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SHORT COMMUNICATIONS

Metabolic Peculiarities of an Obligate Degrader of Ethylenediaminetetraacetate

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Ethylenediaminetetraacetate (EDTA) is widely used in industry due to its ability to form stable water-soluble complexes with di- and trivalent metal ions. The accumulation of EDTA in the environment because of its high persistence [1] leads to the transition of heavy and toxic metal ions into a soluble state that deteriorates drinking water and presents a hazard to human health.

Microbial degradation of EDTA is the most effective way of its removal from the environment. Only few pure cultures of EDTA degraders have been isolated so far: *Agrobacterium* sp. ATCC 55002 [2], which was recently reidentified as *Rhizobium radiobacter* [3]; the gram-negative strain BNC 1 [4], *Pseudomonas* sp. LPM-410 [5], and strain DSM 9103 [6]. All these isolates are facultative EDTA degraders, since they are able to grow on a wide range of organic compounds, including carbohydrates and organic acids. In strain DSM 9103, the first stage of EDTA degradation is catalyzed by a monooxygenase, which consists of two subunits, A (monooxygenase) and B (oxidoreductase) [7]. The pathways for the further conversion of the EDTA degradation products, N,N'-ethylenediaminediacetate (EDDA) and glyoxylate, have not yet been studied.

Recently, a novel EDTA-degrading strain LPM-4 was isolated from activated sludge of the municipal sewage treatment plant in Pushchino, Russia. The cells of this strain are gram-negative colorless rods unable to grow on alcohols, carbohydrates, various organic acids, amino acids, nutrient agar, glucose– potato agar, and casein hydrolysate [8, 9]. Ammonium sulfate and potassium nitrate exerted no effect on the growth of strain LPM-4 in EDTA-containing media, whereas organic nitrogen sources (peptone, casein hydrolysate, and peptides) completely inhibited its growth. Since strain LPM-4 is the only known obligate degrader of EDTA, it was of interest to investigate its taxonomic and metabolic characteristics in more detail.

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The aim of this work was to study the carbon and nitrogen metabolism of strain LPM-4.

The strain was grown in a liquid mineral medium [10] with 0.1% Mg-EDTA as the sole source of carbon, energy, and nitrogen on a shaker (180 rpm) at 28°C. The concentration of ammonium ions in the culture broth was determined using an Ekotest-120 ionomer (EKONIKS, Russia) equipped with an EKOM-NH_4 electrode.

Glyoxylate was analyzed by converting it into a derivative of 1,5-diphenylformazan [11]. Enzyme activities in cell extracts were measured by routine methods [7, 12, 13].

It was found that the ammonium concentration in the culture broth reached a maximum (60 mg/l) after 50 h of cultivation, when the specific growth rate was also at a maximum (0.06 h^{-1}), and then decreased to 10–15 mg/l.

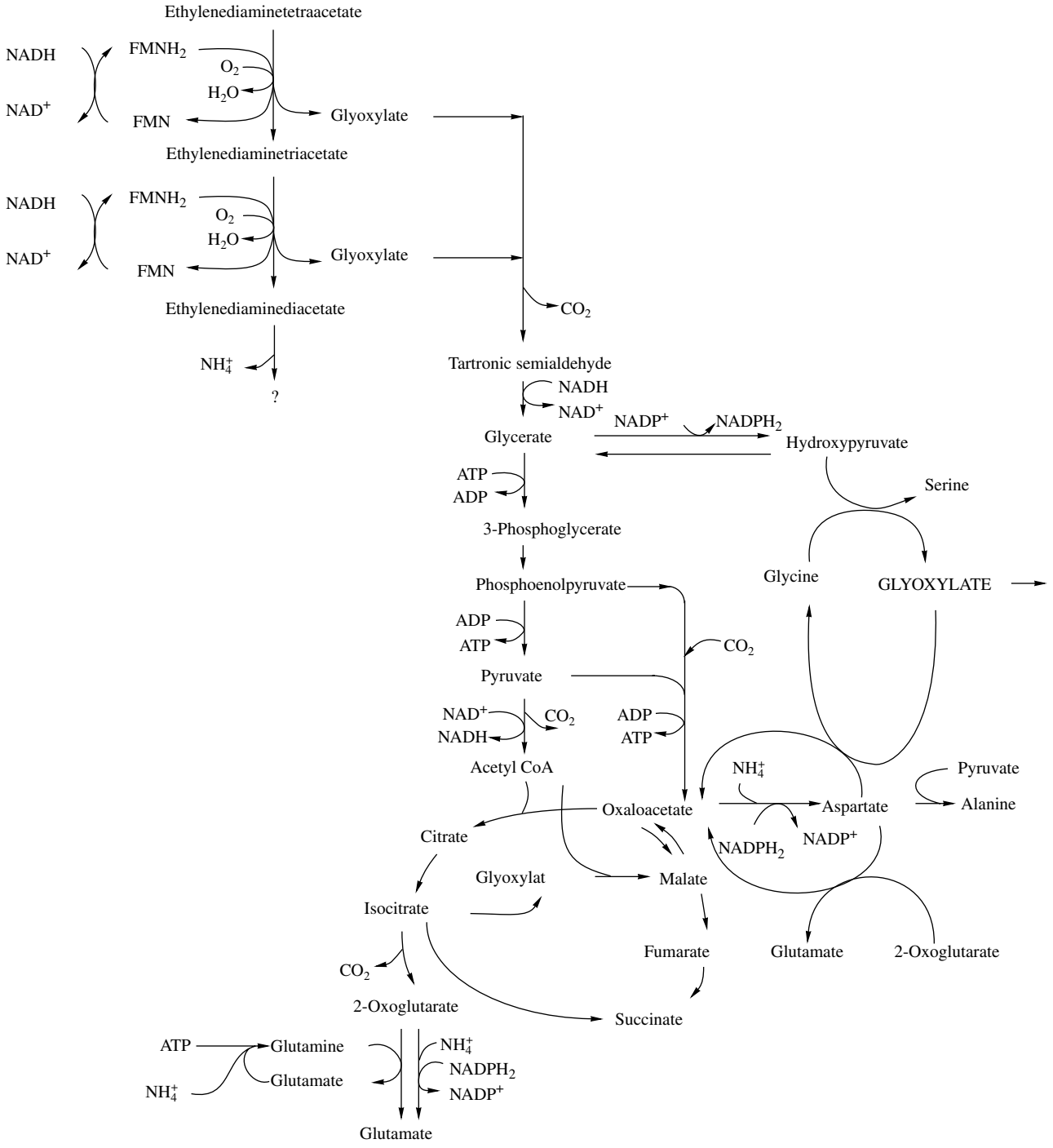
As is evident from the table, strain LPM-4 possesses EDTA monooxygenase, which is active in the presence of $NADH_2$ and is stimulated by flavin mononucleotide (FMN). Polarographic studies showed that the reaction catalyzed by EDTA monooxygenase is accompanied by the O_2 uptake; therefore, this enzyme is similar to the monooxygenase of strain DSM 9103 [7]. It was found that the first product of EDTA degradation in strain LPM-4 is glyoxylate, which becomes involved in the glyoxylate cycle with the aid of malate synthase. The high activities of glyoxylate carboligase, the key enzyme of the glycerate pathway, and glycerate kinase were also detected. Erythro-β-hydroxyaspartate aldolase activity was not detected, which excluded the aldol condensation of glyoxylate and glycine. The latter can be converted to serine by serine thanshydroxymethylase and then to pyruvic acid by serine dehydratase. However, serine dehydratase activity was not found in strain LPM-4. Another possible way for the conversion of serine to hydroxypyruvate involves serine–oxaloacetate aminotransferase; however, this enzyme was not found in strain LPM-4. Hydroxypyruvate reductase required $NADPH_2$ for activity. No transamination of

Enzyme activities in cell extracts of EDTA-grown strain LPM-4

Enzymes	Cofactors	Enzyme activity, nmol/(mg protein · min)
EDTA monooxygenase	NADH	80
	NADH, FMN	156
	NADH, FAD	81
Glyoxylate carboligase	TPP, Mg^{2+}	10
Glycerate kinase	ATP	109
Erythro-β-hydroxyaspartate aldolase		0
Serine dehydratase	NAD(P)H	0
Serine-oxaloacetate aminotransferase	NAD(P)H	0
Hydroxypyruvate reductase	NADH	0
	NADPH	69
Pyruvate kinase	ADP	152
Pyruvate dehydrogenase	NAD^+	9
Citrate synthase		63
Isocitrate dehydrogenase	NAD^+	0
	$NADP^+$	118
α-Oxoglutarate dehydrogenase	NAD^+	0
Malate dehydrogenase	NADH	0
	NADPH	87
Malate synthase		91
Isocitrate lyase		4
Hexokinase	ATP	9
Glucose-6-phosphate dehydrogenase	NAD^+	0
	$NADP^+$	12
6-Phosphogluconate dehydrogenase	NAD^+	0
	$NADP^+$	12
6-Phosphofructokinase	ATP, PP_i	0
Fructose-1,6-bisphosphate aldolase	NADH	0
KDPG aldolase		0
Glutamate dehydrogenase	NADH	0
	NADPH	14
Aspartate dehydrogenase	NADH	26
	NADPH	128
Glutamate synthase	NADH	0
	NADPH	14
Glutamine synthetase	ATP, Mg^{2+}	8

Note: KDPG means 2-keto-3-deoxy-6-phosphogluconate.

hydroxypyruvate to serine or phosphohydroxypyruvate to phosphoserine was detected spectrophotometrically; however, a high activity of serine–glyoxylate aminotransferase was revealed by thin-layer chromatography. Since this reaction is reversible, serine biosynthesis can proceed through the amination of hydroxypyruvate. The high activity of pyruvate kinase is indicative of the conversion of phosphoenolpyruvate (which is formed from 3-phosphoglyceric acid) to pyruvate. The latter is oxidized by pyruvate dehydrogenase to acetyl CoA, which enters the Krebs cycle. The strain under study possesses $NADP^+$-dependent isocitrate dehydrogenase and lacks 2-oxoglutarate dehydrogenase, the glycolytic enzymes 6-phosphofructokinase and fructose-1,6-bisphosphate aldolase, as well as 2-keto-3-deoxy-6-phosphogluconate aldolase. This circumstance explains the inability of this strain to grow on carbohydrates and organic acids.



Carbon and nitrogen metabolism in strain LPM-4.

The activities of enzymes involved in anaplerotic CO_2 fixation, namely, pyruvate carboxylase (both acetyl CoA^- and Mn^{2+}-dependent), PEP carboxylase (Mn^{2+}-dependent), and PEP carboxykinase (ADP-dependent) were also detected.

Ammonium was assimilated through the reductive amination of 2-oxoglutarate and oxaloacetate, as well as via the glutamate cycle enzymes, glutamate synthase and glutamine synthetase. Transamination reactions between the substrate pairs serine–glyoxylate, aspartate–glyoxylate, aspartate–2- oxoglutarate, and aspartate–pyruvate proceeded at rates of 102, 10, 10, and 2 nmol/(mg protein · h), respectively.

The scheme shown in the figure illustrates the primary and intermediate metabolism of EDTA in strain LPM-4. Our future studies will aim at the metabolism

of EDDA, an intermediate of EDTA degradation, and the molecular and genetic causes for the obligate dependence of this unique strain on EDTA.

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