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SHORT COMMUNICATIONS

Metabolism of Methanol and Glucose in *Angulomicrobium tetraedrale*

N. V. Doronina and Yu. A. Trotsenko[1]

Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

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Bacteria of the genus *Angulomicrobium* are aerobic chemoheterotrophs capable of utilizing mono- and/or polycarbon substrates [1, 2]. Unlike the recently described new species *A. amanitiforme*, which is unable to utilize C_1-substrates [2], the type species *Angulomicrobium tetraedrale*^{T} can grow on methanol and formate. These two bacteria, which are distinguished by the radial symmetry of their cells, multiply by budding. Information on the physiology and the metabolism of these bacteria is scarce. This prompted us to perform enzymatic analysis of methanol and glucose metabolism in the type strain *A. tetraedrale* VKM B-1335^T (= DSM 5895^T = Z-2821).

Enzymes were assayed by routine methods [3–5] in the extracts of cells grown on methanol or glucose. Autotrophic growth was studied in an $H_2 : CO_2 : O_2$ (75 : 15 : 10) atmosphere. The vitamin requirements of the strain was determined by growing it on methanol in a medium containing various vitamins (Sigma) at concentrations of 20 μg/l instead of yeast extract.

As can be seen from the table, *A. tetraedrale* possesses a complete set of enzymes necessary for the oxidation of methanol to CO_2 (the dehydrogenases of methanol, formaldehyde, and formate). The high activity of formaldehyde and formate dehydrogenases in the presence of NAD^+ indicates that the first step of oxidation of C_1-substrates produces reduced pyridine nucleotides. The absence of serine–glyoxylate aminotransferase and 3-hexulose-6-phosphate synthase suggests that the serine and ribulose monophosphate pathways are not involved in C_1-metabolism. The higher activity of hydroxypyruvate reductase in glucose-grown cells may indicate that this enzyme is not involved in methanol metabolism. The high activity of phosphoribulokinase and ribulose 1,5-bisphosphate carboxylase shows that methanol is assimilated at the level of CO_2 via the ribulose bisphosphate (RuBP) cycle. Transketolase and transaldolase are shown to be involved in the conversion of glyceraldehyde-3-phosphate to xylulose-5-phosphate, resulting in the regeneration of the primary CO_2 acceptor ribulose-1,5-bisphosphate. The cells grown on methanol or glucose were found to contain glycerate kinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, fructose-1,6-bisphosphate aldolase, 2-keto-3-deoxy-6-phosphogluconate aldolase, and 6-phosphofructokinase (ATP). Therefore, carbohydrates in this bacterium are metabolized via the glycolytic, pentose phosphate, and the Entner–Doudoroff pathways. The cells grown on glucose contained active glucokinase (ATP), pyruvate dehydrogenase, citrate synthase, 2-oxoglutarate dehydrogenase, isocitrate dehydrogenase ($NADP^+$), and malate dehydrogenase. The decreased activity of some enzymes of the tricarboxylic acid (TCA) cycle in the methanol-grown cells suggests that the role of this cycle in methylotrophs is predominantly biosynthetic. The activities of the key enzymes of the glyoxylate cycle (isocitrate lyase and malate synthase) in the methanol-grown cells were also lower than in the glucose-grown cells.

NH_4^+ was assimilated with the involvement of glycine dehydrogenase (NADPH), glutamate dehydrogenase (NAD(P)H), and the glutamate cycle enzymes glutamate synthase and glutamine synthetase. The anaplerotic fixation of CO_2 required pyruvate carboxylase, PEP carboxylase, and PEP carboxykinase. The activity of PEP carboxylase was higher during the methylotrophic growth of the bacterium.

Thus, *A. tetraedrale* assimilates methanol by oxidizing it to CO_2 via formaldehyde and formate, followed by the fixation of the CO_2 in the RuBP pathway. Hence, this bacterium may be considered a facultative autotroph with elements of methylotrophic metabolism. This suggestion is confirmed by the ability of *A. tetraedrale* to grow in an atmosphere of $CO_2 + H_2 + O_2$. The growth of this bacterium was found to be stimulated by biotin. The key enzymes of the autotrophic fixation of CO_2 (phosphoribulokinase and ribulose bisphosphate carboxylase) were induced during bacterial growth on methanol.

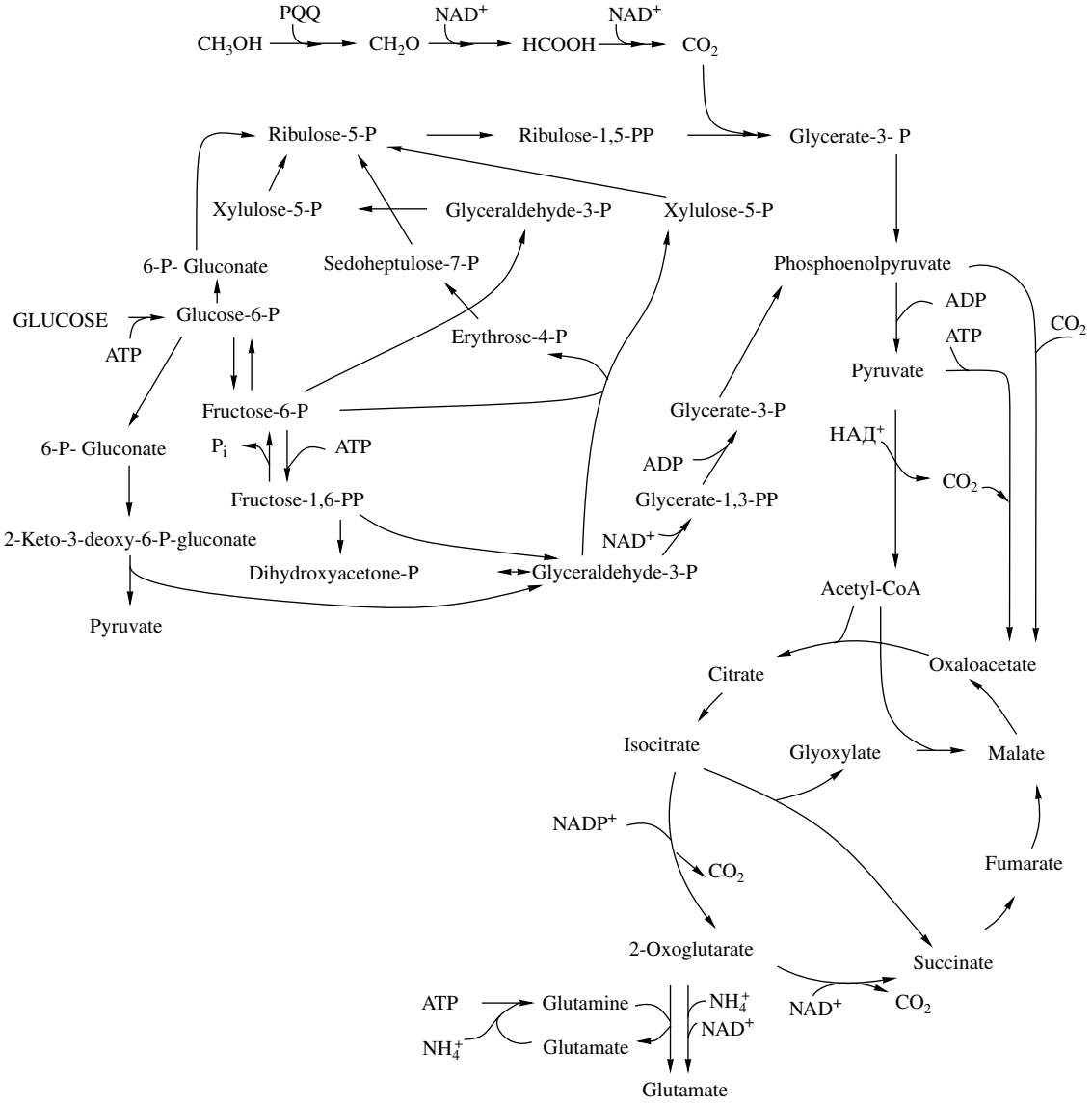
It should be noted that the carbon metabolism of *A. tetraedrale* considerably differs from that of *Beijer-*

1 Corresponding author; e-mail: trotsenko@ibpm.pushchino.ru

Enzyme activities (in nmol/(min mg protein)) in the extracts of *A. tetraedrale* cells grown on methanol or glucose

Enzyme	Cofactor	Activity in cells grown on	
		Methanol	Glucose
Methanol dehydrogenase	PMS	70	0
	NAD^+	0	0
Formaldehyde dehydrogenase	PMS	9	0
	NAD^+	98	10
Formate dehydrogenase	PMS	55	13
	NAD^+	600	0
Hydroxypyruvate reductase	NADH	136	267
	NADPH	43	76
Serine–glyoxylate aminotransferase	NAD(P)H	0	0
Hexulose-3-phosphate synthase		0	0
Phosphoribulokinase	ATP	276	0
Ribulose 1,5-bisphosphate carboxylase		104	0
Glycerate kinase	ATP	92	99
Transaldolase		78	86
Transketolase		60	72
Glucose-6-phosphate dehydrogenase	NAD^+	51	63
	$NADP^+$	25	39
6-Phosphogluconate dehydrogenase	NAD^+	16	31
	$NADP^+$	1	4
Fructose-1,6-bisphosphate aldolase	NADH	50	61
Fructose-1,6-bisphosphatase		25	54
6-Phosphofructokinase	ATP	49	52
2-Keto-3-deoxy-6-phosphogluconate aldolase		23	35
Pyruvate kinase	ADP	64	71
Pyruvate dehydrogenase	NAD^+	19	43
Citrate synthase		83	354
Isocitrate dehydrogenase	NAD^+	0	0
	$NADP^+$	77	163
α-Oxoglutarate dehydrogenase	NAD^+	13	42
Malate dehydrogenase	NADH	754	1549
	NADPH	249	372
Isocitrate lyase		36	75
Malate synthase		29	68
Alanine dehydrogenase	NAD(P)H	0	0
Glycine dehydrogenase	NADH	0	0
	NADPH	9	23
Glutamate dehydrogenase	NADH	20	24
	NADPH	16	17
Glutamate synthase	NADH	0	0
	NADPH	29	27
Glutamine synthetase	ATP, Mg^{2+}	31	40
	ATP, Mn^{2+}	2	2
Glucokinase	ATP	15	198
Pyruvate carboxylase	Acetyl-CoA, Mg^{2+}	23	20
PEP carboxylase	Acetyl-CoA, Mn^{2+}	99	74
PEP carboxykinase	ADP, Mg^{2+}	105	59

Note: PEP, phosphoenolpyruvate; PMS, phenazine methosulfate.



Putative metabolic pathways of methanol and glucose in *A. tetraedrale.*

inckia mobilis, another facultative methylotroph with the RuBP pathway of C_1-metabolism [4]. Indeed, in contrast to *B. mobilis, A. tetraedrale* lacks the serine pathway and possesses the Entner–Doudoroff pathway.

The putative metabolic pathways of methanol and glucose in *A. tetraedrale* are shown in the figure.

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