Transport and Catabolism of D-Fructose by *Spirillum itersonii*

P. B. HYLEMON, N. R. KRIEG, AND P. V. PHIBBS, JR.

Department of Microbiology, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia 23298; and Biology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Spirillum itersonii ATCC 12639 utilized D-fructose but neither D-glucose nor D-gluconate as a sole source of carbon and energy. The substrate saturation kinetics for D-fructose and D-glucose uptake by whole cells indicated the presence of a carrier-mediated transport system for D-fructose but not for D-glucose. The D-fructose uptake activity was induced (10- to 12-fold increase) during growth on D-fructose-Casamino Acids (CA) or D-glucose-CA medium, but not CA alone. D-Fructose uptake activity was stimulated by Na^+ or Li^+, but was inhibited by KCN, NaN_3, 2,4-dinitrophenol, and p-chloromercuribenzoate. High specific activities of glucokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydratase, and 2-keto-3-deoxy-6-phosphogluconate aldolase were detected in extracts of cells cultured on D-fructose-CA medium. These enzymatic activities were undetectable in extracts of cells grown in CA or succinate-CA medium. No decrease in the maximally induced specific activities of these enzymes occurred after the addition of succinate to cells during exponential growth on D-fructose-CA. Fructose 1,6-diphosphate aldolase and glucose-6-phosphate isomerase specific activities were approximately the same irrespective of cultural conditions. These results indicated that D-glucose was not utilized by cells of *S. itersonii* because this bacterium was impermeable to this hexose.

Spirillum itersonii is very restricted in its ability to use hexoses and other carbohydrates as a sole carbon and energy source or to produce detectable acidity from sugars in standard bacteriological growth media (6). D-Fructose is the only hexose known to be utilized by this aerobic, oxidative bacterium. D-Glucose, D-mannose, mannitol, D-galactose, D-gluconate, and sorbitol do not support growth (6). The ability to catabolize D-fructose but not D-glucose has been reported for other bacterial genera (16, 18). This investigation was undertaken to determine why *S. itersonii* can utilize D-fructose but not D-glucose and to characterize the regulation of transport systems and catabolic enzymes associated with D-fructose utilization in this bacterium.

MATERIALS AND METHODS

Microorganism and culture conditions. Stock cultures of *S. itersonii* ATCC 12639 were maintained in peptone-succinate-salts semisolid medium at 30 C as described previously (10). Cells of *S. itersonii* were cultured in a basal salts medium (BSM) of the following composition: 2.8 mM K_2HPO_4, 4.0 mM $MgSO_4 \cdot 7H_2O$, 7.6 mM $(NH_4)_2SO_4$, 7 μM $FeCl_3 \cdot 6H_2O$, and 11 μM $MnSO_4 \cdot H_2O$. The pH was adjusted to 7.0

with 2 N KOH. In all experiments, with the exception of growth studies, Casamino Acids (CA) (Difco) (final concentration = 0.05%) was added to the BSM before sterilizing in an autoclave. The BSM employed in growth studies contained no CA. The appropriate carbon sources were sterilized by filtration and added aseptically to the sterile basal-salts-CA medium.

A 5% (vol/vol) inoculum of cells from a late exponential-phase starter culture containing homologous carbon source was used to initiate growth in all experiments. In growth studies, cells from starter cultures were washed once and suspended in the original culture volume of carbon-free BSM prior to use as inocula. One-liter cultures were shaken in 2.8-liter Fernback flasks (250 rpm) in a New Brunswick gyrotory incubator at a temperature of 30 C. Growth was determined by measuring turbidity with a Klett-Summerson colorimeter equipped with a red (number 66) filter. Culture samples for enzyme assays were removed during the long-phase of growth, were harvested by centrifugation, and washed once in magnesium-saline solution (1 mM $MgCl_2 \cdot 6H_2O$ + 0.15 M NaCl). Washed cell samples were stored frozen overnight at −20 C and supernatant fractions of crude cell extracts were prepared at 105,000 × *g* by using a previously described procedure (5).

Enzyme assays. Glucose dehydrogenase (EC 1.1.99a) activity was assayed in the "particulate" fraction by using a previously described procedure (5). 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldol-

ase (EC 4.1.2.14) activity was determined by using KDPG as the substrate. 6-Phosphogluconate (6-P-G) dehydratase (EC 4.2.1.12) activity was measured by coupling its activity with endogenous KDPG aldolase, by using 6-phosphogluconate as the substrate, as described previously (5). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) specific activities were determined as previously described (5). Fructose-1,6-diphosphate aldolase (EC 4.1.2.13) activity was assayed as described by Rutter and Hunsley (15). The reaction mixture for glucose-6-phosphate isomerase (EC 5.3.1.9) included: 50 mM tris(hydroxymethyl)aminomethane-hydrochloride, 10 mM 2-mercaptoethanol, 10 mM $MgCl_2 \cdot 6H_2O$, 0.3 mM NADP, excess (0.66 international units) exogenous glucose-6-phosphate dehydrogenase (Sigma Type XI, Sigma Chemical Co., St. Louis, Mo.), and 2 mM fructose-6-phosphate (sodium salt), final pH, 8.0. The enzyme reactions were initiated by the addition of crude cell extracts. *Pseudomonas aeruginosa* OSU 64 was used as a positive control for all enzyme assays except fructose-1,6-diphosphate aldolase assay. *Streptococcus lactis* was used as a positive control for fructose-1,6-diphosphate aldolase enzyme assays.

The initial rates of oxidation or reduction of pyridine nucleotides were determined in a Gilford model 2400 recording spectrophotometer at a wavelength of 340 nm (Gilford Instrument Co., Oberlin, Ohio). Protein estimations were performed according to the method of Lowry et al. (8), by using crystalline bovine serum albumin as the protein standard. All assays were performed at 25 C, and specific activities are expressed as nanomoles of substrate converted per minute per milligram of extract protein. All enzyme activities, with the exception of glucose dehydrogenase, were determined in the 105,000 × *g* supernatant fraction of crude extracts prepared as previously described (5).

Uptake of D-fructose and D-glucose by whole cells. Cells from 200-ml portions of cultures of *S. itersonii* were harvested by centrifugation at 22 C, and washed once with 200 ml of 50 mM sodium phosphate buffer, pH 7.0, 22 C. Washed cells were suspended in sufficient volumes of fresh buffer (22 C) to yield a turbidity of 175 Klett units (0.25 to 0.30 mg of cell protein/ml). The cell suspensions were incubated for 5 min in a water bath reciprocal shaker (30 C, 120 cycles/min). Portions of cell suspensions (1.5 ml) were transferred to 50-ml flasks containing 0.5 ml of 150 mM sodium phosphate buffer, pH 7.0. The reaction was initiated by adding 1.0 ml of D-fructose-U-^{14}C (198 Ci/mol) or D-glucose-U-^{14}C (1.55 Ci/mol). The concentration of D-fructose and D-glucose was 50 μM in all studies, except where indicated. Samples (100 μliters) were removed at 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 min, and diluted into 2.0 ml of 30 C buffer overlying membrane filters (0.45 μm pore size; Millipore Corp.). Samples were filtered and washed with 2.0 ml of 30 C buffer. Radioactivity of the filters bearing washed cells was determined by a previously described procedure (12). D-Fructose and D-glucose uptake was recorded as nanomoles per milligram of cell protein.

RESULTS

Growth studies. As shown in Fig. 1, no additional growth occurred in a medium containing 5 mM succinate plus 30 mM D-glucose after the succinate apparently had been depleted from the growth medium. In contrast, additional growth occurred in a medium containing 5 mM succinate plus 30 mM D-fructose beyond what would be expected to occur on 5 mM succinate alone. These results suggest that D-fructose, but not D-glucose, is utilized by cells of *S. itersonii*.

Induction of D-fructose uptake activity. Cells of *S. itersonii* grown in a medium containing 30 mM D-fructose plus CA, accumulated ^{14}C-D-fructose at a rate 12 times that of cells cultured on CA alone (Fig. 2). Interestingly, cells cultured in a medium containing 30 mM D-glucose plus CA accumulated ^{14}C-D-fructose at a rate 10 times that of cells cultured on CA alone (Fig. 2). In contrast, ^{14}C-D-glucose was not taken up by cells of *S. itersonii* cultured in medium containing D-glucose-CA, D-fructose-CA medium, or CA medium alone (Fig. 2). These results suggest that *S. itersonii* has a D-fructose transport system which is induced during growth in medium containing either D-fructose or D-glucose. On the other hand, there appears to be no transport system for D-glucose.

Substrate saturable D-fructose uptake activity stimulated by Na+ or Li+ ions. The data presented in Fig. 3 show a substrate saturable uptake system for D-fructose, but not for D-
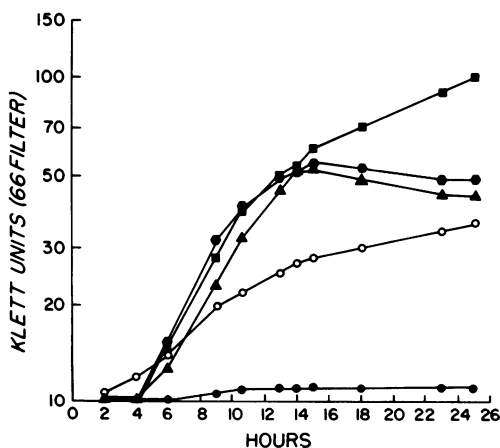

FIG. 1. *Growth of S. itersonii in basal salts media containing: 5 mM succinate (▲); 30 mM M-glucose (●); 30 mM D-fructose (○); 5 mM succinate + 30 mM D-fructose (■); and 5 mM succinate + 30 mM D-glucose (◉). The growth is presented as optical density in Klett units.*
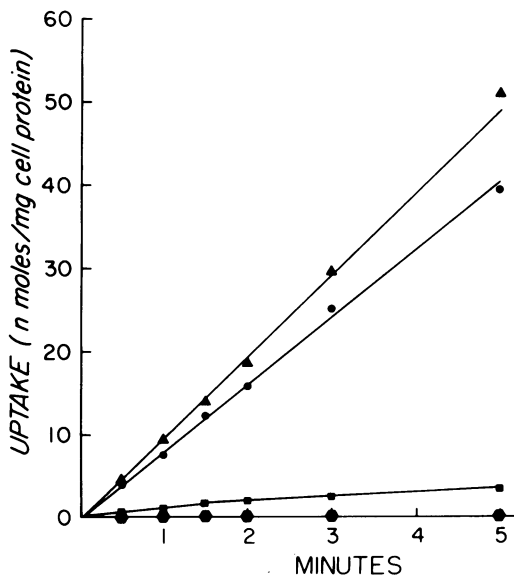
FIG. 2. *Effect of culturing conditions on D-fructose and D-glucose uptake activities. D-fructose uptake activity in log-phase cells grown on 0.3% CA (■), 30 mM D-fructose + 0.05% CA (▲), and 30 mM D-glucose + 0.3% CA (●). D-Glucose uptake activity in cells grown on either 0.3% CA, 30 mM D-fructose plus 0.05% CA, or 30 mM D-glucose + 0.3% CA (●).*
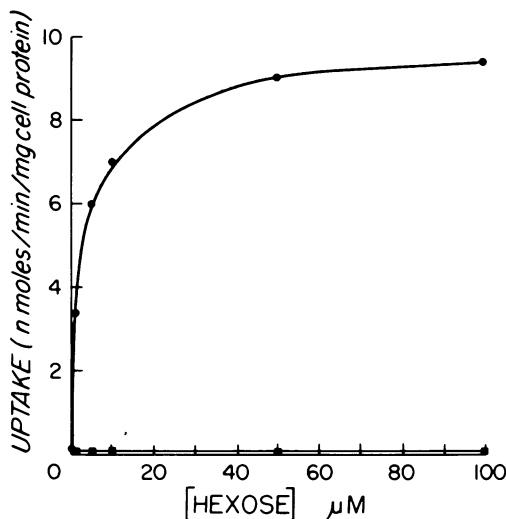

FIG. 3. *Saturation kinetics of D-fructose and D-glucose uptake by cells of S. itersonii. Initial velocity of D-fructose uptake as a function of D-fructose concentration; cells were cultured on 0.05% CA plus 30 mM D-fructose (●). Initial velocity of D-glucose uptake as a function of D-glucose concentration; cells were cultured on 0.3% CA plus 30 mM D-glucose (■).*

glucose in this bacterium. The apparent K_m of the D-fructose uptake system is approximately 2 to 4 μM. In additional studies, the initial

velocity of D-fructose uptake was found to be two- to threefold higher in cells washed in sodium phosphate buffer than in cells washed in potassium phosphate buffer (Fig. 4). Moreover, the addition of various concentrations of NaCl or LiCl to the reaction mixture of cells washed in potassium phosphate buffer resulted in an immediate increase in the initial rates of ^{14}C-D-fructose uptake (Fig. 4 and 5). Cells incubated in potassium phosphate buffer for 45 min or longer showed higher initial rates of ^{14}C-D-fructose uptake activity than cells that were freshly washed in potassium phosphate. The ^{14}C-D-fructose uptake activity continued to increase until it approached the levels for sodium phosphate washed cells (unpublished data). ^{14}C-D-glucose uptake was measured under the same conditions in these experiments and neither NaCl nor LiCl stimulated the rate or extent of glucose uptake (unpublished data).

Further studies of the D-fructose uptake activity established that the uptake of ^{14}C-D-fructose was inhibited by respiratory inhibitors such as KCN and NaN$_3$ as well as uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol (2,4-DNP). Moreover, p-chloromercuribenzoate (p-cmb), a compound that has

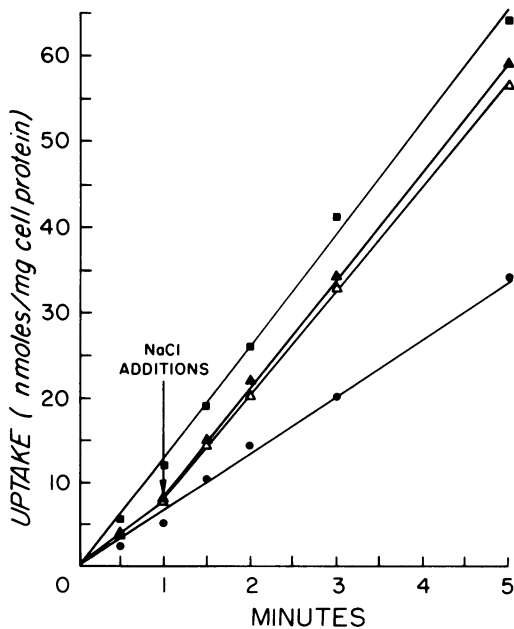FIG. 4. *Effect of sodium ions on ^{14}C-D-fructose uptake activity in S. itersonii. Cells were cultured on 0.05% CA plus 30 mM D-fructose and washed either in potassium phosphate (●) or sodium phosphate buffer (■). The effects on ^{14}C-D-fructose uptake of additions (arrow) of 10^{-5} M NaCl (△) or 10^{-3} M NaCl (▲) to cells initially washed and suspended in potassium phosphate buffer are shown.*
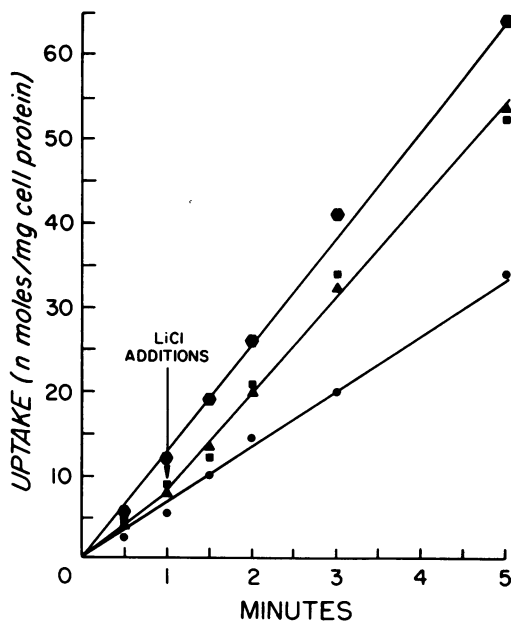
FIG. 5. *Effect of LiCl on ^{14}C-D-fructose uptake in S. itersonii. Cells were cultured on 0.05% CA plus 30 mM D-fructose and washed either in potassium phosphate buffer (●) or sodium phosphate buffer (●). The effects on ^{14}C-D-fructose uptake of additions (arrow) of 10^{-6} M LiCl (▲) or 10^{-3} M LiCl (■) to cells washed and suspended in potassium phosphate buffer are shown.*

high affinity for free sulfhydryl groups, markedly inhibited the uptake of ^{14}C-D-fructose (Fig. 6).

Induction of D-fructose catabolic enzymes. Assays of enzymes that might be involved in hexose metabolism were performed to determine the probable pathway of D-fructose catabolism in this bacterium. When cells of *S. itersonii* were cultured in a medium containing 30 mM D-fructose plus 0.05% CA, several hexose catabolic enzymes were induced (Table 1). The specific activities of glucose-6-phosphate dehydrogenase, glucokinase, and the Entner-Doudoroff enzymes (6-phosphogluconate dehydratase and KDPG aldolase) in cell extracts increased substantially over specific activities of these enzymes in extracts of cells cultured in CA or succinate-CA. Fructose-1,6-diphosphate aldolase and glucose-6-phosphate isomerase specific activities remained essentially constant, irrespective of the carbon source for growth, indicating the constitutive nature of these enzymes in *S. itersonii*. 6-Phosphogluconate dehydrogenase activity was not detectable under our assay conditions, despite exhaustive efforts to measure the activity of this enzyme. For example, no decrease in the activity of commercial

(yeast) 6-phosphogluconate dehydrogenase (Calbiochem) was apparent after mixing with cell extracts of *S. itersonii*. Furthermore, neither dialysis of cell extracts nor the addition of different divalent metal cations, or pyridine nucleotide cofactors (either NADP or NAD) to the reaction mixture in various combinations stimulated 6-phosphogluconate dehydrogenase activity in crude extracts. Membrane-associated glucose dehydrogenase activity was not detectable in this organism; however, this enzyme was easily measured in cell extracts of *P. aeruginosa* OSU 64 by using the same assay procedures.

Lack of catabolite repression of D-fructose catabolic enzymes by succinate. Additional studies were performed to determine if selected enzymes associated with D-fructose catabolism were strongly repressed by the addition of a preferred carbon source, such as succinate or certain other tricarboxylic acid cycle intermediates, to cells initially growing on D-fructose-CA. Succinate was selected because this substrate supports the maximum rate of growth of *S. itersonii* (Fig. 1). The data show (Table 2) no decrease in the maximally induced specific activities of selected carbohydrate catabolizing enzymes for as long as 1.62 cell generations after the addition of succinate. In fact, the specific activities of these enzymes and of D-fructose uptake activity increased slightly during this time period. The results suggest that succinate is not a strong catabolite repressor of these inducible catabolic systems in *S. itersonii*, although succinate supports a more rapid rate of growth of this bacterium than does D-fructose.

DISCUSSION

The inability of *S. itersonii* to grow on D-glucose as a sole source of carbon and energy appears to be due to the impermeability of D-glucose to this organism. Phosphorylation of D-glucose apparently is not a factor because the low, but easily detectable level of glucokinase activity could phosphorylate D-glucose, if it were able to easily permeate the cell envelope of this organism. The initial step of D-fructose catabolism in this bacterium has not been determined. However, we have found that the inducible glucokinase does not catalyze ATP-dependent phosphorylation of D-fructose to D-fructose-6-phosphate (unpublished data). An investigation of the possible presence and function of the phosphoenolpyruvate: hexose phosphotransferase system (PEP-PTS) (14) in this aerobic bacterium might provide some explanation of the mechanisms employed in hexose transport and phosphorylation. However, PEP-
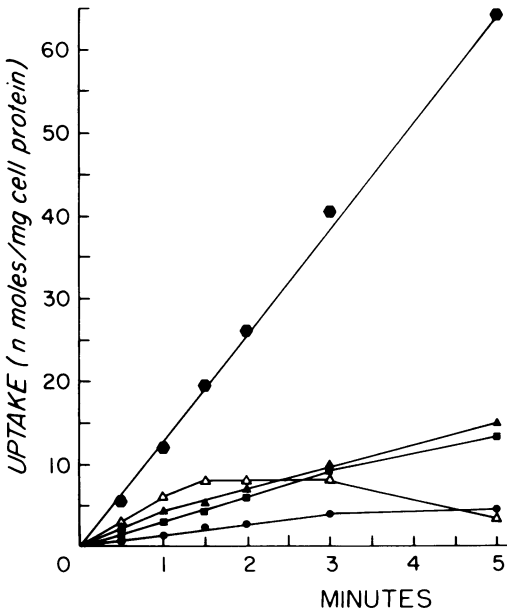
FIG. 6. *Effects of selected metabolic inhibitors on ^{14}C-D-fructose uptake in S. itersonii. Cells were prein-cubated 5 minutes in sodium phosphate buffer plus inhibitor before uptake was initiated by the addition of ^{14}C-D-fructose. No inhibitors ($\bigcirc$), 10 mM sodium azide ($\blacktriangle$), 10 mM potassium cyanide ($\bullet$), 1 mM 2,4-dinitrophenol ($\blacksquare$), and 0.1 mM p-chloromer-curibenzoate ($\triangle$).*

PTS activity is reported lacking in *P. aeruginosa* (12) and several other genera of aerobic oxidative bacteria (13).

The stimulation of D-fructose uptake activity by Na^+ ions was unexpected because added Na^+ ions are neither required in the growth medium nor does addition of NaCl increase the rate of growth of this organism on D-fructose. However, there are several reports of Na^+-stimulated transport systems in bacteria (2, 17, 19, 21). Nevertheless, the observation that potassium phosphate washed cells "regain" ^{14}C-D-fructose uptake activity after incubation indicates per-haps an essential transport factor may be lost during washing with potassium phosphate, but not with sodium phosphate buffer. This obser-vation merits further investigation.

Sobel and Krulwich (18) recently reported that *Arthrobacter pyridomolis*, which usually grows only on D-fructose and not D-glucose, can use D-glucose after prior growth on tricarboxylic acid cycle intermediates. The data presented in Fig. 1 show that no additional growth of *S. itersonii* occurred in a medium containing 5 mM succinate plus 30 mM D-glucose beyond what would be expected to occur on 5 mM

TABLE 1. *Specific activities of carbohydrate catabolizing enzymes in cell extracts of S. itersonii ATCC 12639 when cultured on selected carbon and energy sources[a]*

Enzyme	0.3% CA alone	Carbon source for growth[b]		
		Succi-nate (30 mM)	Fructose (30 mM)	Glycerol (40 mM)
Glucokinase	<1	<1	19	45
Glucose-6-phosphate dehydrogenase (NADP⁺)	<1	<1	32	7
6-Phosphogluconate dehydrogenase (NADP⁺)	<1	<1	<1	<1
6-Phosphogluconate dehydratase	<1	<1	29	8
KDPG aldolase[c]	<1	<1	96	58
Fructose-1,6-diphos-phate aldolase		9	12	
Glucose dehydrogenase (membrane associated)	<1	<1	<1	<1
Glucose-6-phosphate isomerase	65	70	70	66

[a] Specific activities expressed as nanomoles per minute per milligram of extract protein.

[b] All culture media contained 0.05% CA plus indi-cated carbon source.

[c] KDPG, 2-Keto-3-deoxy-6-phosphogluconate.

TABLE 2. *Lack of catabolite repression of certain carbohydrate catabolizing enzymes and D-fructose uptake activity after the addition of succinate to cells initially growing on D-fructose-CA[a]*

No. cell gen-erations after succinate addition	Glucose-6-P dehy-drogenase	6-P-G de-hydratase plus KDPG aldolase[b]	Glucose-6-P iso-merase (NADP)	Fructose uptake activity[c]
0	100	100	100	100
0.38	105	127	112	107
1.00	118	100	100	104
1.62	130	113	105	102

[a] Succinate (20 mM) plus 20 mM additional D-fruc-tose was added to a log-phase culture of *S. itersonii* initially growing on 30 mM fructose-CA. Culture samples were taken at time intervals thereafter and assayed for enzymes and D-fructose uptake activity. The data are expressed as percent of original specific activities remaining after the addition of succinate.

[b] The assay measures the combined activities of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase.

[c] The initial rates of ^{14}C-D-fructose uptake were de-termined as nanomoles per minute per milligram of cell protein.

succinate alone. Hence, it appears that prior growth on tricarboxylic acid cycle intermediates does not enhance the ability of S. itersonii to grow on D-glucose.

Impermeability of bacterial cells to D-glucose but not D-fructose has been reported for other bacterial genera (20). For instance, Hydrogenomonas H 16 is unable to use D-glucose as a sole carbon source, however, good growth occurred on D-fructose (4). D-Fructose is the only carbohydrate utilized by the wild-type strains of the Hydrogenomonas-Alcaligenes group (1). Gottschalk (3) reported that cells of Hydrogenomonas H 16 cannot transport D-glucose into the cell, thus suggesting a possible explanation for the inability of this organism to grow on D-glucose. Although no D-glucose transport activity could be detected in wild-type strains of Hydrogenomonas H 16, a D-glucose-specific system has been described in certain mutant isolates (16). Therefore, the D-glucose and D-fructose transport activities in S. itersonii appear to be similar to those in Hydrogenomonas H 16.

Probable pathway of D-fructose catabolism. The following results suggest that the Entner-Doudoroff Pathway probably is the major pathway for D-fructose catabolism: the specific activities of 6-phosphogluconate dehydratase, KDPG aldolase, and related enzyme glucose-6-phosphate dehydrogenase are much higher in D-fructose-CA cells than in succinate-CA or CA cells. The inability to demonstrate 6-phosphogluconate dehydrogenase in cells grown on D-fructose-CA suggests that the hexose monophosphate shunt is not utilized in this organism. However, a functional Embden-Meyerhof pathway has not been ruled out by the results of the present study. Although the inducers of the D-fructose catabolic enzymes are not known, they were apparently generated during growth on glycerol-CA (Table 1).

Lack of catabolite repression. The apparent lack of catabolite repression control of the synthesis of certain D-fructose catabolic enzymes by succinate in S. itersonii is unlike the carbohydrate regulatory patterns in certain other oxidative aerobic bacteria such as P. aeruginosa (11). Lack of catabolite repression control of inducible synthesis of these enzymes by substrates which yield maximum growth rates is an interesting contrast to those catabolite repression controls described in a variety of other heterotrophic bacteria such as E. coli (9) and P. aeruginosa (5, 11). As another example, the addition of succinate to cultures of P. aeruginosa initially growing on hexoses resulted in strong and immediate repression of a large number of inducible and independently regulated hexose catabolic enzymes (P. B. Hylemon, and P. V. Phibbs, p. 161. Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972).

LITERATURE CITED

1. Davis, D. H., M. Doudoroff, and R. Y. Stainer. 1969. Proposal to reject the genus Hypergenomonas: taxonomic implications. Int. J. Syst. Bacteriol. 19:375.
2. Frank, L., and I. Hopkins. 1969. Sodium-stimulated transport of glutamate in Escherichia coli. J. Bacteriol. 100:329–336.
3. Gottschalk, G. 1964. Verwertung von Fructose durch Hydrogenomonas H 16. Arch. Mikrobiol. 49:96–102.
4. Gottschalk, G., U. Eberhardt, and H. G. Schlegel. 1964. Verwertung von Fructose durch Hydrogenomonas H 16 (I). Arch. Mikrobiol. 48:95–108.
5. 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.
6. 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.
7. Konig, C., I. Sammler, E. Wilde, and H. G. Schlegel. 1969. Constitutive Glucose-6-phosphate dehydrogenase bei Glucose verwertenden Mutanten von einem kryptischen Wildstamm. Arch. Mikrobiol. 67:51–57.
8. 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.
9. Magasanik, B. 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. 26:249–256.
10. McElroy, L. J., J. S. Wells, Jr., and N. R. Krieg. 1967. Mode of extension of cell surface during growth in Spirillum volutans. J. Bacteriol. 93:499–501.
11. Ng, F. M. W., and E. A. Dawes. 1973. Chemostat studies on the regulation of glucose metabolism in Pseudomonas aeruginosa by citrate. Biochem. J. 132:129–140.
12. Phibbs, P. V. Jr., and R. G. Eagon. 1970. Transport and phosphorylation of glucose, fructose, and mannitol by Pseudomonas aeruginosa. Arch. Biochem. Biophys. 138:470–482.
13. Romano, A. H., S. J. Eberhard, S. L. Dingle, and T. D. McDowell. 1970. Distribution of the phosphoenolpyruvate: glucose phosphotransferase system in bacteria. J. Bacteriol. 104:808–813.
14. Roseman, S. 1969. The transport of carbohydrates by a bacterial phosphotransferase system. J. Gen. Physiol. 54:138s–180s.
15. Rutter, W. J., and J. R. Hunsley. 1966. In S. P. Colowick and N. O. Koplan (ed.), Methods in enzymology, p. 137 vol. 9. Academic Press Inc., New York.
16. Schlegel, H. G., and U. Eberhardt. 1972. Regulatory phenomena in the metabolism of knallgas bacteria. In A. H. Rose and J. F. Wilkinson (ed.), Advances in microbiol physiology, vol. 7. Academic Press Inc., New York.
17. Stock, J., and S. Roseman. 1971. A sodium-dependent sugar co-transport system in bacteria. Biochem. Biophys. Res. Commun. 44:132–138.
18. Sobel, M. E., and T. A. Krulwich. 1973. Metabolism of

D-fructose by *Arthrobacter pyridinolis*. J. Bacteriol. **113**:907–913.

19. Thompson, J., and R. A. MacLeod. 1971. Functions of Na$^+$ and K$^+$ in the active transport of a α-aminoisobutyric acid in a marine pseudomonad. J. Biol. Chem. **246**:4066–4074.

20. Wilde, E. Untersuchungen über Wachstum and Spei-cherstoffsynthese von *Hydrogenomonas*. 1962. Arch. Mikrobiol. **43**:109–137.

21. Wong, P. T. S., J. Thompson, and R. A. MacLeod. 1969. Nutrition and metabolism of a marine bacterium. XVII. Ion-dependent retention of α-aminoisobutyric acid and its relation to Na$^+$ dependent transport in a marine pseudomonad. J. Biol. Chem. **244**:1016–1025.