METABOLISM OF PHYTOPATHOGENIC BACTERIA

II. Metabolism of Carbohydrates by Cell-Free Extracts[1]

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Received for publication December 2, 1957

The work presented in this paper is an extension of the studies reported in the first contribution of this series (Katznelson, 1955*b*). It is concerned primarily with the demonstration of the presence in sonic preparations of representative species of phytopathogenic bacteria, of certain key enzymes involved in the glycolytic, and shunt pathways of glucose metabolism. Detailed studies of selected species of the various genera used are underway and will be reported subsquently.

MATERIALS AND METHODS

Unless indicated otherwise, the methods used were the same as those described in previous publications from this laboratory (Katznelson, 1955*b*, 1957; Katznelson and Zagallo, 1957; Mylroie and Katznelson, 1957). Pyruvate production from 6-phosphogluconate or from substrate-coenzyme linked systems yielding 6-phosphogluconate was measured by the direct or double extraction procedure of Friedemann and Haugen (1943). The reaction mixture contained: 0.1 or 0.2 ml sonic extract; 0.1 ml tris(hydroxymethyl)aminomethane buffer (M/1, pH 7.7); 0.1 ml $MgCl_2$ (M/10); 0.2 ml ATP[2] (100 μmoles per ml); 0.2 ml TPN (1.4 μmoles per ml); 0.1 ml substrate (M/20). It was incubated for 30 min at 35 C and the reaction stopped by the addition of 1 ml of a 0.1 per cent solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. For the spectrophotometric tests, reduction of TPN or DPN was followed at 340 mμ in a Beckman DU spectrophotometer. The cuvette contained: 0.01 to 0.1 ml sonic extract; 0.4 ml glycylglycine buffer (M/4 pH 7.4); 0.3 ml $MgCl_2$ (M/10); 0.2 ml TPN or DPN (1.4 μmoles per ml); 0.2 ml ATP (100 μmoles per ml); 0.1 ml substrate (M/20); water to 3.0 ml. Glyceraldehyde-3-phosphate dehydrogenase activity was determined in the following reaction mixture: 0.1 ml sonic extract; 2.0 ml sodium pyrophosphate (M/25, pH 8.5); 0.2 ml reduced glutathione (60 μmoles per ml); 0.4 ml DPN (1.4 μmoles per ml); 0.2 ml sodium arsenate (90 μmoles per ml) and 0.1 ml fructose-1,6-diphosphate (100 μmoles per ml). Aldolase and phosphohexokinase activity was determined by the method of Sibley and Lehninger (1949); fructose-1,6-diphosphate was used as substrate for the aldolase determination whereas fructose-6-phosphate, ATP and $MgCl_2$ were used in the test for phosphohexokinase.

The following organisms were used: *Xanthomonas phaseoli*, *X. begoniae*, *X. pruni*, *X. cucurbitae*, *X. campestris*, *X. juglandis*, *X. cerealis*, *X. malvacearum*, *X. vesicatoria*, *X. pelargonii*, *X. carotae*, *X. corylina*, *X. maculafoliagardeniae; Pseudomonas angulata*, *P. coronafaciens*, *P. tabaci*, *P. pisi*, *P. syringae; Erwinia amylovora*, *E. carotovora; Agrobacterium rubi*, *A. radiobacter*, *A. tumefaciens; Corynebacterium michiganense*, *C. flaccumfaciens*, *C. fascians*. They were obtained from the culture collection of Mr. M. D. Sutton, Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa.

RESULTS

Pyruvate was produced from glucose + TPN +ATP, glucose-6-phosphate +TPN, fructose-6-phosphate + TPN and 6-phosphogluconate by extracts of *Xanthomonas*, *Pseudomonas*, and *Agrobacterium* species shown in table 1 as well as by the remaining xanthomonads listed above, but not by extracts of the *Erwinia* and *Corynebacterium* species. Pyruvate was produced also from 2-keto-3-deoxy-6-phosphogluconate, the intermediate in the 6-phosphogluconate-splitting reaction of Entner and Doudoroff (1952) (MacGee and Doudoroff, 1954), by extracts of the rep-

[1] Contribution no. 449.

[2] The following abbreviations are used throughout this paper: ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

TABLE 1

Pyruvate production from different substrates and cofactors by certain species of phytopathogenic bacteria (μmoles)

Species	Substrates and Cofactors				
	Glucose, ATP, TPN	Gluconate, ATP	Glucose-6-phosphate, TPN	Fructose-6-phosphate, TPN	6-Phosphogluconate
Xanthomonas vesicatoria	0.5	0	1.0	1.1	1.8
Xanthomonas pelargonii	0.7	0	1.0	1.2	1.6
Xanthomonas malvacearum	1.4	0	1.7	1.5	1.8
Xanthomonas begoniae	0.9	0	1.3	1.2	1.5
Pseudomonas angulata	1.75	3.3	1.6	0.4	1.5
Pseudomonas coronafaciens	0.45	2.0	1.5	0.7	1.9
Pseudomonas tabaci	2.10	3.3	1.8	0.5	1.6
Pseudomonas syringae	0.63	1.5	1.6	0.5	1.8
Agrobacterium rubi	1.3	2.9	1.5	1.0	1.3
Agrobacterium tumefaciens	0.92	2.9	0.5	0.5	1.8
Agrobacterium radiobacter	0.8	3.4	0.6	—	1.8

resentatives of the first three genera. In addition, extracts of *Pseudomonas* and *Agrobacterium* species actively formed pyruvate from gluconate + ATP. The results suggest therefore the presence in the xanthomonads, pseudomonads and agrobacteria of hexokinase (glucose + TPN + ATP), glucose-6-phosphate dehydrogenase (glucose-6-phosphate + TPN), phosphohexose isomerase (fructose-6-phosphate + TPN) and the 6-phosphogluconate-splitting system; the latter two groups of organisms also possess an active gluconokinase (gluconate + ATP); however a 2-ketogluconokinase was not demonstrable by the pyruvate method with 2-ketogluconate as substrate and ATP as cofactor (Narrod and Wood, 1956).

Aldolase and phosphohexokinase activity in sonic extracts of representative species of the 5 genera studied is shown in table 2. A very active aldolase was found in the extracts of all the organisms listed. However, only extracts of *E. carotovora* and *E. amylovora* showed strong phosphohexokinase activity.

Spectrophotometric studies were also carried out with the extracts of most of the species used but were limited to the demonstration of glucose-6-phosphate and 6-phosphogluconate dehydrogenases (TPN- or DPN-linked) phosphohexose isomerase, hexokinase, and glyceraldehyde-3-phosphate dehydrogenase. The results obtained are summarized in table 3. It is noteworthy that with one exception all the species tested possessed a TPN-linked glucose-6-phosphate dehydrogenase and the majority a phosphohexose isomerase. Hexokinase activity (glucose + ATP) was demonstrated in all species except *Pseudomonas* and *Corynebacterium* and glyceraldehyde-3-phosphate dehydrogenase in the majority with the exception of the corynebacteria. TPN-linked 6-phosphogluconate dehydrogenase was present in a number of the extracts. No regu-

TABLE 2

*Relative intensity of aldolase and phosphohexokinase activity in various species of phytopathogenic bacteria**

Species	Aldolase Activity	Phosphohexokinase Activity
Xanthomonas phaseoli	3	Trace
Xanthomonas pruni	4	0
Pseudomonas angulata	4	0
Pseudomonas coronafaciens	4	0
Pseudomonas tabaci	4	0
Agrobacterium rubi	3	0
Agrobacterium tumefaciens	3	1
Erwinia carotovora	4	3
Erwinia amylovora	4	3
Corynebacterium michiganense	3	0
Corynebacterium flaccumfaciens	4	0
Corynebacterium fascians	4	1

* Numbers 0–4 = increasing order of activity (intensity of chromogen formation as determined by the method of Sibley and Lehninger (1949)).

TABLE 3

*Summary of spectrophotometric studies with sonic preparations of phytopathogenic bacteria**

Species	In Presence of TPN				In Presence of DPN	
	Glucose-6-phosphate	6-Phosphogluconate	Fructose-6-phosphate	Glucose + ATP	Glucose-6-phosphate	Glyceraldehyde-3-phosphate (Fructose-1,6-diphosphate)
Xanthomonas phaseoli	+		+	+	−	+
Xanthomonas begoniae	+		+	+	+	+
Xanthomonas pruni	+		+	+	−	+
Xanthomonas cucurbitae	+		+	+	+	
Xanthomonas campestris	+		+	+	−	+
Pseudomonas angulata	+		+	−	−	+
Pseudomonas coronafaciens	+		+	−	−	
Pseudomonas tabaci	+		+	−	−	+
Pseudomonas pisi	+		−	−	+	−
Pseudomonas syringae	+		−	−	+	−
Erwinia carotovora	+	+	+	+	−	+
Erwinia amylovora	+	−	+	+	+	+
Agrobacterium rubi	+	+	+	+	+	+
Agrobacterium tumefaciens	+	+	+	+	−	+
Corynebacterium michiganense	−	−			−	−
Corynebacterium flaccumfaciens	+	+		−	−	−
Corynebacterium fascians	+	+	+	−	−	−

* + = reduction of TPN or DPN; − = no reduction.

larity in the distribution of DPN-linked glucose-6-phosphate dehydrogenase was noted.

DISCUSSION

The above data are in general agreement with the manometric results presented in the preceding paper of this series (Katznelson, 1955*b*). It may be concluded, therefore, that the majority of the bacterial plant pathogens are strongly aerobic organisms possessing alternate systems for glucose oxidation. The previously observed difference in the metabolic pattern of the 5 genera tested (Katznelson, 1955*a*) has been verified in this study, as has been the observation (Katznelson, 1955*a*) that only species of *Erwinia* possess an intact glycolytic system. The critical difference between this group of organisms and those of the remaining genera seems to be in the possession, by the former, of an active phosphohexokinase. This enzyme is either absent, relatively inactive, or very labile in the species of *Xanthomonas*, *Pseudomonas*, *Agrobacterium*, and *Corynebacterium* used. However, members of these genera may achieve virtually the same end by the shunt pathway, the xanthomonads, pseudomonads, and agrobacteria through the 6-phosphogluconate-splitting system which yields pyruvate and triose phosphate, and possibly through the pentose cycle and the corynebacteria probably by the latter pathway. Zajic *et al.* (1956) have shown that *Corynebacterium insidiosum* metabolizes glucose by the shunt pathway, that the 6-phosphogluconate-splitting system is absent and that the pentose cycle is operative. Ghiretti and Barron (1954) have also reported the existence of an active system for hexose metabolism via pentose and sedoheptulose in *Corynebacterium creatinovorans*.

SUMMARY

Studies with sonic preparations of representative species of phytopathogenic bacteria have demonstrated the presence in these organisms of certain key enzymes involved in the glycolytic and shunt pathways of glucose metabolism. The evidence suggests that species of the genera *Xanthomonas*, *Pseudomonas*, and *Agrobacterium* metabolize glucose by the shunt pathway and either by the 6-phosphogluconate splitting system, the pentose cycle or both. Species of *Erwinia* may use either the glycolytic or the oxidative route whereas those of the genus *Corynebacterium* appear to utilize the latter pathway, possibly via the pentose cycle.

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