Microbiology, Vol. 74, No. 5, 2005, pp. 615–618. Translated from Mikrobiologiya, Vol. 74, No. 5, 2005, pp. 707–710.

SHORT COMMUNICATIONS

Methanol and Glucose Metabolism in *Beijerinckia mobilis*

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Received March 3, 2005; in final form, April 21, 2005

Bacteria of the genus *Beijerinckia* are nonsymbiotic aerobic chemoheterotrophs and diazotrophs that are able to utilize a broad range of polycarbon substrates, preferably sugars [1]. Due to their acidotolerance (ability to survive at pH 3.0–4.0), these bacteria are widespread in acidic soils, although they also occur in neutral soils. There are four *Beijerinckia* species with a suitable taxonomic description: *B. mobilis, B. indica, B. derxii*, and *B. fluminensis.* However, their physiology and metabolism are as yet poorly understood [2–4]. Our recent investigations have shown that *B. mobilis* can grow autotrophically on methanol and formate [5]. This prompted us to study the enzymes involved in the metabolism of methanol and glucose in this facultative methylotroph.

Enzyme activity was measured, as described in [6, 7], in extracts of *B. mobilis* cells grown on methanol and glucose. As is evident from the table, *B. mobilis* possesses all the enzymes necessary for oxidation of methanol to CO_2 via formaldehyde and formate, i.e., for methylotrophic growth. The presence of highly active phosphoribulokinase and ribulose 1,5-bisphosphate carboxylase/oxygenase suggests that the methanol carbon is mainly assimilated at the level of CO_2 via the ribulose bisphosphate (RuBP) pathway. Transketolase and transaldolase are involved in reactions that convert glyceraldehyde-3-phosphate into xylulose-5-phosphate, which results in the regeneration of ribulose-1,5-bisphosphate, the primary acceptor of CO_2 (see figure). The reaction catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase produces, in addition to 3-phosphoglycerate, phosphoglycolate, which is then dephosphorylated to glycolate. The last compound transforms into glyoxylate, glycine, and serine via a shunted variant of the serine pathway. Since the activity of NADH-dependent hydroxypyruvate reductase, serine–glyoxylate aminotransferase, and glycerate kinase is low, the serine pathway plays a minor role in assimilation of the methanol carbon at the level of formaldehyde. *B. mobilis* does not have 3-hexulose-6-phosphate synthase, suggesting that the ribulose monophosphate pathway is not involved in the primary assimilation of formaldehyde.

In contrast to fructose-1,6-bisphosphate aldolase and fructose-1,6-bisphosphatase, which are involved in gluconeogenesis, some enzymes involved in carbon metabolism (ATP-dependent 6-phosphofructokinase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase) were suppressed in the methanol-grown culture. Growth on glucose was found to activate ATP-dependent glucokinase, 6-phosphofructokinase, fructose-1,6-bisphosphate aldolase, and 6-phosphogluconate dehydrogenase ($NADP^+$-dependent). This circumstance suggests that *B. mobilis* catabolizes glucose with the involvement of both the glycolytic and the pentose phosphate oxidative pathways. At the same time, the absence of 2-keto-3-deoxy-6-phosphogluconate aldolase activity indicates that hexose phosphates are not metabolized via the Entner–Doudoroff pathway.

The *B. mobilis* cells grown on methanol exhibited low activity with of pyruvate dehydrogenase and tricarboxylic acid cycle enzymes (such as citrate synthase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase), indicating that the Krebs cycle mainly performs a biosynthetic function during methylotrophic growth of *B. mobilis*. The low activity of isocitrate lyase and malate synthase in the cells grown on methanol as compared to those grown on glucose suggests that the glyoxylate bypass plays a minor role in methylotrophic growth of *B. mobilis*.

Ammonium is assimilated by *B. mobilis* with the involvement of NADPH-dependent glutamate dehydrogenase, alanine dehydrogenase, and the glutamate cycle. The presence of highly active phosphogluco-

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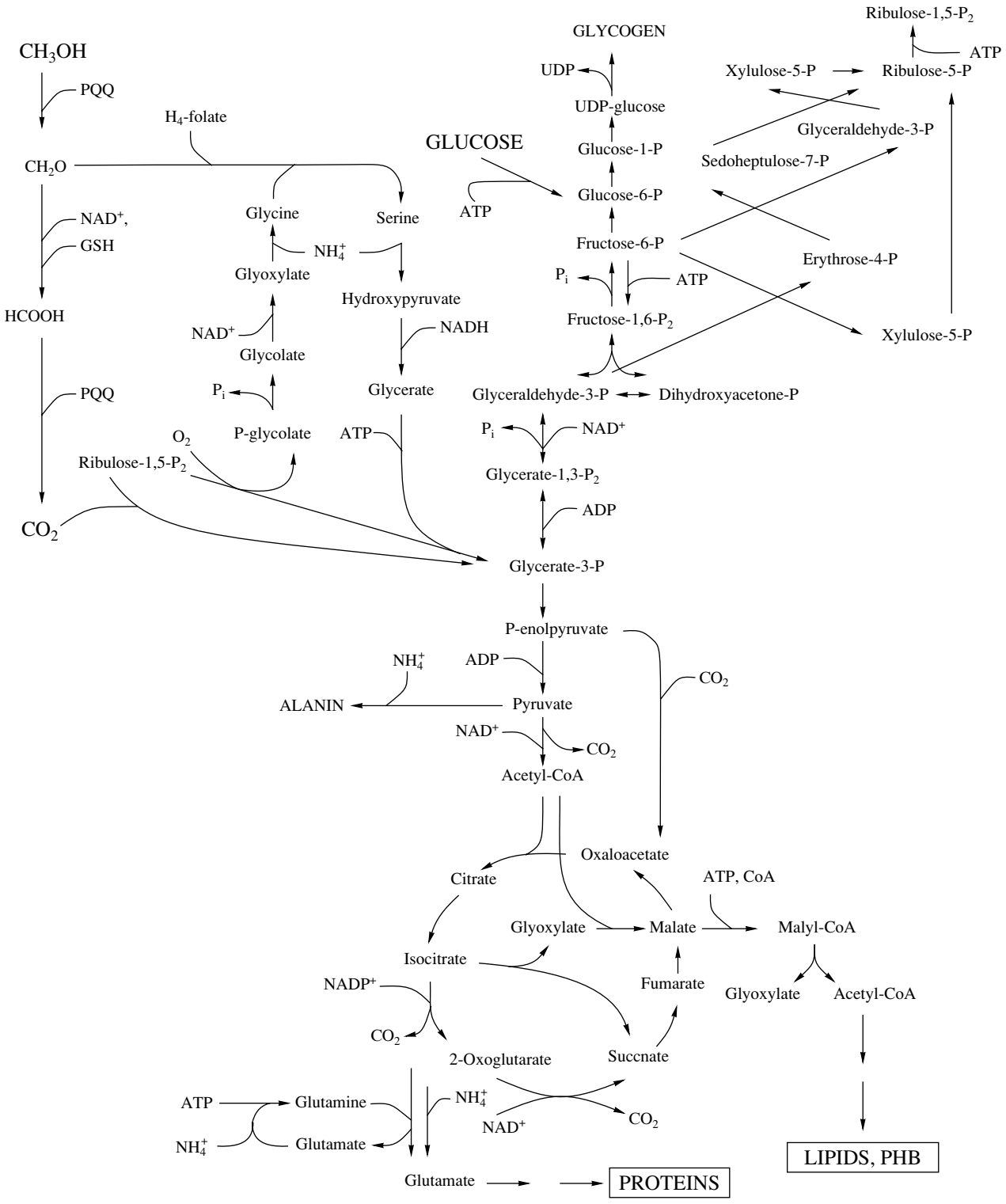
Activity of the enzymes involved in the metabolism of methanol and glucose in *B. mobilis*

Enzyme	Cofactor	Activity with	
		methanol	glucose
Methanol dehydrogenase	PMS	27	0
Formaldehyde dehydrogenase	PMS	17	0
	NAD^+, GSH	210	0
	$NADP^+$, GSH	108	0
Formate dehydrogenase	PMS	32	0
	NAD^+	0	0
Phosphoribulokinase	ATP	103	3
Ribulose 1,5-bisphosphate carboxylase		160	30
Phosphoenolpyruvate carboxylase		103	47
3-Hexulose-6-phosphate synthase		0	0
Hydroxypyruvate reductase	NADH	18	10
	NADPH	0	0
L-Serine–glyoxylate aminotransferase		63	40
Phosphoglycolate phosphatase		25	12
Malyl-CoA synthase/Malyl-CoA lyase	ATP, CoA	49	56
Glycerate kinase	ATP	64	40
Transaldolase		86	94
Glucose-6-phosphate dehydrogenase	HAD^+	10	322
	$HADP^+$	24	349
6-Phosphogluconate dehydrogenase	NAD^+	0	0
	$NADP^+$	16	345
Fructose-1,6-bisphosphate aldolase	NADH	133	215
Fructose-1,6-bisphosphatase		31	64
6-Phosphofructokinase	PP_i	0	0
	ATP	54	41
2-Keto-3-deoxy-6-phosphogluconate aldolase		0	0
Pyruvate dehydrogenase	NAD^+	143	212
Pyruvate kinase		100	96
Citrate synthase		22	130
Isocitrate dehydrogenase	NAD^+	0	0
	$NADP^+$	68	67
2-Oxoglutarate dehydrogenase	NAD^+	286	295
Malate dehydrogenase	NADH	250	700
	NADPH	357	296
Isocitrate lyase		35	225
Malate synthase		20	188
Glutamate dehydrogenase	NADPH	0	0
	NADH	49	201
Alanine dehydrogenase	NADPH	206	188
Glutamate synthase	NADH	78	362
	NADPH	0	0
Glutamine synthetase	ATP, Mg^{2+}	48	44
Glucokinase	ATP	97	104
Phosphoglucomutase		101	131
UDP-glucopyrophosphorylase		140	81
Glycogen synthase		72	199
Acetoacetyl-CoA reductase	NADPH	73	120



Pathways of methanol metabolism in *Beijerinckia mobilis*

mutase, UDP-glucopyrophosphorylase, glycogen synthase, and NADPH-dependent acetoacetyl-CoA reductase confirms their involvement in the synthesis of reserve biopolymers, such as glycogen and polyhydroxybutyrate.

The comprehensive metabolic characteristics of *B. mobilis* schematically presented in the figure are important for understanding ecophysiology and the role of this facultative methylotroph in natural microbial communities [1, 5].

REFERENCES

1. Becking, J.H., The Genus *Beijerinckia*, in The Prokaryotes: *An Evolving Electronic Resource for the Microbiological Community*, Dworkin, M. *et al.*, Eds., New York: Springer, 1999 (http://141.150.157.117:8080/prokRUB/index.htm).
2. Becking, J.H., Studies on Nitrogen-Fixing Bacteria of the Genus *Beijerinckia*: I. Geographical and Ecological Distribution in Soils, *Plant Soil*, 1961, vol. 14, pp. 49–81.
3. Derx, H.G., Further Researches on *Beijerinckia, Annales Bogorienses*, 1950, vol. 1, pp. 1–12.
4. Tchan, Y.T., Studies of Nitrogen-Fixing Bacteria: VI. A New Species of Nitrogen-Fixing Bacteria, *Proceedings of the Linnean Society of New South Wales*, 1957, vol. 82, pp. 379–385.
5. Dedysh, S.N., Smirnova, K.V., Khmelenina, V.N., Suzina, N.E., Liesack, W., and Trotsenko, Y.A., Methylotrophic Autotrophy in *Beijerinckia mobilis, J. Bacteriol.*, 2005, vol. 187, no. 11, pp. 3884–3888.
6. Kalyuzhnaya, M., Khmelenina, V.N., Kotelnikova, S., Holmquist, L., Pedersen, K., and Trotsenko, Y.A., *Methylomonas scandinavica* sp. nov., a New Methanotrophic Psychrotrophic Bacterium Isolated from Deep Igneous Rock Ground Water of Sweden, *Syst. Appl. Microbiol.*, 1999, vol. 22, no. 3, pp. 565–572.
7. Trotsenko, Y.A., Doronina, N.V., and Govorukhina, N.I., Metabolism of Non-Motile Obligately Methylotrophic Bacteria, *FEMS Microbiol. Letts.*, 1986, vol. 33, no. 2, pp. 293–297.