JOURNAL OF BACTERIOLOGY, Sept. 1974, p. 691-697
Vol. 119, No. 3
Printed in U.S.A.
Copyright © 1974 American Society for Microbiology

Sugar Catabolism in *Aquaspirillum gracile*

BARBARA E. LAUGHON AND NOEL R. KRIEG

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received for publication 28 May 1974

Aquaspirillum (*Spirillum*) *gracile* is one of the few spirilla that cause acidification of the medium when cultured with sugars. Acidic reactions have been reported only for D-glucose, D-galactose, and L-arabinose, and the mode of attack of these sugars has not been previously investigated. The soluble portion of extracts of glucose-cultured cells of *A. gracile* ATCC 19624 was found by spectrophotometric methods to contain enzyme activities characteristic of the Entner-Doudoroff and Embden-Meyerhof-Parnas pathways. No activity for 6-phosphogluconate dehydrogenase (EC 1.1.1.44) was detected. Pyridine nucleotide-linked dehydrogenase activities for L-arabinose and D-galactose (EC 1.1.1.46 and EC 1.1.1.48) occurred in the soluble fraction of cells cultured with either sugar. Glucose-cultured cells contained not only glucokinase (EC 2.7.1.2) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activities but also glucose dehydrogenase (EC 1.1.1.47) activity. Enzymes capable of oxidizing gluconate were not detectable, but gluconokinase (EC 2.7.1.12) activity was present. Paper chromatographic analysis of the spent culture supernatant media from glucose-cultured cells indicated an accumulation of gluconic acid, and this was confirmed by enzymatic methods. Evidence is presented for the production of D-galactonic and L-arabonic acids in cultures containing D-galactose or L-arabinose, respectively.

Among the fresh water spirilla, *Aquaspirillum* (*Spirillum*) *gracile*, *Aquaspirillum* (*Spirillum*) *itersonii*, and *Aquaspirillum* (*Spirillum*) *peregrinum* are unusual in their ability to produce acidification of the medium when cultured with any of a limited variety of carbohydrates (16). Using *A. itersonii*, Hylemon et al. (14) demonstrated enzymatic activities characteristic of the Entner-Doudoroff (ED) pathway and also much lower activities characteristic of the Embden-Meyerhof-Parnas (EMP) pathway. Activities characteristic of the hexose monophosphate pathway were not detected. *A. gracile*, the smallest of the spirilla, has been shown to produce acidic reactions only from D-glucose, D-galactose, and L-arabinose, and is the only species of *Aquaspirillum* reported to be capable of attacking a pentose (16). The present investigation was undertaken to characterize its carbohydrate catabolism by examination of cell-free extracts for certain key enzymatic activities and by identification of the products responsible for the acidic reactions.

MATERIALS AND METHODS

Microorganism and culture conditions. Stock cultures of *A. gracile* ATCC 19624 were maintained by weekly subculture at 30 C in a modified peptone-succinate-salts semisolid medium described previously (1). For extracts, cells were pooled at mid-logarithmic growth (ca. 12 h) at 30 C from seven 2.8-liter cotton-stoppered Fernbach flasks, each containing 1 liter of a casein hydrolysate-yeast extract-salts medium. This medium (pH = 7.0) consisted of defined basal medium as described previously (16) with the K_2HPO_4 decreased to 0.005% and with the addition of 0.1% vitamin-free, salt-free casein hydrolysate (Nutritional Biochemical Corp., Cleveland, Ohio). The medium was sterilized for 20 min in an autoclave. Carbon sources (D-glucose, D-galactose, D-gluconate, or L-arabinose at a final concentration of 1%) were sterilized by filtration and added aseptically; however, succinic acid (0.2%) was incorporated before sterilization of the medium. A 5% (vol/vol) inoculum of cells from a 24-h starter culture containing homologous carbon sources was used to initiate growth for cell-free extracts. Cultures were incubated at 30 C and agitated by magnetic stirring until the mid-logarithmic phase.

For chromatographic identification of acidic products, cotton-stoppered 1-liter Erlenmeyer flasks, each containing 200 ml of an inoculated hydrolysate-yeast extract-salts medium and a suitable carbon source, were incubated statically at 30 C for 1 week. Cells were removed by centrifugation at 7,000 × *g* for 10 min, and portions of the supernatant fraction were either concentrated 10-fold at 30 C by flash evaporation for paper chromatography or prepared for gas-liquid chromatographic analysis.

Preparation of cell-free extracts. Cells were harvested at 7,000 × g and washed twice in 0.1 M tris-(hydroxymethyl)aminoethane chloride (Tris) buffer, pH 8.0. The final suspension was prepared in 20 ml of cold Tris buffer containing 10 mM β-mercaptoethanol. Cells were ruptured at 10 kHz (sonic oscillator, model DF-101, Raytheon Manufacturing Co., Waltham, Mass.) for 30 s at 0 C. Whole cells and large particles were removed at 7,000 × g for 10 min at 0 C. The resulting supernatant fraction was then centrifuged at 100,000 × g for 2 h at 0 C to minimize levels of reduced nicotinamide adenine dinucleotide (NADH) oxidase in the soluble fraction. The clear supernatant fraction was used for the assay of soluble enzymes. The particulate fraction was washed once in Tris buffer containing β-mercaptoethanol.

Enzyme assays. Glucokinase (EC 2.7.1.2) activity was measured as described previously (15). The reaction mixture for assay of gluconokinase (EC 2.7.1.12) was the same as previously described for glucokinase except for substitution of 25 mM sodium gluconate for glucose and excess (0.12 IU) exogenous 6-phosphogluconate dehydrogenase (EC 1.1.1.44, Sigma Type V, Sigma Chemical Co., St. Louis, Mo.) for glucose-6-phosphate dehydrogenase (EC 1.1.1.49). Fructose 1,6-diphosphate aldolase (EC 4.1.2.6) activity was determined by the method of Rutter and Hunsley (23). The assay of phosphofructokinase (EC 2.7.1.11) activity was a modification of the procedure of Ling et al. (17); the reaction mixture contained 50 mM Tris buffer (pH 8.0), 5 mM β-mercaptoethanol, 5 mM adenosine triphosphate, 10 mM $MgCl_2 \cdot 6H_2O$, 0.12 mM NADH, excess (0.012 IU) exogenous fructose 1,6-diphosphate aldolase (Sigma), excess (3.4 IU) exogenous α-glycerophosphate dehydrogenase (EC 1.1.1.8), excess (21.1 IU) exogenous triose phosphate isomerase (EC 5.3.1.1, Calbiochem Co., Los Angeles, Calif.), and 10 mM fructose-6-phosphate. Glucose-6-phosphate dehydrogenase activity was measured by the method of DeMoss (8), and 6-phosphogluconate dehydrogenase activity was determined as described by Pontremoli and Grazi (22). 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14) activity was assayed with KDPG as the substrate as described by Meloche et al. (20). 6-Phosphogluconate dehydratase (EC 4.2.1.12) activity was determined by coupling its activity with endogenous KDPG aldolase as described by Wood (31). For assay of glucose dehydrogenase (EC 1.1.1.47) activity, the reaction mixture contained 50 mM Tris buffer (pH 8.0), 0.17 mM nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP), and 20 mM glucose. Glucose dehydrogenase (EC 1.1.99.a) activity was assayed as described by Hauge (12) with both the soluble and particulate preparations. L-Arabinose dehydrogenase (EC 1.1.1.46) and D-galactose dehydrogenase (EC 1.1.1.48) activities were determined with a reaction mixture containing 50 mM Tris buffer (pH 8.6), 5 mM β-mercaptoethanol, 0.17 mM NAD, and 20 mM L-arabinose or D-galactose. 2-Ketogluconate reductase (EC 1.1.1 group) or 5-ketogluconate reductase (EC 1.1.1.69) activities were assayed by the method of DeLey (6) using both the soluble and particulate preparations; since NADP-linked gluconate oxidation forms the basis of this method, an increase in absorbance at 340 nm would indicate the formation of one and/or the other keto-sugar acid.

The initial rates of oxidation or reduction of pyridine nucleotides were determined at 340 nm with a Hitachi-Perkin-Elmer model 139 recording spectrophotometer (Perkin-Elmer Co., Norwalk, Conn.) or a Gilford model 240 recording spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio). For assays which monitored the disappearance of NADH (phosphofructokinase, fructose diphosphate aldolase, 6-phosphogluconate dehydratase, and KDPG aldolase), corrections were made for NADH oxidase activity. Corrections for NADH oxidase activity were not made for assays which monitored the rate of formation of NADH (glucose, galactose, and arabinose dehydrogenases, and glucose-6-phosphate dehydrogenase); NADH oxidase activity was very low, however. All assays except those for glucose dehydrogenase and 2-keto- or 5-ketogluconate reductase (where the particulate fraction was also assayed) were performed at 25 C with the soluble extract fraction. Specific activities were expressed as micromoles of substrate converted per minute (international units) per milligrams of protein. Protein estimations were performed by the method of Lowry et al. (18) with crystalline bovine serum albumin as a standard. All enzyme assays were linear with respect to time; linearity with respect to protein concentration was not determined.

Gas-liquid chromatography. The spent media from centrifuged cultures were analyzed for alcohols (C_1 to C_5), and for volatile (C_2 to C_6) and non-volatile (C_1 to C_4) organic acids by the methods of the Virginia Polytechnic Institute Anaerobe Laboratory (13). A Hewlett-Packard model 700 dual-column gas chromatograph equipped with flame-ionization detector and temperature programmer (Hewlett-Packard/Analytical Instruments, Avondale, Pa.) was used. Glass columns (6 ft × ¼ inch diameter [1.828 m by .635 cm]) were packed with Resoflex LAC-1-R-296 (standard concentration, Burrell Corp., Pittsburgh, Pa.). Samples (5 μliters) were analyzed at an attenuation setting of 20, with temperature programmming from 50 to 123 C.

Paper chromatography. Paper chromatographic analyses for sugar acids were performed on concentrated spent media. Samples were neutralized and spotted in 10-μliter amounts on Whatman no. 1 chromatography paper for one-dimensional descending chromatography. Solvent systems employed were (i) methanol-ethanol-water (45:45:10) (21) and (ii) pyridine-ethyl acetate-acetic acid-water (5:5:1:3) with equilibration of the tank by pyridine-ethyl acetate-water (11:40:6) (10). Detectors were alkaline silver nitrate (25), modified by the use of 1% $Na_2S_2O_3$ to prevent extensive darkening of the background (29), and aniline hydrogen phthalate (11). Papers sprayed with the latter reagent were examined for color reactions under ultraviolet light (365 nm).

Enzymatic identification of gluconic acid. Portions of the D-glucose, D-galactose, and L-arabinose cultural supernatant preparations (concentrated 10 times) were assayed for the presence of gluconic acid.

The reaction mixtures were identical to the assay mixture for gluconokinase except for the omission of gluconate and the addition of 0.2 IU of gluconokinase purified from *Escherichia coli* (Sigma Chemical Co., St. Louis, Mo.). The reactions were monitored spectrophotometrically at 340 nm for 5 min.

RESULTS

Enzyme assays. The specific activities of various carbohydrate-catabolizing enzymes present in crude cell-free extracts are listed in Table 1. In certain cases, activities were so much greater for cells cultured in the presence of certain carbohydrates compared to cells cultured with succinate as to suggest a possible inducible or derepressible system.

Glucokinase activity occurred in all extracts. The assay procedure was modified by substitution of various buffers in an attempt to determine if a pH other than 8.0 was optimal. With the extract from cells cultured with glucose, no appreciable increase in activity occurred over a range of pH 5.6 to 9.0, nor after dialysis for 48 h against cold 0.1 M Tris-hydrochloride buffer (pH 8.0) and 10 mM β-mercaptoethanol.

Fructose diphosphate aldolase activity was detectable in all extracts. Attempts to increase activity by addition of Fe^{2+} or Co^{2+} to the reaction mixture or by dialysis of the crude soluble extract against 0.1 M Tris buffer (pH 8.0) were unsuccessful.

For glucose-6-phosphate dehydrogenase activity, both NAD and NADP were effective as electron acceptors (Table 1). The NADP-associated activity was 10-fold higher in cells cultured with glucose compared to cells cultured with succinate, and the NAD-associated activity was 8-fold higher.

The 6-phosphogluconate dehydratase of *A. gracile* was apparently quite labile, as activity could be detected only when the assay was performed immediately after preparation of the extract. Cells cultured with glucose possessed a seven-fold higher activity than cells cultured with succinate.

The representative enzyme of the hexose monophosphate pathway, 6-phosphogluconate dehydrogenase, was not detectable in any extract, even when high protein concentrations were used and with incubation periods of 15 min. Some endogenous formation of reduced nicotinamide adenine dinucleotide phosphate did occur in the absence of substrate, especially when a large volume of cell extract was used.

NAD-linked glucose dehydrogenase was detected only in cells cultured with glucose, galactose, or arabinose. The activity was specific for NAD as an electron acceptor; NADP or 2,6-

TABLE 1. *Enzymatic activities of crude extracts of Aquaspirillum gracile*

Enzyme	Assay performed with soluble (S) or particulate (P) fraction	Sp act[a] of cells cultured in CYS medium with the following carbon sources:				
		Succinate	D-Glucose	D-Gluconate	D-Galactose	L-Arabinose
Glucokinase	S	0.0022	0.0080	0.0030	0.0018	0.0009
Phosphofructokinase	S	0.0322	0.0921	0.0087	0.0024	0.0047
Fructose diphosphate aldolase	S	0.0040	0.0143	0.0034	0.0011	0.0130
Glucose-6-phosphate dehydrogenase (NADP)	S	0.0137	*0.1331*[b]	0.0498	0.0235	0.0051
Glucose-6-phosphate dehydrogenase (NAD)	S	0.0075	*0.0587*	0.0128	0.0087	0.0021
6-Phosphogluconate dehydratase	S	0.0247	*0.1679*	0.0620	<0.0001	0.0166
2-Keto-3-deoxy-6-phosphogluconate aldolase	S	0.0834	*0.2886*	0.2094	0.0868	*0.2656*
Gluconokinase	S	0.0014	0.0078	0.0055	0.0006	0.0008
6-Phosphogluconate dehydrogenase	S	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Glucose dehydrogenase (DIP)[c]	S, P		<0.0001	<0.0001		
Glucose dehydrogenase (NAD)	S	<0.0001	*0.0183*	<0.0001	0.0036	0.0074
Galactose dehydrogenase (NAD)	S	0.0037	0.0059	<0.0001	*0.1310*	*0.1605*
L-Arabinose dehydrogenase (NAD)	S	0.0052	0.0273	<0.0001	*0.1605*	*0.1220*
2-Keto- or 5-ketogluconate reductase	S, P		<0.0001	<0.0001		

[a] Micromoles of substrate converted per min (international units) per mg of protein. CYS, Hydrolysate-yeast extract-salts.

[b] The italicized values represent possible induced or derepressed activities (compared to data for cells cultured with succinate).

[c] DIP, 2,6-Dichlorophenol indophenol.

dichlorophenol indophenol could not be substituted. No dichlorophenol indophenol-linked glucose dehydrogenase activity was detected in the soluble or particulate fractions of glucose- or gluconate-cultured cells.

Activities for D-galactose and L-arabinose dehydrogenases were strikingly greater in cells cultured with either sugar compared to cells cultured with succinate. Activities of D-galactose dehydrogenase were 22- to 27-fold greater than for cells cultured with glucose, whereas L-arabinose dehydrogenase activities were 4.5- to 6-fold greater.

Gas-liquid chromatography. The gas-liquid chromatographic analyses of glucose, galactose, or arabinose cultural supernatant media failed to detect any volatile fatty acids, alcohols, or nonvolatile organic acids such as pyruvic, lactic, fumaric, or succinic acids.

Paper chromatography. The results for paper chromatography of glucose, galactose, or arabinose cultural supernatant media are shown in Table 2. The glucose cultural supernatant medium yielded two spots with both solvent systems: one corresponding in R_f and $R_{glucose}$ to the glucose of the medium, and the other corresponding to either gluconate, 2-ketogluconate, or 5-ketogluconate. (In the case of the 5-ketogluconate standard [Sigma Chemical Co., St. Louis, Mo.], two spots were detected. Norris and Campbell [21] also found two spots when using standards of 5-ketogluconate. Because 5-ketogluconate has been reported to yield a brown color under ultraviolet light with aniline hydrogen phthalate reagent [11], the spot we obtained having an R_f of 0.12, or an $R_{glucose}$ of 0.52, was presumed to represent the 5-ketogluconate. The identity of the second spot [R_f = 0.19, $R_{glucose}$ = 0.60] is not known.) Aniline hydrogen phthalate was found to successfully differentiate among the three sugar acids: gluconate produced no color reaction, 2-ketogluconate produced a red color under ultraviolet light and 5-ketogluconate produced a brown color under ultraviolet light. The detection of the unknown spot with silver nitrate, together with its lack of reaction with aniline hydrogen phthalate, implied that it was an aldonic acid, presumably gluconate. By similar deductions, the unknown spots on chromatograms of galactose and arabinose cultural supernatant media appeared to be identifiable as galactonic acid and arabonic acid, respectively. This conclusion was reinforced when it was found that gluconate was not present in these two supernatant media by enzymatic assay (see below).

Enzymatic assay for gluconate. That gluconate was produced by *A. gracile* in complex media containing glucose was confirmed by the results shown in Table 3. The phosphorylation of gluconate, if the latter is present in the samples, by gluconokinase (Sigma Chemical Co., St. Louis, Mo.) was coupled to 6-phosphogluconate dehydrogenase. The large change in absorbance at 340 nm when the glucose cultural supernatant medium was tested indicated the presence of gluconate. No change in absorbance occurred when galactose or arabinose cultural supernatant media were tested, in accordance with the hypothesis that galactonic and arabonic acids were occurring in these media. The specificity of the test system for gluconate was demonstrated by the omission of gluconokinase from otherwise complete reaction mixtures containing the glucose cultural supernatant medium; no change in absorbance occurred.

TABLE 2. *Paper chromatography of glucose, galactose, and arabinose cultural supernatant media*

Substance applied	Migration in solvent systems[a]		Reaction with $AgNO_3$	Color with aniline hydrogen phthalate[b]
	A	B		
Glucose	0.48[c]	1.00[d]	+	Y-O
Gluconate	*0.21*[e]	*0.55*	+	—
2-Ketogluconate	0.19	0.47	+	R[f]
5-Ketogluconate	0.12	0.52	+	Br[f]
	0.19	0.60	+	G[f]
Glucose cultural supernatant medium	0.44	1.00	+	Y-O
	0.22	*0.53*	+	--
Galactose	0.46	1.00[g]	+	Y-O
Galactonate	*0.16*	*0.51*	+	—
Galactose cultural supernatant medium	0.43	0.92	+	Y-O
	0.19	*0.48*	+	—
Galactonate + galactose cultural supernatant medium	0.43	0.85	+	Y-O
	0.18	*0.46*	+	—
Arabinose	0.66	1.00[h]	+	Y-O
Arabonate	*0.30*	*0.58*	+	—
Arabinose cultural supernatant medium	0.52	0.84	+	Y-O
	0.40	*0.56*	+	—
Arabonate + arabinose cultural supernatant medium	0.51	0.83	+	Y-O
	0.38	*0.56*	+	—

[a] Solvent systems: (A) methanol-ethanol-water (45:45:10); (B) pyridine-ethyl acetate-acetic acid-water (5:5:1:3).

[b] Y-O, Yellow-orange; R, red; Br, brown; G, pale green; —, no reaction.

[c] R_f.

[d] $R_{glucose}$.

[e] Italicized values represent migration rates of especial interest for identification of compounds in cultural supernatant media.

[f] Color observed under ultraviolet light.

[g] $R_{galactose}$.

[h] $R_{arabinose}$.

TABLE 3. *Enzymatic assay of cultural supernatant media for presence of gluconic acid*[a]

Substrate	Absorbance[b]/5 min
Glucose cultural supernatant medium	0.230
Galactose cultural supernatant medium	0.005
Arabinose cultural supernatant medium	0.000
Glucose (0.2 M)	0.002
Sodium gluconate (0.2 M)	0.360
Glucose cultural supernatant medium[c]	0.000

[a] The procedure employed was identical to the procedure for assay of gluconokinase, except for the omission of gluconate and the addition of gluconokinase.

[b] 340 nm, 1-cm light path, 25 C.

[c] Gluconokinase was omitted from the reaction mixture.

DISCUSSION

Enzymatic activities characteristic of the ED pathway (i.e., 6-phosphogluconate dehydratase and KDPG aldolase) occurred in *A. gracile*. When the organism was cultured with glucose, the activity of these enzymes, as well as glucose-6-phosphate dehydrogenase, was 3.5- to 10-fold higher than the activities when cultured with succinate. This suggests that the ED pathway may possibly be inducible with glucose. Derepression of the enzymes by the absence of succinate is another possibility; however, the relatively low activities of most of the ED pathway enzymes in cells cultured with arabinose or galactose does not appear to support this possibility, since succinate was not present under these cultural conditions.

Enzymatic activities characteristic of the EMP pathway (i.e., phosphofructokinase and fructose diphosphate aldolase)were also present. Adenosine triphosphate is a substrate for phosphofructokinase and was therefore included in the reaction mixture. In phosphofructokinase from yeast (24) or cardiac muscle (19), adenosine triphosphate is also a powerful inhibitor of the enzyme; the addition of ammonium ions was reported to counteract this inhibition in cardiac muscle enzyme. Consequently, ammonium ions were incorporated into the reaction mixture for the phosphofructokinase of *A. gracile*, but no enhancement of activity occurred. With regard to fructose diphosphate aldolase, the relatively low activity found in *A. gracile* suggested that the EMP pathway may not be of great importance to the organism. The extent of contribution of the ED and EMP pathways to glucose catabolism in whole cells, however, can be determined only by such methods as isotope labeling in substrates and products. It is clear that the hexose monophosphate pathway is absent in *A. gracile*, by virtue of the lack of detectable 6-phosphogluconate dehydrogenase activity regardless of the carbon source used for growth.

The existence of an additional mechanism for glucose catabolism tends to complicate the situation just described. A soluble NAD-linked glucose dehydrogenase was present in cells cultured with glucose but was not detected in cells cultured with succinate or gluconate. This enzyme is apparently responsible for the production of gluconic acid in complex media containing glucose; furthermore, it appears to be inducible (unless both succinate and gluconate are metabolite repressors). That gluconic acid can be metabolized further is suggested by the presence of gluconokinase activity. Since the product of this phosphorylation reaction is 6-phosphogluconate, the next steps in its breakdown could be catalyzed by the 6-phosphogluconate dehydratase and KDPG aldolase of the ED pathway, as suggested by Wood (30) for *Pseudomonas fluorescens*. Alternatively, some pseudomonads are also capable of oxidizing gluconic acid to 2-keto- or 5-ketogluconic acid (7). The latter compounds were not demonstrable in paper chromatograms of glucose cultural supernatant media for *A. gracile*. This is not in itself conclusive evidence for the lack of formation of ketogluconic acids, since the latter merely may not be excreted into the medium; however, the lack of detectable 2-keto- or 5-ketogluconate reductase activity, in both the soluble and particulate fractions, strongly indicates that ketogluconic acids are indeed not formed from gluconic acid by the organism.

The dissimilation of D-galactose and of L-arabinose follow very similar patterns with respect to the following. (i) Acidic reactions in complex media were produced during growth with both sugars. (ii) When cultured in a medium containing galactose, *A. gracile* formed and excreted a compound with migration values and a lack of color reaction with aniline hydrogen phthalate characteristic of galactonic acid; similarly, in the case of L-arabinose, a compound with the characteristics of L-arabonic acid was formed. (iii) Both sugars served as substrates for soluble NAD-linked dehydrogenase activities in crude cell-free extracts. (iv) Both enzymatic activities attained high (and

remarkably similar) levels in cells cultured with either galactose or arabinose but not with succinate, glucose, or gluconate. These observations suggest that at least the initial step in the catabolism of galactose and arabinose is either catalyzed by similar enzymes with overlapping specificities, or catalyzed by the same enzyme whose synthesis is induced by either sugar (or metabolic derivative thereof). Although D-galactose is a hexose and L-arabinose is a pentose, the configurations of the hydroxyl groups about the first four carbon atoms are identical. In view of this similarity in structure, it seems possible that a dehydrogenase active for one sugar could exhibit cross-specificity for the other. This is indeed the case with various pseudomonads (2, 4, 9, 26).

One anomaly in the enzyme spectrum for arabinose-cultured cells can be seen in Table 1: an unusually high level of KDPG aldolase activity (a key ED pathway enzyme) was detected in the crude extracts. Since this enzyme is involved in hexose catabolism, its presence was surprising in arabinose-cultured cells; e.g., in *Pseudomonas saccharophila* (28) and *Pseudomonas fragi* (27), L-arabinose is first oxidized to L-arabonate which undergoes dehydration to 2-keto-3-deoxy-L-arabonate. These organisms subsequently oxidize this intermediate further to α-ketoglutarate. Another pathway has recently been reported in an unidentified pseudomonad which also forms 2-keto-3-deoxy-L-arabonate, but possesses an aldolase for cleavage of this intermediate to pyruvate and glycolaldehyde (3). If this latter mechanism was present in *A. gracile*, it is possible that the aldolase might also be active with 2-keto-3-deoxy-6-phosphogluconate as a substrate, accouting for the KDPG aldolase activity detected; Dahms and Anderson found no evidence of such cross-specificity, however, in their studies of a pseudomonad (5).

It should be borne in mind that enzymes of other pathways for galactose and arabinose metabolism were not assayed in our study. However, on the basis of the large increases in specific activity of the dehydrogenases, it seems likely that these sugars are dissimilated by mechanisms which parallel the ED pathway, mechanisms which have been previously demonstrated in pseudomonads (3, 27).

ACKNOWLEDGMENTS

We thank W. A. Wood for his generosity in supplying KDPG for the assays of KDPG aldolase.

LITERATURE CITED

1. Caraway, B. H., and N. R. Krieg. 1972. Uncoordination and recoordination in *Spirillum volutans*. Can. J. Microbiol. **18**:1749–1759.
2. Cline, A. L., and A. S. L. Hu. 1965. Some physical properties of three sugar dehydrogenases from a pseudomonad. J. Biol. Chem. **240**:4498–4502.
3. Dahms, A. S., and R. L. Anderson. 1969. 2-Keto-3-deoxy-L-arabonate aldolase and its role in a new pathway of L-arabinose degradation. Biochem. Biophys. Res. Commun. **36**:809–814.
4. Dahms, A. S., and R. L. Anderson. 1972. D-fucose metabolism in a pseudomonad. II. Oxidation of D-fucose to D-fucono-γ-lactone by an L-*araino*-aldose dehydrogenase and hydrolysis of the lactone by a lactonase. J. Biol. Chem. **247**:2228–2232.
5. Dahms, A. S., and R. L. Anderson. 1972. D-fucose metabolism in a pseudomonad. IV. Cleavage of 2-keto-3-deoxy-D-fuconate to pyruvate and D-lactaldehyde by 2-keto-3-deoxy-L-arabonate aldolase. J. Biol. Chem. **247**:2238–2241.
6. DeLey, J. 1966. 2-Ketogluconic acid reductase, p. 196–203. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
7. DeLey, J., and J. DeFloor. 1959. 2-Ketogluconoreductase in microorganisms. Biochim. Biophys. Acta **33**:47–54.
8. DeMoss, R. D. 1955. Glucose-6-phosphate and 6-phosphogluconic dehydrogenases from *Leuconostoc mesenteroides*, p. 328. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 1. Academic Press Inc., New York.
9. Doudoroff, M. 1962. L-arabinose dehydrogenase from *Pseudomonas saccharophila*, p. 342–344. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 5. Academic Press Inc., New York.
10. Fischer, F. G., and H. Dorfel. 1955. Die papier chromatographische Trennung und Bestimmung der Uronsauren. Z. Physiol. Chem. **301**:224–234.
11. Hais, I. M., and K. Macek (ed.). 1963. Paper chromatography—a comprehensive treatise, p. 793. Academic Press Inc., New York.
12. Hauge, J. G. 1966. Glucose dehydrogenases—particulate. I. *Pseudomonas* species and *Bacterium anitratum*, p. 92–98. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
13. Holdeman, L. V., and W. E. C. Moore (ed.). 1973. V. P. I. anaerobe laboratory manual, p. 113–115. The Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.
14. Hylemon, P. B., N. R. Krieg, and P. V. Phibbs, Jr. 1974. Transport and catabolism of D-fructose by *Spirillum itersonii*. J. Bacteriol. **117**:144–150.
15. Hylemon, P. B., and P. V. Phibbs, Jr. 1972. Independent regulation of hexose catabolizing enzymes and glucose transport activity in *Pseudomonas aeruginosa*. Biochem. Biophys. Res. Commun. **48**:1041–1048.
16. Hylemon, P. B., J. S. Wells, Jr., N. R. Krieg, and H. W. Jannasch. 1973. The genus *Spirillum*: a taxonomic study. Int. J. Syst. Bacteriol. **23**:340–380.
17. Ling, K. H., V. Paetkau, F. Marcus, and H. A. Lardy. 1966. Phosphofructokinase. I. Skeletal muscle, p. 425–429. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. Mansour, T. E. 1966. Phosphofructokinase. II. Heart muscle, p. 430–436. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
20. Meloche, H. P., J. M. Ingram, and W. A. Wood. 1966. 2-Keto-3-deoxy-6-phosphogluconic aldolase (crystalline), p. 520–524. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
21. Norris, F. C., and J. J. R. Campbell. 1949. The intermediate metabolism of *Pseudomonas aeruginosa*. III. The application of paper chromatography to the identification of gluconic and 2-ketogluconic acids, intermedi-

ates in glucose oxidation. Can. J. Res. **27**(c):253-261.

22. Pontremoli, S., and E. Grazi. 1966. 6-Phosphogluconate dehydrogenase-crystalline, p. 137. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
23. Rutter, W. J., and J. R. Hunsley. 1966. Fructose diphosphate aldolase. I. Yeast, p. 480. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
24. Sols, A., and M. L. Salas. 1966. Phosphofructokinase. III. Yeast., p. 436. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
25. Trevelyan, W. E., D. P. Proctor, and J. S. Harrison. 1950. Detection of sugars on paper chromatograms. Nature (London) **166**:444-445.
26. Wallenfels, K., and G. Kurz. 1962. Über die Spezifitat der Galaktose-dehydrogenase aus *Pseudomonas saccharophila* und deren Anwendung als analytiches Hilfsmittel. Biochem. Z. **335**:559-572.
27. Weimberg, R. 1961. Pentose oxidation by *Pseudomonas fragi*. J. Biol. Chem. **236**:629-635.
28. Weimberg, R., and M. Doudoroff. 1955. The oxidation of L-arabinose by *Pseudomonas saccharophila*. J. Biol. Chem. **217**:607-624.
29. Wheat, R. W. 1966. Analysis of hexosamines in bacterial polysaccharides by chromatographic procedures, p. 60-78. *In* E. F. Neufeld and V. Ginsburg (ed.), Methods in enzymology, vol. 8. Academic Press Inc., New York.
30. Wood, W. A. 1955. Pathways of carbohydrate degradation in *Pseudomonas fluorescens*. Bacteriol. Rev. **19**:222-233.
31. Wood, W. A. 1971. Assay of enzymes representative of metabolic pathways, p. 421. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 6A. Academic Press Inc., New York.