Comparative Glucose Catabolism of Xanthomonas Species

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Glucose catabolism in eight Xanthomonas species has been comparatively examined by means of the radiorespirometric method. The basic mechanisms for the respective xanthomonads closely resembled each other. The Entner-Doudoroff pathway, in conjunction with the tricarboxylic acid cycle pathway, was the predominant mechanism for glucose catabolism. A small portion (8 to 16%) of substrate glucose was routed into the pentose phosphate pathway. The hexose cycle pathway did not appear to play any significant role in glucose catabolism of these xanthomonads. The results are also consistent with the well-recognized close phylogenetic relationship between xanthomonads and pseudomonads.

The species included under the genus Xanthomonas form a well-known group of plant pathogens and are of great interest to phytopathologists. At present, there are more than 60 recognized species described in Bergey's Manual of Determinative Bacteriology (7th ed.), although the justification for the recognition of these species is questionable. There are close similarities among some of the species with respect to their morphology, physiology, and biochemistry (2, 8, 15, 16), and there is a high degree of cross-reaction between Xanthomonas species and their antisera (5-7, 14).

Host specificity has been considered chiefly, if not exclusively, the principal criterion for the separation of the species, but classification on this basis has not proven to be very sound in practice. Dye (3) successfully demonstrated the possibility of cross-infection; species isolated from 20 different hosts had characteristically infected the same bean plant (Phaseolus vulgaris, L.). These findings thus undermined the concept hereto considered valid that “there is no possibility of error in using the host specificity as a species determiner” (22). Moreover, a thorough investigation with regard to a complete host range of xanthomonads has never been carried out (3, 5-7).

Colwell and Liston (1), applying Adansonian numerical taxonomy, concluded that Xanthomonas species and Pseudomonas species were clearly distinct at the genus level, and that the Xanthomonas species could be divided into two subgroups. However, under each subgroup, many species appear to be indistinguishable.

Limited information is known on the carbohydrate metabolism of Xanthomonas species. Katznelson (9, 10) reported that certain intact xanthomonad cells oxidize glucose readily, but that they are not capable of utilizing gluconate as a carbon source. Sonic extracts of X. pruni as well as of X. campestris oxidized gluconate, but X. phaseoli did not. The author suggested that phosphohexokinase may be absent, inactive, or very labile in Xanthomonas organisms and that 6-phospho-gluconate is readily degraded to yield pyruvate and triose-3-phosphate.

Hochster and Katznelson (11), working with cell-free extracts of X. phaseoli, demonstrated the operation of the “hexose cycle” (HC) pathway for the metabolism of glucose-6-phosphate in the organism. Firstly, glucose is catabolized via the Entner-Doudoroff (ED) pathway, thus giving rise to pyruvate and glyceraldehyde-3-phosphate. The latter compound, in turn, gives rise to the formation of fructose-1 (F-1), 6-diphosphate by the action of triosephosphate isomerase and aldolase. In the absence of an active glycolytic pathway, F-1, 6-diphosphate is readily converted to fructose-6-phosphate (F-6-P) and then to glucose-6-phosphate (G-6-P), thus completing the HC cycle sequence. Madsen and Hochster (12) also demonstrated the presence of most of the enzymes of the tricarboxylic acid cycle and the glyoxylate cycle pathways in X. phaseoli.

The present work concerns a comparative study of the pathways of glucose catabolism in eight species of Xanthomonas, by use of the radiorespirometric method (18, 20). The study also aims at a better understanding of the phylogenetic relationships among these organisms.
Materials and Methods

Eight species of Xanthomonas were selected to represent a broad spectrum with respect to their respective pathogenic host specificity. The organisms are: X. hyacinthi, ATCC 12612; X. translucens, ATCC 10770; X. juglandis, ATCC 11329; X. pruni, ATCC 10016; X. malvacearum, ATCC 12131; X. carotae, ATCC 10547; X. pelargonii, ATCC 11057; X. phaseoli, ATCC 9563.

The stock cultures of these organisms prepared from the original lyophilized cultures were maintained on agar slants at approximately 18°C and transferred to fresh medium every 2 months. When cultures were kept in a refrigerator, their viability was markedly reduced. The slants were made up of the same medium used for the growth of the cells, with the addition of 1.8% Difco agar. This medium was essentially that described by Payne (13); however, the concentration of MgSO4·7H2O was reduced by half, and the inorganic nutrients (0.1 mg/100 ml) and yeast extract (10 mg/100 ml) added. The basal medium was adjusted to pH 6.8 with 0.1 N NaOH, after sterilization. Glucose and yeast extract were sterilized separately and were added aseptically to provide a final concentration of 0.25 and 0.07%, respectively.

Three transfers of small inocula were made at convenient intervals starting from the slants. The xanthomonads were then grown under aerobic conditions in 130 ml of medium contained in 250 ml Erlenmeyer flasks. The flasks with X. hyacinthi, X. juglandis, and X. carotae were incubated at 30°C, and the others, at 27°C in a rotary shaker. The cells were harvested by centrifugation at the late exponential phase of growth (18-22 hr). The cells grown under these conditions were resuspended in a fresh cultural medium, containing no carbohydrates. Cell concentrations, determined turbidimetrically, were adjusted to a desirable level. The optimal cell concentration was determined on the basis that a reasonable amount of substrate glucose can be completely utilized within 4 to 5 hr. The cell suspensions were then transferred to flasks for radiorespirometric experiments.

The labeled substrates glucose-1-14C, glucose-2-14C, glucose-3-14C, and glucose-6-14C were obtained from the National Bureau of Standards. Glucose-3,4-14C was obtained in this laboratory from liver glycogen of rats metabolizing 14C according to the method of Wood, Lifson, and Lorber (24).

The radiorespirometric experiments were carried out according to the procedures previously described by Wang (18, 20). Generally, 10 ml of cell suspension was placed in each incubation flask so as to utilize a chosen amount of the labeled substrate aerobically in the growth medium. Usually five incubation flasks were used in an experiment, to study the utilization of five specifically labeled glucose-14C substrates. Respiratory 14CO2 released by the cells was swept through 10 ml of an ethanolamine-ethyl alcohol solution (2:1, v/v), which was replaced at hourly intervals. The trap solution, containing respiratory 14CO2 evolved during the hour, was processed for radioactivity measurement. The experiment was terminated when the rate for the production of respiratory 14CO2 in a given experiment declined to an insignificant level, thus indicating that the administered substrate was exhausted from the medium. Upon termination of the experiment, the vessels containing the cell suspensions were cooled in ice for about 10 min, after which the cells were separated from the medium by centrifugation. Both the washed cells and the supernatant medium thus obtained were processed for measurement of their radioactivity.

The radioactivity in respiratory 14CO2 was assayed by liquid scintillation counting with a Tri-Carb spectrometer (model 314-EX2; Packard Instrument Co., Inc., Downers Grove, Ill.) in the manner described by Wang (18, 20). The radioactivity of the cells and the excreted compounds in the media was determined by liquid scintillation counting by use of thioctropic gel techniques (23).

The counting efficiency of the liquid scintillation counter for different types of samples was determined by the internal standard technique. Countings were carried out over a sufficient period of time to give a relative standard deviation no greater than 2%.

Results

Experimental conditions and substrate inventories of the radiorespirometric experiment on the utilization of glucose by eight xanthomonads are given in Table 1. The data on substrate inventory are those collected at the end of the time course of utilization. The data given in Table 1 represent the average of four replica experiments, with deviation among replica experiments being less than 3%. The data on the peak rate of production of respiratory 14CO2 provide kinetic information on the conversion of individual glucose carbon atoms to 14CO2 in lieu of graphic presentation.

The rates and the extents of production of respiratory 14CO2 by X. phaseoli utilizing various specifically labeled glucose-14C substrates are given in Fig. 1 as a typical example. The graph provides information for the amount of respiratory 14CO2 evolved by the organism during each hourly period of the incubation experiment. The maximum of each of the curves hence provides information on the time and the extent of peak rate of 14CO2 evolution. The data used in constructing the graph represent the average of four replica experiments, with reproducibility better than 3%. The radiorespirometric patterns observed with the other xanthomonads of the present study are basically the same as that shown in Fig. 1 with only minor differences.

Discussion

The radiorespirometric pattern for the utilization of specifically labeled glucose-14C substrates by X. phaseoli (Fig. 1) resembles to a large extent that observed previously in similar experiments.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Peak rate of production of respiratory CO₂</th>
<th>Substrate inventory (%)</th>
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<tbody>
<tr>
<td></td>
<td>Time occurred</td>
<td>Extent (% per hr)</td>
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<tr>
<td>X. hyacinthi, 15 mg (dry wt)/10 ml</td>
<td></td>
<td></td>
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<tr>
<td>X. translucens, 20 mg (dry wt)/10 ml</td>
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<tr>
<td>X. juglandis, 12 mg (dry wt)/10 ml</td>
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<tr>
<td>X. pruni, 14 mg (dry wt)/10 ml</td>
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<tr>
<td>X. malvacearum, 16 mg (dry wt)/10 ml</td>
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<tr>
<td>X. carotae, 13 mg (dry wt)/10 ml</td>
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<tr>
<td>X. pelargonii, 22 mg (dry wt)/10 ml</td>
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<tr>
<td>X. phaseoli, 15 mg (dry wt)/10 ml</td>
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</table>

* Level of the substrates for the various species are (mg/10 ml): X. hyacinthi, 3.0; X. translucens, 3.0; X. juglandis, 5.0; X. pruni, 4.0; X. malvacearum, 4.0; X. carotae, 3.5; X. pelargonii, 1.0; X. phaseoli, 3.0.

* Data pertaining to the substrate glucose-4,14C are calculated from the experimental findings in the glucose-3,14C and glucose-3,4,14C experiments.

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with _Pseudomonas reptilvora_, _P. fluorescens_ KB1, and _P. aeruginosa_ (17, 19, 21). This is not surprising in view of the recognized close relationship between _Xanthomonas_ species and _Pseudomonas_ species. The present radiorespirometric data can be interpreted in much the same manner as that reported for pseudomonads.

As shown in Fig. 1, the conversion of C-1 of glucose to CO₂ is significantly greater than that of C-4, with both being considerably greater in rates and extents than other carbon atoms of glucose. Catabolic equivalence with regard to the formation of respiratory CO₂ is observed between C-3 and C-6 of glucose. These facts suggest that in _X. phaseoli_ glucose is catabolized primarily by way of the ED pathway, with the pentose phosphate (PP) pathway also playing a role, although minor. Since the conversion of C-2 of glucose to CO₂ is considerably less in rate and extent than that of C-1, pentose phosphate formed via the PP pathway does not appear to be catabolized further via the pentose cycle (PC) pathway to any great extent. It is also interesting to note that the observed equivalence of catabolic behaviors between C-3 and C-6 of glucose implies that the HC pathway is not playing an important role in this organism. Otherwise, one would expect to observe a substantially higher yield of C-6 in the respiratory ¹⁴CO₂. The operation of the HC pathway in _X. phaseoli_ has been previously demonstrated by Hochster and Katzenelson (11); however, the role played by the HC pathway relative to other catabolic routes in the overall glucose catabolism has not been indicated by these authors.

The relative yields of respiratory CO₂ derived from C-2, C-3, and C-6 of glucose indicate that the pyruvate, derived either from glucose directly or from glyceraldehyde-3-phosphate formed via the PP pathway, is further catabolized by way of the tricarboxylic acid cycle pathway (12).

The data interpretation of the radiorespirometric findings with _X. phaseoli_ is generally applicable to the other seven species of xanthomonads discussed here. In short, in all the eight species of xanthomonads examined in this study, glucose is catabolized primarily by way of the ED pathway with the PP pathway playing only a minor role. The HC pathway does not appear to be an important mechanism insofar as the overall glucose catabolism is concerned. However, with _X. pelargonii_, the CO₂ yield from C-6 of glucose is noticeably higher than that from C-3; some investigation may indicate that the HC pathway is operative in this organism to some extent.

The estimate of the relative participation of the ED pathway and the PP pathway can be made by the method previously described for studies with pseudomonads (17), assuming that substrate glucose does not engage in the anabolic process to any great extent. The amount of glucose carbon atoms incorporated into cellular constituents (Table 1) provides evidence that a small amount of substrate glucose may have been routed into anabolic processes, although the magnitude is such that it would not introduce serious inaccuracy in the estimated values of relative pathway participation. This conclusion is drawn from two considerations. First, with microorganisms relying on the ED and PP pathways as major routes of glucose catabolism, the extent of conversion of C-1 of substrate glucose to respiratory CO₂ and fermentation products reflects the magnitude of the fraction of substrate glucose that has participated in catabolism; with xanthomonads included in the present study, the extent of such conversion ranges from 70 to 80%. Second, it is known that C-1 of substrate glucose can be incorporated into cellular constituents by way of intermediates derived from glucose catabolism; thus, pyruvates derived from substrate glucose by way of the ED pathway, with its carboxyl carbon atom corresponding to C-1 and C-4 of glucose, can be converted to alanine or to other amino acids without decarboxylation. Moreover, pyruvates can also participate in CO₂ fixation.
reactions to provide C₃ units for cellular biosynthesis.

The relative participation of the two glucose pathways in eight species of xanthomonads is given in Table 2. As shown by the results given in Table 2, the PP pathway plays only a minor role in contrast to the predominant role played by the ED pathway in the overall glucose catabolism in all eight xanthomonads. There exists a close resemblance among these xanthomonads with regard to their basic mechanisms for glucose utilization. It is practically impossible to distinguish one species from the other on this basis. One can also conclude from the results presented in this study that the host pathogenic specificities of the xanthomonads are not derived from variations among their respective catabolic mechanisms for glucose utilization. The findings render strong support to the thesis of Starr (15) that of Dye (4) which express their belief that most of the so-called "species" under the genus Xanthomonas are merely formae specialae of one or of a few principal species. There is indeed a great need to consolidate some of the species to avoid undue confusion insofar as taxonomical principles are concerned. It is true there are some minor differences in catabolic behavior among the xanthomonads studied. However, the findings cannot be correlated to the immunological groupings proposed by Elrod and Braun (5).

The findings in the present work support the well-recognized close phylogenetic relationship between xanthomonads and pseudomonads (2, 17, 21). Previously, on the basis of work by Hochster and Katzenelson (11), the operation of the HC pathway in xanthomonads appeared to be a notable distinction between these two genera, since the HC pathway was not playing any significant role in glucose catabolism of pseudomonads. On the basis of the findings described in the present work, the HC pathway is not an important pathway in the xanthomonads.

ACKNOWLEDGMENT

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LITERATURE CITED


| Table 2. Relative participation of major glucose pathways in Xanthomonas species |
|-----------------|-----------------|
| Species        | Per cent substrate glucose catabolized |
|                | Entner-Doudoroff | Pentose phosphate |
| X. hyacinthi   | 84              | 16               |
| X. translucens | 90              | 10               |
| X. juglandis   | 87              | 13               |
| X. pruni       | 81              | 19               |
| X. malvacearum | 92              | 8                |
| X. carotae     | 90              | 10               |
| X. pelargonii  | 85              | 15               |
| X. phaseoli    | 93              | 7                |