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Pseudomonas cepacia Mutants Blocked in the Direct Oxidative Pathway of Glucose Degradation

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Glucose dehydrogenase-deficient strains of *Pseudomonas cepacia* grew normally with glucose as carbon source, indicating that the direct pathway of glucose oxidation does not play an essential role in this bacterium.

In pseudomonads, glucose is metabolized via the Entner-Doudoroff pathway, which requires its conversion to the key compound 6-phosphogluconate by enzymes of the phosphorylative or direct oxidative routes, or both (3, 5–8, 13). Four steps intervene in the conversion of glucose to 6-phosphogluconate via the direct oxidative route. Glucose and gluconate dehydrogenase catalyze the pyridine nucleotide-independent oxidation of glucose to gluconate and 2-ketogluconate (7, 10, 12). ATP-dependent phosphorylation of the latter leads to formation of 2-keto,6-phosphogluconate, which is in turn reduced in a reduced nicotinamide adenine dinucleotide phosphate-dependent step to 6-phosphogluconate (8). Formation of 6-phosphogluconate via the phosphorylative route requires only two steps: ATP-dependent phosphorylation of glucose to produce glucose-6-phosphate (mediated by glucokinase) and pyridine nucleotide-dependent oxidation of glucose-6-phosphate (mediated by glucose-6-phosphate dehydrogenase). The relative importance of the two routes of 6-phosphogluconate formation in different pseudomonads is not entirely clear. Mutant strains of *Pseudomonas putida* deficient in glucose dehydrogenase fail to grow on glucose, suggesting that in this species the phosphorylative route is unimportant (11). In contrast, the phosphorylative route appears to play a critical role in *P. aeruginosa,* where, under conditions of low glucose concentration (12) or anaerobic growth with nitrate as electron acceptor (J. C. Hunt and P. V. Phibbs, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, K5, p. 187), it represents the primary route of conversion of glucose to 6-phosphogluconate.

The pathway of glucose degradation in *P. cepacia* (*P. multivorans*) is more complex than in other pseudomonads, particularly with respect to its unusual complement of glucose-6-phosphate and 6-phosphogluconate dehydrogenase isoenzymes (1, 2, 4, 9). The unusual prominence of 6-phosphogluconate dehydrogenase in this bacterium suggests that the Entner-Doudoroff and pentose shunt pathways share a major role in the metabolism of 6-phosphogluconate. The present data indicate that, in *P. cepacia,* loss of glucose dehydrogenase activity does not impair growth on glucose, suggesting that the complex phosphorylative route found in this bacterium is the essential pathway of glucose catabolism.

Glucose dehydrogenase-deficient mutants of *P. cepacia* were obtained readily by screening for atypically pigmented colonies on solid medium consisting of 1% (wt/vol) yeast extract and 1% (wt/vol) glucose supplemented with eosin (200 μg/ml) and methylene blue (33 μg/ml). Wild-type bacteria formed dark purple colonies with a characteristic green sheen. Glucose dehydrogenase-deficient (EMB-negative) mutants formed white to pink colonies. The glucose dehydrogenase-deficient mutants grew at the same rate as the wild type (generation time, ca. 70 min at 37°C) in medium containing 0.5% glucose as sole carbon source.

Extracts of the EMB-negative strains 84 and 85 had less than 1% of the level of glucose dehydrogenase present in extracts of the wild-type strain 249 (Table 1). A third mutant, strain 21, had about 10% of the normal activity of this enzyme. All three mutant strains had normal levels of glucokinase and glucose-6-phosphate dehydrogenase, which accounts for their ability to form 6-phosphogluconate and utilize glucose as carbon source (results not shown). The same mutant strains had normal levels of gluconate dehydrogenase when grown with gluconate as carbon source, but only low levels of this enzyme when grown with glucose (see Table 1). We interpret the results as indicating that gluconate is the physiological inducer of gluconate dehydrogenase, and that failure of EMB-negative mutants to express gluconate dehydrogenase normally during growth on glucose is due to the inability of these strains to form adequate

TABLE 1. *Activities of enzymes of the direct oxidative pathway of glucose degradation in wild-type and EMB-negative strains of P. cepacia*[a]

Carbon source (0.5%, wt/vol)	Strain	Phenotype	Sp act[b]	
			Glucose dehydrogenase	Gluconate dehydrogenase
Glucose	249	Wild type (EMB positive)	219	216
	21	EMB negative	21	31
	84[c]	EMB negative	<1	6
	85[c]	EMB negative	<1	3
Gluconate	249	Wild type	345	345
	21	EMB negative	19	297
	84	EMB negative	<1	245
	85	EMB negative	<1	306

[a] Bacteria were grown in minimal salts medium (2) supplemented with the indicated carbon source and where appropriate 40 μg of L-isoleucine per ml. The bacteria were collected by centrifugation, suspended in 0.015 M phosphate buffer (pH 6.5) containing 0.1% (wt/vol) sodium dodecyl sulfate (see text), and disrupted by sonic treatment (4). The disrupted cell suspensions were centrifuged for 10 min at 12,000 × *g* to remove unbroken cells and cell debris, and the supernatant fractions were assayed for glucose and gluconate dehydrogenase. The assay mixtures (1 ml) contained 0.15 M phosphate buffer (pH 6.5), 3×10^{-4} M dichloroindophenol, 10^{-2} M glucose or gluconate, and between 10 and 100 μg of crude extract protein. Reduction of dichloroindophenol was monitored by measuring the decrease in absorbance of the assay mixtures at 600 nm.

[b] Values represent nanomoles of glucose or gluconate oxidized per minute per milligram of protein, determined at 24°C.

[c] Strain 21 was derived from strain 249. Strains 84 and 85 were derived from an isoleucine auxotroph of strain 249 (249-13).

amounts of inducer. The results shown in Table 2 indicate that, in the wild-type strain 249, glucose dehydrogenase is not regulated coordinately with gluconate dehydrogenase, but expressed constitutively.

Optimal activities of glucose and gluconate dehydrogenase in cell-free extracts of *P. cepacia* were obtained when the bacteria were disrupted in buffers containing 0.1% (wt/vol) sodium dodecyl sulfate as in Table 1. Under these conditions, the specific activities of the enzymes were increased between three- and fourfold compared to extracts prepared in the absence of sodium dodecyl sulfate. Experiments not reported here indicate that both glucose and gluconate dehydrogenase are membrane associated, and that sodium dodecyl sulfate promotes their release from membrane material without loss of enzyme activity.

TABLE 2. *Noncoordinate expression of glucose and gluconate dehydrogenase during growth of P. cepacia 249 on different carbon sources*

Carbon source[a]	Sp act[b]	
	Glucose dehydrogenase	Gluconate dehydrogenase
Mannitol	215	26
Glucose	284	252
Gluconate	318	305
2-Ketogluconate	300	151
Citrate	264	20
Succinate	242	38
Phthalate	280	48

[a] The concentration of carbon source was 0.5% (wt/vol), except for 2-ketogluconate and phthalate, which were each supplied at 0.2% (wt/vol).

[b] Values represent nanomoles of glucose or gluconate oxidized per minute per milligram of protein, determined as in Table 1.

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