Glucose Metabolism in *Xanthomonas campestris*

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(Received 10 September 1981)

The pathways of glucose metabolism in *Xanthomonas campestris* closely resemble those described for *Pseudomonas aeruginosa*, in which two discrete systems exist for the uptake of glucose, one periplasmic and oxidative requiring glucose dehydrogenase activity, the second intracellular and phosphorylative. These systems lead to the production of 6-phosphogluconate, which may be metabolized via the Entner–Doudoroff pathway and the pentose phosphate pathway. Terminal oxidation is mediated by the tricarboxylic acid cycle. A mucoid strain of *X. campestris* lacked glucose dehydrogenase activity and thus the oxidative pathway was not functional; the pentose phosphate pathway was also inoperative in this strain, as evidenced by the absence of 6-phosphogluconate dehydrogenase activity. Non-mucoid mutants appeared identical to the parent strain; however, a second group of mutants (crenated mutants) were derepressed for both glucose dehydrogenase and 6-phosphogluconate dehydrogenase. Gluconate kinase was not detected in any of the *X. campestris* strains studied when grown on glucose; however, growth of the parent strain on gluconate resulted in the induction of this enzyme.

INTRODUCTION

Katznelson (1955, 1957) studied the metabolism of gluconate by *Xanthomonas* species; although sonic lysates of *X. campestris* and *X. pruni* (but not *X. phaseoli*) were active, intact cells were not capable of metabolizing gluconate. Employing radiorespirometric techniques, Zagallo & Wang (1967) showed that most glucose (80–90%) was catabolized via the Entner–Doudoroff pathway, with the pentose phosphate pathway fulfilling a minor role in several *Xanthomonas* species. These reports suggest a possible similarity to the glucose metabolizing system of *Pseudomonas aeruginosa* (Ng & Dawes, 1973) which possesses two coupled, yet discrete, pathways (Fig. 1). The oxidative pathway, operating in the periplasm, with a characteristically high K_m for glucose and an absolute requirement for glucose dehydrogenase activity (Midgley & Dawes, 1973), is linked to an intracellular phosphorylative pathway by a transport system with a low K_m for glucose and a broad specificity. Subsequent metabolism occurs via the pentose phosphate and Entner–Doudoroff pathways and terminal oxidation.

We now report preliminary data concerning glucose metabolism in a series of *X. campestris* mutants selected for alterations in surface polysaccharides (Whitfield *et al.*, 1981).

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Fig. 1. Pathways of glucose catabolism and related transport systems in *Pseudomonas aeruginosa*.

METHODS

Growth of bacteria. The bacterial strains and cultural conditions have been described elsewhere (Whitfield *et al.*, 1981).

Preparation of cell-free extracts. Bacteria were harvested in mid- to late-exponential phase and washed twice in 67 mM-sodium phosphate buffer, pH 7.0 and in 120 mM-glycyl-glycine buffer, pH 7.0. They were then resuspended in 60 mM-glycyl-glycine buffer, pH 7.0 and disrupted by sonication. Debris was removed by centrifuging at 7000 *g* for 15 min; the supernatant was used as a crude cell extract.

Enzyme assays. All enzymes were assayed in freshly prepared extracts at 30 °C under optimal conditions of pH, substrate and cofactor concentration. Measurements were made in a Gilford model 240 spectrophotometer. Reaction mixtures, in cuvettes of 1 cm path length contained a final volume of 1 ml. Specific activities are expressed as μmol substrate converted $(\text{mg protein})^{-1} \text{ min}^{-1}$. Reproducibility of the assays was within $\pm 10\%$. The assumed ϵ_{340} for NADPH was $6.22 \times 10^3 \text{ litre mol}^{-1} \text{ cm}^{-1}$ and ϵ_{600} for dichlorophenol indophenol was $19.5 \times 10^3 \text{ litre mol}^{-1} \text{ cm}^{-1}$ at pH 6.6 and $9.1 \times 10^3 \text{ litre mol}^{-1} \text{ cm}^{-1}$ at pH 5.5 (Ng & Dawes, 1973).

The following enzymes were assayed according to the methods of Ng & Dawes (1973): hexokinase (EC 2.7.1.1); glucokinase (EC 2.7.1.12); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); 6-phosphogluconate dehydrogenase (decarboxylating 6-phospho-D-gluconate:NADP⁺ oxidoreductase) (EC 1.1.1.44); isocitrate dehydrogenase (EC 1.1.1.42); glucose dehydrogenase (EC 1.1.1.47); gluconate dehydrogenase (EC 1.1.99.3); 6-phosphogluconate dehydratase (EC 4.2.1.12) and 3-deoxy-2-oxo-6-phosphogluconate aldolase (EC 4.1.2.14) (Entner–Doudoroff enzymes); aconitase (EC 4.2.1.3). 2-Oxogluconate kinase (EC 2.7.1.13) and 2-oxogluconate-6-phosphate reductase (oxogluconate-metabolizing enzymes) were assayed as described by Roberts *et al.* (1973). Phosphofructokinase was detected by the method of Racker (1947).

Protein was estimated by the Lowry method.

Gluconate was detected by paper chromatography with butan-1-ol/pyridine/water (6:4:3, by vol.) as solvent (Whistler & Conrad, 1954). Portions of culture filtrate were lyophilized before chromatographic comparison with authentic gluconic acid.

Biochemicals. All chemicals, of the purest grade available, were obtained from BDH. Biochemicals and enzymes were from Boehringer, and D-[U-¹⁴C]glucose (230 mCi μmol^{-1}), D-[U-¹⁴C]galactose (50–200 mCi μmol^{-1}) and D-[U-¹⁴C]mannose (2–5 mCi μmol^{-1}) were from Amersham (1 mCi = 37 MBq).

RESULTS

Growth of *X. campestris* was accompanied by a marked decrease in the pH of the medium; the decrease was greater in cultures of the crenated strains 646D, 646E and 646KR than in the wild type. On plates of bromothymol blue/glucose medium, a colour change indicated acid production. Under identical conditions, growth of *X. campestris* T646 and non-mucoid mutants 646NM1 and 646NM2 did not induce a colour change. Following growth of crenated strains in glucose minimal medium, gluconate was detected in the culture filtrates.

Table 1. *Specific activities of glucose-metabolizing enzymes of Xanthomonas campestris strains*

Cells were grown in 1-litre batch cultures containing minimal medium supplemented with D-glucose unless otherwise indicated; all carbon sources were at 2% (w/v). The values given are the average of two determinations.

Enzyme	Specific activity [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]								
	Strains grown on glucose:						T646 grown on:		
	T646	646NM1	646NM2	646D	646E	646KR	Gluconate	Citrate	Glycerol
Glucose dehydrogenase	ND	ND	ND	10.7	7.8	4.6	ND	ND	ND
Hexokinase	23.0	37.3	21.3	17.9	21.3	4.6	26.3	20.3	16.1
Glucose-6-phosphate dehydrogenase	144	253	252	129	116	107	153	77.6	114
Gluconate kinase	ND	ND	ND	ND	ND	ND	8.3	ND	ND
6-Phosphogluconate dehydrogenase	ND	ND	ND	112	118	265	ND	ND	ND
Gluconate dehydrogenase	6.1	5.1	7.3	11.4	10.9	11.6	15.3	4.2	ND
2-Oxogluconate metabolizing enzymes*	14.3	16.9	38.3	40.2	56.1	41.3	19.1	6.4	4.9
Entner-Doudoroff enzymes†	11.4	7.8	6.3	14.5	12.1	7.7	6.6	5.5	11.5
Aconitase	119	88.5	52.6	46.2	43.7	40.2	140	55.2	83.1
Isocitrate dehydrogenase	245	231	290	231	221	210	128	115	77.3

ND, Not detected.

* 2-Oxogluconate kinase + 2-oxogluconate reductase.

† 6-Phosphogluconate dehydratase + 2-oxo-3-deoxygluconate aldolase.

No 2-oxogluconate was found. Crenated mutants and, to a lesser extent, strains T646, 646NM1 and 646NM2, grew in media containing gluconate or 2-oxogluconate as sole carbon source. This indirect evidence suggested that an oxidative pathway similar to that described for *P. aeruginosa* (Ng & Dawes, 1973; Roberts *et al.*, 1973) (Fig. 1) may function in the crenated *X. campestris* strains.

The activities of enzymes potentially involved in glucose metabolism by *X. campestris* strains were measured in cell lysates (Table 1). The enzymes tested were representative of the oxidative, phosphorylative and oxidative pentose phosphate pathways; isocitrate dehydrogenase and aconitase were measured to monitor tricarboxylic acid cycle activity. Differences in enzyme activity were apparent between the wild-type organism and mutants derived from it. Neither *X. campestris* T646 nor its non-mucoid mutants possessed detectable amounts of glucose dehydrogenase activity under the assay conditions used, whereas activity of this enzyme was present in extracts from crenated bacteria. Furthermore, gluconate dehydrogenase activity was higher in crenated strains than in wild-type bacteria. Enzymes responsible for the metabolism of 2-oxogluconate were detected in all strains examined.

Although the enzymes of the phosphorylative pathway were detected in all the strains studied, the activities of hexokinase and glucose-6-phosphate dehydrogenase varied.

Enzymes of the Entner-Doudoroff pathway were assayed in cell-free extracts by measuring pyruvate production using lactate dehydrogenase. Each strain studied produced pyruvate. No glycolysis occurred in these strains, since no phosphofructokinase activity was detected. The validity of the enzyme assay was verified with extracts of *Escherichia coli*.

Extracts prepared from crenated mutants of *X. campestris* had detectable activity of 6-phosphogluconate dehydrogenase, a key enzyme of the oxidative pentose phosphate pathway. No activity of this enzyme was detected in extracts prepared from *X. campestris* T646 or non-mucoid bacteria. Since the assay depends upon the conversion of NADP⁺ into NADPH, absolute values of enzyme activity should be regarded with caution. In a study of carbohydrate catabolism in mutant strains of *P. aeruginosa* (Blevins *et al.*, 1975), increased 6-phosphogluconate dehydrogenase activity was detected only in those strains containing elevated levels of Entner–Doudoroff enzymes. The generation of glyceraldehyde 3-phosphate by this pathway may provide substrate for endogenous glyceraldehyde-3-phosphate dehydrogenase, thus creating errors in the assay. This problem was not found in the present study as values for the specific activity of 6-phosphogluconate dehydrogenase in crenated mutants were high, whereas no activity was detected in either parent or non-mucoid bacteria. The variations in amounts of Entner–Doudoroff enzymes should not account for the observed differences. All strains tested showed variable amounts of the representative enzymes of the tricarboxylic acid cycle.

Enzymes were also assayed in extracts from *X. campestris* T646 cells grown in minimal medium with different carbon sources (Table 1). No glucose dehydrogenase activity was detected. Growth on gluconate resulted in a fourfold increase in the activity of gluconate dehydrogenase when compared with values from glucose-grown cells; glucokinase activity was also present. The activity of 2-oxogluconate metabolizing enzymes was also higher in gluconate-grown cells. Growth on citrate resulted in a decrease in the specific activity of gluconate dehydrogenase, and no activity was detected in glycerol-grown bacteria. Activities of 2-oxogluconate metabolizing enzymes were lower in cells grown on either glycerol or citrate. The activities of glucose-6-phosphate dehydrogenase and, to a lesser extent, hexokinase were higher in extracts prepared from glucose- and gluconate-grown cells than in extracts of cells grown in glycerol or citrate media. The activity of enzymes of the Entner–Doudoroff pathway varied with growth substrate. Neither 6-phosphogluconate dehydrogenase nor phosphofructokinase activity was detected in any of these extracts. The activity of tricarboxylic acid enzymes was decreased in bacteria grown in media containing citrate and glycerol.

DISCUSSION

Estimation of the amounts of glucose-metabolizing enzymes demonstrated differences between the *X. campestris* strains investigated. Crenated mutants appear to have higher specific activities of the enzymes involved in the oxidation of glucose to gluconate and subsequently to 2-oxogluconate. Operation of this essentially periplasmic system (see Fig. 1) may account for the observed decrease in pH of the medium during growth and the presence of gluconate after growth on glucose. Wild-type *X. campestris* T646 grew on gluconate but, unlike the crenated bacteria, grew only poorly on 2-oxogluconate. The apparent lack of glucose dehydrogenase activity in the wild-type and non-mucoid bacteria precludes operation of the periplasmic oxidative pathway. The ability to grow on gluconate, and the synthesis of detectable gluconokinase and gluconate dehydrogenase suggests that the defect in the periplasmic pathway does indeed lie at the glucose dehydrogenase step.

Although these results are in accord with those expected from an extracellular system analogous to that described in *P. aeruginosa* (Fig. 1), we have not attempted to describe the membrane location or orientation of the enzymes with regard to the periplasmic space in *X. campestris*.

Xanthomonas campestris strains possess the enzymes necessary for the intracellular phosphorylative pathway. Thus, these bacteria resemble *P. aeruginosa* in the possession of two coupled, yet discrete, systems for glucose uptake and subsequent metabolism, although it is apparent that both systems operate only in the crenated mutants and not in either the

parent or non-mucoid strains. In *P. aeruginosa*, the intracellular pathway is inhibited by the presence of high concentrations of gluconate produced periplasmically. Since *X. campestris* T646 lacks glucose dehydrogenase activity, such inhibition would not occur during growth on glucose. In *P. aeruginosa*, the presence of two pathways may perform a regulatory function since, under carbon-limiting conditions, *P. aeruginosa* transports glucose and metabolizes it via the intracellular pathway (Whiting *et al.*, 1973).

Growth of *P. aeruginosa* on organic acids (e.g. citric, succinic) repressed enzymes of glucose metabolism (Hamilton & Dawes, 1960; Hamlin *et al.*, 1967). This occurs to a slight extent in *X. campestris* T646. Growth of *P. aeruginosa* on glycerol derepressed enzymes of glucose metabolism (Hamlin *et al.*, 1967); this did not happen in *X. campestris*.

The inability of *X. campestris* to utilize the glycolytic pathway results from the lack of active phosphofructokinase, confirming results from *X. phaseoli* (Hochster & Katznelson, 1958).

C. W. acknowledges receipt of an SRC CASE award.

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