Carbohydrate Catabolism of Selected Strains in the Genus Agrobacterium

LARRY O. ARTHUR, LAWRENCE K. NAKAMURA, GRANT ST. JULIAN, AND LEE A. BULLA, JR.

Northern Regional Research Laboratory, Agricultural Research Service, Peoria, Illinois 61604

Received for publication 3 July 1975

Radiorespirometric and enzyme analyses were used to reveal the glucose-catabolizing mechanisms functioning in single strains of seven presumed Agrobacterium species. The Enterob-Doudoroff and pentose cycle pathways functioned in _A. radiobacter_, _A. tumefaciens_, _A. rubi_, and _A. rhizogenes_. Whereas both catabolic pathways were utilized to an almost equal degree in the _A. radiobacter_ and _A. tumefaciens_ strains, use of the Enterob-Doudoroff pathway predominated in the _A. rubi_ and _A. rhizogenes_ strains. _A. stellulatum_ catabolized glucose almost solely through the Enterob-Doudoroff pathway. In _A. pseudotsugae_ and _A. gypsophilae_, glucose was metabolized mainly through the Emden-Meyerhof-Parnas pathway; the pentose phosphate pathway was also utilized.

According to Berger's *Manual of Determinative Bacteriology*, 7th ed. (3), the genus *Agrobacterium* is heterogenous and contains five phytopathogenic species (_A. tumefaciens_, _A. rubi_, _A. rhizogenes_, _A. gypsophilae_, and _A. pseudotsugae_) and two nonphytopathogenic ones (_A. radiobacter_ and _A. stellulatum_). In addition to pathogenicity, classification is based on indole production, congo red absorption, and nitrate reduction. Modified taxonomic schemes based on deoxyribonucleic acid (DNA) composition and homology, flagellation, 3-ketolactose production, phytopathogenicity, and numerical analyses of nutritional requirements (5, 7, 9, 24) group _A. radiobacter_, _A. tumefaciens_, _A. rhizogenes_, and _A. rubi_ as closely related organisms separate and apart from _A. gypsophilae_, _A. pseudotsugae_, and _A. stellulatum_. In fact, some taxonomists suggest that _A. tumefaciens_, _A. rhizogenes_, and _A. rubi_ are pathogenic variants of _A. radiobacter_. However, results of other taximetric DNA compositional analyses and hybridization tests (5, 9) place in question this proposed relatedness of _A. rubi_ and _A. rhizogenes_ to _A. radiobacter_. The validity of including _A. stellulatum_, _A. gypsophilae_, and _A. pseudotsugae_ in the genus *Agrobacterium* can be challenged directly from results of these latter experiments. Berger's *Manual*, 8th ed. (4), now places these three species as *incertae sedis*. Obviously, additional characterization of the agrobacteria would facilitate establishing and delineating their position in any taxonomic scheme.

Although the DNA, serological properties, and protein similarities of the agrobacteria have been extensively investigated, only limited work has been done on determining the carbohydrate-catabolizing mechanisms of these organisms. The work done indicated that _A. tumefaciens_ utilizes D-glucose by strictly aerobic mechanisms involving pentose cycling (PC) reactions and the Entner-Doudoroff (ED) pathway (1, 22). In the present study we identified and quantitated by radiorespirometry and enzyme analyses the glucose-catabolizing pathways functionings in selected strains of seven species in the genus _Agrobacterium_. (This paper was presented in part at the 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., 23-28 April 1972.)

**MATERIALS AND METHODS**

Organisms and cultural conditions. _A. radiobacter_ NRRL B-164, _A. tumefaciens_ NRRL B-36, _A. rubi_ NRRL B-4017, _A. rhizogenes_ NRRL B-193, _A. pseudotsugae_ NRRL B-4016, and _A. gypsophilae_ NRRL B-4015 were obtained from the Agricultural Research Service culture collection maintained at the Northern Regional Research Laboratory. _A. stellulatum_ TS101 (NRRL B-4018) was kindly furnished by M. P. Starr, Department of Bacteriology, University of California, Davis.

All cultures, except _A. stellulatum_, were maintained on yeast-malt agar slants (8). _A. stellulatum_ was transferred biweekly on artificial seawater agar consisting of 5.0 g of peptone, 1.0 g of yeast extract, 0.1 g of iron citrate, 19.45 g of NaCl, 5.0 g of MgCl2, 3.74 g of Na2SO4, 1.8 g of CaCl2, 0.22 g of KCl, 0.16 g of NaHCO3, 80.0 mg of KBr, 34.0 mg of SrCl2, 2.0 mg of H3BO3, 4.0 mg of Na silicate, 2.4 mg of NaF, 1.6

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1 Present address: Frederick Cancer Research Center, Frederick, Md. 21701.
2 Present address: U. S. Grain Marketing Research Center, Agricultural Research Service, Manhattan, Kan. 66502.
mg of NH₄NO₃, 8.0 mg of Na₂HPO₄, 20.0 g of agar, and 1.0 liter of water.

For enzyme analyses and radiorespirometric experiments, A. radiobacter, A. tumefaciens, A. pseudoalga, and A. glycosolae strains were cultured in a chemically defined medium described by Kane

329 shiro (11). The strains A. rubi and A. rhizogenes grew actively in the chemically defined medium with yeast-malt broth (20:1 [vol/vol]), and A. stellatum in the chemically defined medium with seawater broth (10:1 [vol/vol]). All cultures were incubated at 25°C on a rotary shaker (250 rpm).

Preparation of cell extracts. Midexponential phase cells were collected by centrifugation at 4°C. After being washed once in an equal volume of distilled water, the cells were suspended in 0.02 M phosphate buffer (pH 7.4) and disrupted in a French pressure cell. Nearly all the cells were ruptured after the suspensions were processed twice. The mixtures were centrifuged at 12,000 × g for 30 min at 4°C to obtain crude extracts; in turn, the extracts were separated into soluble and particulate fractions by centrifugation at 105,000 × g for 120 min at 4°C. Protein in the extracts was determined by the method of Lowry et al. (16).

Enzyme assays. Enzymes were assayed spectrophotometrically with a Beckman Acta III doublebeam, multiple-sample absorbance recorder by standard procedures (25) unless otherwise indicated. Specific activities are expressed as enzyme units per milligram of protein in the cell-free extracts. One enzyme unit is defined as the quantity of enzyme that converted 1 μmol of substrate per min.

Phosphoenolpyruvate-glucose phosphotransferase was assayed by the procedure of Ghosh and Ghosh (6). Glucokinase (adenosine triphosphate [ATP]:α-glucose 6-phosphotransferase, EC 2.7.1.2) was measured by observing nicotinamide adenine dinucleotide (NAD) reduction in the presence of excess glucose 6-phosphate dehydrogenase. Glucose 6-phosphate dehydrogenase (α-glucose-6-phosphate:nicotinamide adenine dinucleotide phosphate [NADP] oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluco-

nate:NAD oxidoreductase [decarboxylating], EC 1.1.1.44) were determined by assays of NADP reduction.

Transketolase (α-sedoheptulose-7-phosphate:α-glyceraldehyde-3-phosphate glycoaldehyde transferase, EC 2.2.1.1) activity was determined by following reduced NAD (NADH) oxidation in a triose isomerase-α-glycerophosphate dehydrogenase-coupled reaction that forms glyceraldehyde-3-phosphate from ribose-5-phosphate. Transaldolase (sedoheptulose-7-phosphate:α-glyceraldehyde-3-phosphate dihydroxyacetone transferase, EC 2.2.1.2) activity was determined by following NADH oxidation in the presence of fructose-6-phosphate, erythrose-4-phosphate, and excess triose phosphate isomerase and α-glycerophosphate dehydrogenase.

The assay for 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (6-phospho-2-keto-3-deoxy-D-glu-

conate:α-glyceraldehyde-3-phosphate lyase, EC 4.12.14) was accomplished by observing NADH-dependent reduction of pyruvate in the presence of excess lactic dehydrogenase. KDPG was the generous gift of W. A. Wood, Department of Biochemistry, Michigan State University, East Lansing.

Glucosephosphate isomerase (α-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) was measured in the reverse direction by monitoring the reduction of NADP in the presence of fructose-6-phosphate and excess glucose 6-phosphate dehydrogenase. Phospho-

fructokinase (ATP:α-fructose 6-phosphate-1-phos-

photransferase, EC 2.7.1.11) was assayed by coupl-

ing with fructose-1, 6-diphosphate aldolase, triose-

phosphate isomerase, and α-glycerophosphate dehy-

drogenase and following NADH oxidation. Fructose-

1,6-diphosphate aldolase (Fructose-1, 6-diphosphate: α-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13) activity was determined by following NADH oxidation during formation of triose phosphates from fructose-1,6-diphosphate in the presence of excess glycerophosphate dehydrogenase and triosephosphate isomerase.

Fructose-6-phosphate phosphoketolase (EC 4.1.2.22) was measured by two different methods. In the first method, acetyl phosphate formed from fructose-6-phosphate was determined as a derivative of hydroxamic acid (20). The reaction mixture consisted of 0.5 ml of 6 mM fructose-6-phosphate, 0.1 ml of 100 mM MgCl₂, 0.1 ml of 25 mM 1,2-ethanediol, 0.1 ml of 45 mM Na₂HPO₄, 0.1 ml of 1 mM thiamine pyrophosphate, and 0.8 ml of 45 mM tris(hydroxymethyl)aminomethane - hydrochloride buffer (pH 7.2) plus cell-free extract and distilled water. Final volume was 2 ml. This reaction mixture was incubated at 30°C, and at appropriate intervals fractions were removed and combined with equal volumes of hydroxylamine solution (4 M hydroxylamine-hydrochloride and 3.5 M NaOH) and 0.1 M acetate buffer (pH 5.4). The hydroxamic acid derivative of acetyl phosphate formed in this reaction mixture was assayed spectrophotometrically at 505 nm after addition to the above mixture of equal volumes of ferric chloride (5% FeCl₃·6H₂O in 0.1 N HCl), 3.86 N HCl, and 12% trichloroacetic acid. The second method involved the determination of glycer-

aldehyde-3-phosphate by following at 340 nm the oxidation of NAD in the presence of fructose-6-

phosphate, transaldolase, excess triose isomerase, and α-glycerophosphate dehydrogenase. Pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) was assayed by following the formation of pyruvate from phosphoenolpyruvate via NADH oxida-

tion in a coupled enzyme system containing ex-

cess lactic dehydrogenase. Lactic dehydrogenase (t-

lactate:NAD oxidoreductase, EC 1.1.1.27) was mea-

sured by following NADH oxidation in the presence of pyruvate.

Radiorespirometric method. Cells for radiorespirometric experiments were harvested by centrifugation at 4°C. The cells were washed three times in 0.05 M phosphate buffer (pH 7.4) and then sus-
pended without substrate in phosphate buffer at a cell concentration of 2 to 3 mg/ml (dry weight).

Radiorespirometric experiments, each done in triplicate, were performed as described earlier (1). All radiochemicals used in this study were obtained from New England Nuclear Corp., Boston, Mass.
Respirometer flasks containing 60 to 100 mg of cells were incubated at 28°C in a modified Gilson differential respirometer. Specifically labeled substrate (0.25 µCi) was added from a side arm; each flask contained 0.5 to 1 mg of radioactive substrate. Final volume was 30 ml. Flasks were shaken while air was passed through at a rate of 60 ml/min. To decrease endogenous metabolism, cultures were incubated for 60 to 90 min before addition of substrate. Respiratory ¹⁴CO₂ was trapped in 10 ml of a mixture of absolute ethyl alcohol and monoethanolamine (2:1 [vol/vol]); the trapping solution was removed and a fresh supply was provided at hourly intervals.

Trapping solutions containing ¹⁴CO₂ were adjusted to 15 ml with absolute ethyl alcohol; a 5-ml portion of each was mixed with 10 ml of toluene containing 6 µg of 2,5-diphenyloxazole per ml and 0.1 mg of 1,4-bis-[2]-(5-phenyloxazolyl)benzene per ml. The mixtures were placed into scintillation vials, and radioactivity was measured at balance-point conditions with an automatic liquid scintillation spectrometer. Standard deviation of radioactive measurements was no greater than 2%

At the end of each experiment, the culture flasks were chilled quickly and cells were separated by centrifugation at 4°C. Cells were homogenized in NCS solubilizer (Amersham/Searle Corp., Des Plaines, Ill.) and incubated at 37°C for 12 h; the quantity of radioactive carbon incorporated was then determined by suspending the cells in a scintillation cocktail containing (vol/vol): toluene-2,5-diphenyloxazole-1,4-bis-[2-(5-phenyloxazolyl)]benzene: Triton X-100:ethyl alcohol (8:4:3). Samples of the phosphate buffer also were mixed with this scintillation mixture and analyzed. Counting efficiency for each sample was determined by appropriate internal standards.

Calculations. Total ¹⁴CO₂ recoveries for [4-¹⁴C]glucose were calculated by comparing total recoveries from both [3-¹⁴C]glucose and [3,4-¹⁴C]glucose. Quantity of labeled C₄ recovered was calculated from the following relationship: C₄ = 2(C3,4) – (C3). Relative participation of carbohydrate catabolic pathways involving simultaneous operation of the Embden-Meyerhof-Parnas (EMP) and pentose phosphate (PP) pathways and concurrent activity of the ED and PC pathways was estimated according to the methods of Wang et al. (23) and of Arthur et al. (1), respectively.

**RESULTS**

Identifying and estimating the percent participation of pathways involved in glucose catabolism in the various organisms required (i) comparing cumulative ¹⁴CO₂ generated from specifically labeled D-glucose, (ii) analyzing maximal interval rates of ¹⁴CO₂ released from specifically labeled D-glucose, and (iii) assaying for the presence in extracts of key glucose-catabolizing enzymes. Recorded in Table 1 is an accounting of the various quantities of labeled radioisotope recovered in experiments measuring glucose utilization by the seven agrobacteria. Maximal interval releases of respiratory ¹⁴CO₂ by each species utilizing specifically labeled D-glucose are plotted in Fig. 1. Estimates of the relative extent of utilization of the various pathways by each organism are presented in Table.
2. A catalog of key enzymes for glucose catabolism is recorded in Table 3.

**A. radiobacter** and **A. tumefaciens.** In terms of rate and extent of conversion, the preferential order of oxidation of the glucose carbon skeleton was C1 > C2 > C4 > C3 > C6 for **A. radiobacter** (Fig. 1a). **A. tumefaciens** displayed so similar a pattern that separate graphs illustrating the activities of both **A. tumefaciens** and **A. radiobacter** are not presented (see reference 1). Active evolution of C1, C2, and C4 as CO₂ indicated that the major mechanisms for catabolizing glucose were the ED and PC pathways (1), utilized to the extent of 56 and 44%, respectively, in **A. radiobacter** (Table 2) and 55 and 44%, respectively, in **A. tumefaciens** (1).

**A. rubi** and **A. rhizogenes.** The preferred order of oxidizing glucose carbons by **A. rubi** was C1 > C4 > C2 > C3 > C6 (Fig. 1b), suggesting concurrent operation of the ED and PP pathways (28). In contrast to the activity displayed by **A. radiobacter** and **A. tumefaciens**, the greater and more rapid oxidation of C4 than C2 by **A. rubi** and **A. rhizogenes** revealed that catabolism proceeded predominantly through the ED pathway. Participation of the ED and PC pathways was 77 and 23%, respectively (Table 2); for **A. rhizogenes** (Fig. 1c), the ED was 88% and the PC was 12%. Significantly, **A. rubi** utilized PC reactions extensively as evidenced by its release of a greater quantity of C2 as CO₂ (Fig. 1b) than **A. rhizogenes** achieved (Fig. 1c). The possibility that **A. radiobacter**, **A. tumefaciens**, **A. rubi**, and **A. rhizogenes** could operate the ED and PC pathways was confirmed by demonstration of the presence in ex-
tracts of the necessary enzymes (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, transketolase, transaldolase, and KDPG aldolase). Detection of no more than minimal quantities of phosphofructokinase and fructose-1,6-diphosphate aldolase is consistent with the hypothesis based on results from radiorespirometry that the EMP pathway did not operate in the four organisms.

A. stellulatum. A. stellulatum oxidized C1 and C4 to CO₂ at approximately equal rates whereas C2, C3, and C6 were oxidized less rapidly (Fig. 1d). Equivalence of CO₂ evolution from C1 and C4 and the presence of KDPG aldolase (Table 3) strongly suggest that glucose was metabolized almost exclusively via the ED pathway. Although PP pathway enzymes were detected, they appeared not to function during primary glucose metabolism in nonproliferating cells. The apparent inactivity of the PP pathway and absence of EMP enzymes leave the tricarboxylic acid (TCA) cycle as the most likely mechanism for oxidizing C2, C3, and C6 to CO₂. Further evidence for involvement of the TCA cycle is the delayed release of C2, C3, and C6 relative to C1 and C4. Preliminary unpublished radiorespirometric assays of release of CO₂ from specifically labeled TCA cycle intermediates indicate that in the seven species of

| Identity | Participation of glucose pathways (%) | ED | PC | EMP |
|----------|---------------------------------------|----|----|-----|
| A. radiobacter NRRL B-164 | 56 | 44 |
| A. tumefaciens NRRL B-36 | 55 | 45 |
| A. rubi NRRL B-4017 | 77 | 23 |
| A. rhizogenes NRRL B-193 | 88 | 12 |
| A. stellulatum NRRL B-4018 | 100 |
| A. pseudotsugae NRRL B-4016 | 22 | 78 |

* Estimated by method of Arthur et al. (1).
* Estimated by inspection of Fig. 1d.
* Estimated by method of Wang et al. (23).
* Represents PP pathway rather than PC activity.
* Because three pathways may function, percent participation calculations were not made for this organism.

Table 3. Activity of enzymes of glucose catabolism in Agrobacterium strains

| Enzyme | A. radiobacter NRRL B-164 | A. tumefaciens NRRL B-36 | A. rubi NRRL B-4017 | A. rhizogenes NRRL B-193 | A. stellulatum NRRL B-4018 | A. pseudotsugae NRRL B-4016 | A. pseudotsugae NRRL B-4016 |
|--------|---------------------------|--------------------------|------------------|--------------------------|-----------------------------|-----------------------------|-----------------------------|
| Phosphoenolpyruvate-glucose phosphotransferase | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Gluokinase | 85 | 84 | 66 | 100 | 10 | 83 | 23 |
| Glucose-6-phosphate dehydrogenase | 19 | 28 | 46 | 24 | 5 | 22 | 20 |
| Phosphoglucoisomerase | 89 | 81 | 35 | 51 | 16 | 24 | 71 |
| Fructose-6-phosphate phosphoketolase | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Phosphofructokinase | <0.1 | <0.1 | <0.1 | <0.1 | 5 | 15 | 18 |
| Fructose-1,6-diphosphate aldolase | <0.8 | <0.8 | <0.8 | <0.8 | <0.8 | 23 | 18 |
| 6-Phosphogluconate dehydrogenase | 19 | 23 | 46 | 24 | 8 | 22 | 20 |
| KDPG aldolase | 195 | 185 | 185 | 205 | 23 | <0.2 | 55 |
| Pyruvic kinase | 56 | 83 | 69 | 23 | 16 | 23 | 25 |
| Lactic dehydrogenase | <0.5 | <0.5 | 28 | 56 | 23 | 16 | 28 |
| Transaldolase | 120 | 240 | 40 | 250 | 63 | 160 | 106 |
| Transketolase | 123 | 106 | 129 | 110 | 11 | 12 | 32 |
| Enolase | 191 | 153 | 177 | 174 | 35 | 137 | 34 |

* Cells were cultured as described in Materials and Methods and harvested during midexponential growth.
* Values are expressed as 10⁻² enzyme unit per mg of protein and are the average of three separate measurements. To calculate net specific activities of enzyme reactions involving either NADPH or reduced NADP oxidation, NADH oxidase and reduced NADP oxidase activities were subtracted from the respective total activity values.
* Data previously reported in reference 1.
agrobacteria it is utilization of the TCA cycle for oxidation of the catabolic intermediates during and after depletion of glucose that gives the observed order and timing of CO₂ labeling.

A. pseudotsugae and A. gypsophilae. That the EMP pathway predominated in A. pseudotsugae and A. gypsophilae was evidenced by the preferential oxidation of C3 and C4 to CO₂ (Fig. 1e, f). Furthermore, selective decarboxylation of C1 over C6 and C4 over C3 indicates operation of the PP pathway in these bacteria. The respective extent of operation of the EMP and PP pathways in A. pseudotsugae was 78 and 22% (Table 2). Because three pathways may be operative, the fractional contribution of each by A. gypsophilae was not calculated. Recovery of C2 and C6 and CO₂ suggests extensive cycling of carbons via pentoses. The redistribution of carbon atoms presumably followed the pattern described by Beevers (2). Extracts of A. pseudotsugae exhibited no KDPG aldolase activity, whereas those of A. gypsophilae did (Table 2).

In fact, the specific activity for this enzyme was as high as that of other enzymes assayed in A. gypsophilae. Therefore, PP and ED pathways possibly provide supplementary mechanisms for glucose catabolism in A. gypsophilae; in A. pseudotsugae, C1 and C2 of glucose was catabolized only through the PP and EMP pathways.

**DISCUSSION**

From the foregoing studies, the following groupings appear valid: (i) in the strains of A. radiobacter and A. tumefaciens used, glucose catabolism was attributable almost equally to the activity of the enzymes of the ED and PC pathways, whereas the A. rhizogenes and A. rubi strains utilized the ED pathway predominantly and the PC pathway to a lesser degree; (ii) the A. stellulatum strain catabolized glucose solely via the ED pathway; and (iii) the A. gypsophilae and A. pseudotsugae strains catabolized glucose mainly by the EMP pathway with minor involvement of the PP or ED pathways or both.

In recent taxonomic schemes based on biochemical and physiological criteria, A. radiobacter, A. tumefaciens, A. rhizogenes, and A. rubi are the only four species recognized as agrobacteria (5, 24). Furthermore, it has been suggested that A. tumefaciens, A. rhizogenes, and A. rubi are merely pathogenic variants of A. radiobacter (5, 7, 9, 13). Accordingly, it is not surprising that representative strains of these four organisms displayed similar mechanisms for glucose catabolism, although fractional concurrent degrees of utilization of the pathways varied. Nor is it unexpected that A. stellulatum, A. gypsophilae, and A. pseudotsugae utilized different ones. Previous radiorespirometric studies demonstrated that utilization of a common set of mechanisms for glucose catabolism may be heavily relied upon to characterize species of well-defined genera (19, 27, 28).

Numerical taxonomic analyses (7, 24) and DNA composition (5) and homology (9) reveal similarities between A. radiobacter and A. tumefaciens and serve as bases for arguments favoring the designation of A. tumefaciens as a pathogenic variant of A. radiobacter. That strains representative of these two presumed species used similar mechanisms of glucose metabolism is consistent with this hypothesis. Although there are contradicting opinions (12, 17), most workers have concluded (as we have also on the basis of the current radiorespirometric studies) that A. rhizogenes can be distinguished from A. tumefaciens and A. radiobacter, and from A. rubi as well.

The species status of A. rubi is not as clear as that of A. rhizogenes. Some taxonomists argue that A. rubi and A. tumefaciens are identical because: (i) A. rubi is not specifically pathogenic for the Rubus plant; (ii) in terms of guanine-cytosine and homology, the DNA of A. rubi is very similar to that of A. tumefaciens; and (iii) nitrite and indole production by each is approximately equivalent (5, 9, 14, 17). In contrast, numerical analyses align A. rubi more closely to the other agrobacteria but designate it as a separate entity (24). Our study reveals similar mechanisms of glucose catabolism for A. rubi and A. tumefaciens but demonstrates that the metabolic relationship between these two organisms is not as close as that between A. radiobacter and A. tumefaciens.

The following factors heavily weigh against including A. stellulatum in the genus Agrobacterium: (i) polar flagellation, (ii) preferential growth on seawater medium, (iii) non-pathogenicity for the tomato and Datura plants, (iv) failure to produce 3-ketolactose, and (v) compositional characteristics of its DNA (5, 9). Marked use of the ED pathway in A. stellulatum (see Fig. 1) is consistent with De Ley's suggestion (5) that this bacterium is more closely related to the marine pseudomonads than to the agrobacteria.

The emergence of the EMP pathway as the principal means for glucose utilization in A. pseudotsugae and A. gypsophilae represents a clear divergence of these bacteria from the metabolic pattern exhibited by A. radiobacter, A. tumefaciens, A. rubi, and A. rhizogenes. Evidence based on differences in DNA properties, lack of tumorigenicity, pigment formation, and
numerical analyses opposed the inclusion of *A. pseudotsugae* and *A. gypsophilae* in the genus *Agrobacterium* (5, 9, 24).

Catabolism of glucose by the ED pathway without EMP pathway involvement (as in the *A. radiobacter, A. tumefaciens, A. rubi, A. rhizogenes, and A. stellulatum* strains used here) is characteristic of other aerobic bacteria such as *Pseudomonas aeruginosa* (15, 21), *P. stutzeri* (19), and several strains of *Neisseria gonorrhoeae* (18). In the absence of EMP capacity, carbon cycling via pentoses could be a significant feature in those bacteria that utilize the ED pathway as the major respiratory pathway and as the primary mechanism for glucose assimilation as well as for production of biosynthetic intermediates. Albeit, glucose was observed to induce hexose-catabolizing enzymes in *P. aeruginosa* (10), we observed no such enhancement of activity in glycerol-grown cells of *A. tumefaciens* (1), and furthermore, no affect by 3-ketoglucose-synthesizing system on the relative participation of ED and PC pathways.

ACKNOWLEDGMENTS

We thank W. A. Wood, Michigan State University, for his helpful advice during this investigation. Special appreciation is given to L. Adams for his able technical assistance in this work.

L. O. A. was the recipient of a postdoctoral resident research association established by the Agricultural Research Service, U.S. Department of Agriculture, in association with the National Academy of Sciences-National Research Council, 1970–1972.

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