinelandii* from the inhibitory effects of glycine (T. Melton, unpublished data).

Results of previous studies in our laboratory have shown that acetate transport under diauxie conditions is expressed during both the glucose and acetate phases of growth. However, the glucose transport system is inhibited during the acetate phase of diauxie and is expressed only as acetate concentrations decrease in the media. Wild-type cells recover from the acetate inhibition and grow on glucose on the depletion of acetate from the acetate-glucose media. We found that FA-resistant cells of *A. vinelandii*, i.e., mutant AM6, fail to accumulate acetate at a rate which would support normal growth on this substrate (Fig. 3). However, this mutant exhibits a hypersensitive response to acetate in that under diauxie conditions, growth of this strain on glucose is completely inhibited (Fig. 1B). If these cells are harvested and washed free of the acetate, they will grow on glucose. The utilization of other substrates by strain AM6 does not appear to be hypersensitive to the presence of acetate. These results suggest that either acetate or an intermediate of acetate metabolism inhibits glucose utilization. Senior and Dawes (27) have demonstrated that the formation of pyruvate and glyceraldehyde 3-phosphate from 6-phosphogluconate by the action of the Entner-Doudoroff enzymes is inhibited in *Azotobacter beijerinckii* by citrate, isocitrate, and *cis*-aconitate. The acetate hypersensitivity of glucose-grown FA-resistant cells might be due to acetate directly rather than to an intermediate of acetate metabolism because these strains lack both acetate kinase and phosphotransacetylase and cannot grow on this substrate. Because these strains lack these enzymes, acetate cannot be depleted from the acetate-glucose media, and as a consequence, the cell cannot utilize glucose. Revertants which have regained acetate kinase and phosphotransacetylase activities can utilize acetate and undergo acetate-glucose diauxie.

Results of this study also indicate that there may be an alternative route for acetate metabolism in *A. vinelandii* that is independent of both acetate kinase and phosphotransacetylase. The revertant strains AM34 and AM32 lack both of these enzymes (Table 3) but can grow on acetate, as can the wild-type strain. Brown et al. (3) found that *E. coli* mutants impaired for both acetate kinase and phosphotransacetylase activities were capable of growing on acetate due to the induction of an alternative enzyme system known as acetyl-CoA synthetase (acetate:CoA ligase [AMP-forming]; EC 6.2.1.1]. This enzyme used by eucaryotic cells and some bacteria effects the acetylation of CoA concomitant with the cleavage of ATP to AMP and PP_i (2, 4). It has been suggested that this enzyme might play a primary role in scavenging acetate at low concentrations (3). Currently, we are investigating the possible presence of this enzyme system in *A. vinelandii*.

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