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METABOLISM OF GLUCOSE AND GLUCONATE IN FAST- AND SLOW-GROWING RHIZOBIA

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Abstract—Enzymatic evidence was sought for the operation of pathways involved in glucose and gluconate catabolisms in fast- and slow-growing *Rhizobium* species including members of the cowpea group. Enzymes of the Entner–Doudoroff pathway, pentose phosphate pathway and tricarboxylic acid cycle were detected in fast-growing rhizobia but the pentose phosphate pathway was absent in slow-growers, regardless of the carbon source used. When analysed for enzymes of the Embden–Meyerhof–Parnas and Entner Doudoroff pathways in glucose-grown cells, the pathways were found to operate simultaneously in rhizobia.

INTRODUCTION

Previous investigations have shown that all rhizobia irrespective of their growth properties possess the Entner–Doudoroff (ED) pathway and the tricarboxylic acid (TCA) cycle for glucose oxidation [1–5]. Using cells cultured on glucose, the occurrence of the pentose phosphate (PP) pathway has been demonstrated in fast-growing rhizobia [4] which possess NADP-linked 6-phosphogluconate dehydrogenase (6-PGD), the key enzyme for this pathway. Lack of the enzyme 6-PGD in glucose-grown slow-growing rhizobia laid the foundation of the enzymatic basis for differentiation of fast- and slow-growing groups of *Rhizobium* [4]. Evidence for the presence of the Embden–Meyerhof–Parnas (EMP) pathway, however, is contradictory. There have been a few reports [1, 4, 6–8] of the measurement of significant levels of the glycolytic enzymes phosphofructokinase (PFK) and fructose-1,6-diphosphate aldolase in these organisms while others failed to do so. Little attention has been directed towards the study of gluconate catabolism in rhizobia. The radiorespirometric method indicated that in *R. japonicum*, gluconate was degraded primarily by the ED pathway and possibly also by an ancillary ketogluconate pathway and by the TCA cycle [3]. The purpose of the present investigation was to compare the enzyme systems and hence the pathways involved in the catabolism of glucose and gluconate in fast- and slow-growing rhizobia, including members of the cowpea group. Cowpea rhizobia are agronomically very important because of their symbiotic nitrogen fixation.

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RESULTS AND DISCUSSION

The carbon metabolism of rhizobia was initially explored by checking the growth response of the organisms to various carbon sources including glucose and gluconate. The results (data not shown) are in general agreement with the findings of others [9–11]. From a survey of the key enzymes of the EMP, ED, PP and TCA cycle pathways (Table 1) it is apparent that significant levels of enzymes of the EMP, ED and TCA cycle pathways are present for both glucose and gluconate dissimilation in both fast- and slow-growers.

The high level of the PFK and glyceraldehyde-3-phosphate dehydrogenase (GL3PD) activities suggested the presence of the EMP pathway in the glucose-grown rhizobia (Table 1). The 'Entner–Doudoroff' enzyme (6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase) activities attested to the operation of the ED pathway. The activities of malate dehydrogenase and isocitrate dehydrogenase indicated operation of the TCA cycle in these cells. Disagreement as to the presence of fructose diphosphate aldolase and PFK, the key enzymes of the EMP pathway, in rhizobia could be noted in the observations of previous investigators [1, 2, 5–8, 12]. The reason for such a discrepancy is not clear. It could have been due to the difference in age of the cultures used. Some suggest that utilization of the EMP pathway may be related to the nitrogen-fixing efficiency of the rhizobial strains [8].

All the enzymes for an operational ED pathway are present in the glucose-grown rhizobia (Table 1). The specific activities of 6-PGD and the ED enzymes are several-fold higher in the cell-free extracts of the fast-growers. The ED pathway is known to account for the major proportion of the glucose catabolism in both slow- and fast-growing species of *Rhizobium* in culture [2–4]. However, the reason for the higher activity of the enzymes

Table 1. Specific activities* of key enzymes of carbohydrate metabolism in cell-free extracts of rhizobia when cultured on glucose or gluconate

	Carbon source										
	Glucose							Gluconate			
Strain	G6PD	ED enzymes	6PGD	PFK	GL3PD	MDH	ISDH	Glucono-kinase	ED enzymes	6PGD	MDH
Fast-growers											
Rhizobium leguminosarum SU391†	120	30	80	80	70	480	240	65	105	94	336
R. phaseoli CC365	130	45	105	64	65	260	210	50	92	115	160
R. trifolii CC224	217	26	104	48	52	256	216	80	80	100	260
R. meliloti SU216	190	50	65	85	55	450	236	40	100	91	300
R. loti NZP2213	140	35	76	50	75	400	260	43	80	70	140
Rhizobium sp. (*Sesbania bispinosa*) BICC610	180	50	135	40	100	206	194	35	96	130	200
Rhizobium sp. (*Arachis hypogaea*) BICC606	100	24	96	70	80	320	220	64	80	120	255
Rhizobium sp. (*Dolichos lablab*) BICC607	110	14	130	80	90	255	210	80	102	100	200
Rhizobium sp. (*Cassia sophera*) BICC609	108	32	64	40	96	320	256	48	80	70	196
Slow-growers											
R. japonicum CC409†	24	6.2	1.4	48	50	200	160	20	100	2.3	200
R. lupini RL3001	10	2.0	1.3	40	35	420	130	13	66	1.6	260
Rhizobium sp. (*Vigna radiata* var. *mungo*) U8	12	6.4	1.2	40	50	200	112	10	96	0.9	160
Rhizobium sp. (*Cicer arietinum*) Ca181	8	3.1	0.8	41	42	200	100	12	72	1.5	175
Rhizobium sp. (*Cajanus cajan*) BICC616	16	4.1	1.4	64	50	290	120	16	64	1.6	250
Rhizobium sp. (*Desmodium gangeticum*) BICC611	8	4.8	0.6	50	24	160	130	16	64	0.8	200
Rhizobium sp. (*Clitoria ternatea*) BICC614	16	4.0	1.1	32	48	224	160	15	62	1.2	196
Rhizobium sp. (*Tephrosia purpurea*) BICC613	20	2.4	1.6	29	46	160	140	13	68	1.3	180

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; ED enzymes, combined activity of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase; 6PGD, 6-phosphogluconate dehydrogenase; PFK, phosphofructokinase; GL3PD, glyceraldehyde-3-phosphate dehydrogenase; MDH, malate dehydrogenase; ISDH, isocitrate dehydrogenase.

*Specific activity expressed as nmol of product formed per min per mg of protein at 30°.

†Values quoted from our previous observations [23].

of the ED pathway in the fast-growing rhizobia than in the slow-growing ones as observed in the present investigation is not clear.

In the fast-growing cells cultured on glucose, significant activity of NADP-linked 6-PGD, the key enzyme for operation of the PP pathway, was detected. There was no evidence for the presence of the PP pathway for glucose metabolism in slow-growers (Table 1). This is in confirmation of earlier observations [2, 4].

Gluconate is an inducer of the ED pathway [13] and dissimilation of this compound has been considered confirmatory for its operation [3, 14]. Gluconate is thought not to be metabolized by the EMP pathway [3]. Therefore no attempt was made to study the key enzymes of the EMP pathway in gluconate-grown cells. For gluconate metabolism, the enzymes gluconokinase, ED enzymes and malate dehydrogenase were detected in all the strains (Table 1). The specific activity of the enzyme gluconokinase was found to be several-fold higher in the cell-free extracts of fast-growers than in the cell-free extracts of slow-growers. The specific activities of the ED enzymes and malate dehydrogenase were not significantly different in the two groups of rhizobia. Such an observation is suggestive, although indirectly, of the operation of an ancillary, probably the ketogluconate, pathway in slow-growers [3], where gluconate is thought to be converted into 6-ketogluconate via the formation of 2-ketogluconate. In these cells, NADP-linked 6-PGD activity is detected in appreciable quantity in the extracts of fast-growers and only in negligible quantity in the extracts of slow-growers, indicating the absence of the PP pathway for gluconate metabolism in slow-growing rhizobia. It is now clear that the PP pathway is absent in both glucose-grown and gluconate-grown cells of slow-growing rhizobia. It has been suggested that in the absence of the PP pathway, pentose required for cellular growth could be obtained from fructose-6-phosphate; as such. The PP pathway is not mandatory for biosynthetic purposes [3]. The synthesis of fructose-6-phosphate from gluconate would, however, involve anabolic glycolysis, the presence of which needs to be explored in gluconate-grown rhizobia.

EXPERIMENTAL

The *Rhizobium* strains used in this study were obtained from different sources throughout the world. Strains with the prefix BICC were isolated in this laboratory. The strains were maintained on yeast mannitol agar slopes [15] between expts. Carbon source utilization was studied as described in ref. [16]. All enzyme assays were carried out in a final vol. of 1 ml. Activities were determined at room temp. ($28 \pm 2°$) by measuring A_{340}. Each assay had two controls, one without extract and the other without substrate. Published procedures were used to assay the enzymes. The precise compositions of the assay mixtures were as follows: Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) [17, 18]: 100 mM Tris HCl (pH 7.6), 2 mM $MgCl_2$, 25 mM glucose-6-phosphate and 0.4 mM NAD^+ or $NADP^+$. *The Entner-Doudoroff enzymes* (combined activities of 6-phosphogluconate dehydratase (EC 4.2.1.12) and 2-keto-3-deoxy-6-phosphogluconate aldolase (EC 4.1.2.14) [11]: 100 mM Tris-HCl (pH 8.5), 10 mM β-mercaptoethanol, 0.4 mM NADH, 5 mM 6-phosphogluconate and 100 μg lactate dehydrogenase (Sigma) per ml. 6-*Phosphogluconate dehydrogenase* (EC 1.1.1.44) [17]: 100 mM Tris HCl (pH 7.6), 2 mM $MgCl_2$, 5 mM 6-phosphogluconate and 0.4 mM NAD^+ or $NADP^+$. 6-*Phosphofructokinase* (EC 2.7.1.11) [19]: 67 mM Tris-HCl (pH 8.0), 50 mM KCl, 17 mM Na_2HAsO_4, 5 mM $MgCl_2$, 2.5 mM ATP, 0.25 mM NAD^+, 2.5 mM fructose-6-phosphate, and 5 U fructose biphosphate aldolase and 10 U glyceraldehyde-3-phosphate dehydrogenase per assay. *Glyceraldehyde-3-phosphate dehydrogenase* (EC 1.2.1.12) [20]: 50 mM Tris-HCl (pH 7.6), 6 mM D,L-glyceraldehyde-3-phosphate, 10 mM cysteine HCl, 17 mM Na_2HAsO_4, 20 mM NaF and 0.25 mM NAD^+. *Malate dehydrogenase* (EC 1.1.1.37) [21]: 100 mM phosphate buffer (pH 7.4), 0.25 mM NADH and 1 mM oxalacetate. *Isocitrate dehydrogenase* (EC 1.1.1.42) [21]: 300 mM Tris-HCl (pH 7.6) 10 mM $MgCl_2$, 0.2 mM $NADP^+$ and 10 mM D,L-isocitrate. *Gluconokinase* (EC 2.7.1.12) [20]: 50 mM Tris HCl (pH 8.0), 10 mM $MgCl_2$, 2.5 mM ATP, 0.25 mM $NADP^+$, 2.5 mM sodium gluconate and 0.1 U 6-phosphogluconate dehydrogenase per assay. Protein was determined in cell-extract according to ref. [22].

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