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SHORT COMMUNICATIONS

Effect of Exogenous Indoleacetic Acid on the Activity of the Central Metabolism Enzymes in *Methylobacterium extorquens* AM1

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Pink-pigmented aerobic facultative methylotrophic bacteria of the genus *Methylobacterium* are widespread phyllosphere symbionts. Phytosymbiosis of methylobacteria is stipulated by the methanol released into the environment by plants through stomata and consumed by methylobacteria as a carbon and energy source. In turn, methylobacteria stimulate plant growth and development through synthesis of bioactive substances, or phytohormones (namely, auxins and cytokinins) [1–3].

Despite of the fact that indole-3-acetic acid (IAA) is a major plant auxin and a key phytosymbiosis factor, the stimulating activity of auxins upon the metabolism of bacterial producers was not reported until recently [4]. Transcriptome and enzymological analysis revealed that treatment of an *Escherichia coli* culture with IAA resulted in increased levels of mRNA and increased the activity of central metabolic enzymes [5] as well as improved resistance of bacterial cells to various stress factors [6]. Therefore, bacter;ia producing IAA may be themselves a subject of its activity. Moreover, several up-regulated IAA genes were found in *Azospirillum brasilense* and *Rhizobium etli* which are known to synthesize the auxin [7, 8]. However, no data has been produced so far concerning the effect of IAA upon the metabolism of IAA-producing bacteria, including methylotrophs. The aim of the present work was therefore to investigate the effect of IAA added into the growth medium on the activity of central metabolic enzymes in the *M. extorquens* strain AM1 and its mutant Δ*ipdC*, twice less active in IAA synthesis.

M. extorquens AM1 (VKM V-2067 = NCIB 9133 = ATCC 14718) and its mutant strain Δ*ipdC* were grown in 2 l conical flasks on a K medium containing the following (g/l): KH_2PO_4, 2; $(NH_4)_2SO_4$, 2; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.025; and $FeSO_4 \cdot 7H_2O$, 0.002; pH 7.2; it was supplemented with 1% (by vol.) methanol or 5 g/l succinate. Cultures were grown on a shaker (180 rpm) at 29°C until the mid-exponential growth phase ($OD_{600} = 0.8$). Then, cultures were divided in two parts; 0.25 mM IAA (Sigma) dissolved in methanol was added to the first part, and an equal volume of methanol, to the second part in order to exclude the stimulating activity of methanol added with IAA. By the end of the exponential growth phase ($OD_{600} = 1.4$), the cells were precipitated by centrifugation (6000 *g*, 30 min, 4°C), washed with the buffer according to the procedure for determination of the enzyme activity, and resuspended in the same buffer. The cells were disintegrated using an MSE ultrasound disintegrator (England) with a capacity of 150 W at 20 kHz (4 × 30 s) at 4°C. Whole cells and cell fragments were separated by centrifugation (10000 *g*, 45 min) and the supernatant was used for spectrophotometric determination of the enzymatic activity (Shimadzu Pharmaspec UV-1700, Japan) as was described previously [9]. In the extracts, protein was determined by the Lowry method [10]. Enzyme activity was measured in three independent experiments in three repeats. Statistical processing of the data was performed with the Systat Sigmaplot 10.0 software package (http://www.sigmaplot.com/home.php). In the table, the average results of three independent experiments are presented (standard error did not exceed ±5%).

The results of enzymological analysis of cell extracts of the strain AM1 and Δ*ipdC* mutant grown on a mineral medium supplemented with either methanol or succinate with or without IAA are given in the table. It was demonstrated that the activity of the first enzyme of methanol oxidation, methanol dehydrogenase (MDH), did not change in comparison to the samples which were not treated with IAA in the case of both strain AM1, and the mutant. The result is logical, in view of recent evidence that the expression of MDH structural genes is not affected by the shift from a methanol to succinate-containing medium [12]. By contrast with MDH structural genes, those responsible for bio-

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Activity of the enzymes of central metabolism (nmol/min mg protein) in the extracts of *Methylobacterium extorquens* strain AM1 and its *ΔipdC* mutant grown on mineral medium containing methanol or succinate with or without addition of 0.25 mM IAA

Enzyme	Cofactor	AM1 strain				Δ*ipdC* strain			
		Methanol		Succinate		Methanol		Succinate	
		–IAA	+IAA	–IAA	+IAA	–IAA	+IAA	–IAA	+IAA
Methanol dehydrogenase	PMS	169	175	4	3	165	168	16	15
Formaldehyde dehydrogenase	PMS	11	15	7	6	18	14	8	17
	NAD^+	1	4	0	0	1	3	0	0
	NAD^+, GSH	2	3	0	0	3	4	0	0
Formate dehydrogenase	PMS	2	1	0	0	2	3	0.2	0.5
	NAD^+	43	42	0	0	34	50	0	0
Oxypyruvate reductase	NADH	244	250	151	126	201	247	93	171
	NADPH	16	18	50	47	13	14	51	88
Serine–glyoxylate aminotransferase	NADH	137	147	17	22	76	100	26	32
	NADPH	10	11	1	1	7	15	2	2
Glyoxylate reductase	NADH	42	50	25	17	27	38	25	20
Isocitrate dehydrogenase	NADPH	20	17	67	76	24	19	54	95
Citrate synthase	DTNB	5	7	29	32	25	29	34	56
α-Ketoglutarate dehydrogenase	NAD^+	9	9	30	19	28	33	30	43

PMS, phenazine methosulfate; GSH, reduced glutathione; DTNB, dithionitrobenzoate.

synthesis of an MDH cofactor, pyrroloquinoline quinone, were suppressed in the cells transferred to succinate medium [12]. This explains a decrease in MDH activity in the extracts of succinate-grown cells.

On the other hand, expression of the structural genes coding for the enzymes of further methanol oxidation (formaldehyde and formate dehydrogenases) and formaldehyde assimilation (oxypyruvate reductase and L-serine-glyoxylate aminotransferase) decreased significantly in the presence of succinate [12]. Apparently, due to a higher variability in the regulation of these enzymes, only a slight stimulation of their activity may be observed in the case of methylotrophic growth of strain AM1 (see the table). By analogy with *E. coli* experiments [5], increased activity of the enzymes in the extracts of *M. extroquens* AM1 cells treated with IAA may probably be due to an increase in the mRNAs of corresponding proteins.

As *M. extorquens* AM1, unlike *E. coli*, is capable of IAA synthesis from tryptophan, we assumed that the stimulating effect of IAA on AM1 cells should be less pronounced. Therefore, a *M. extorquens* mutant with deletion of the *ipdC* gene coding for the key enzyme of the indolepyruvate pathway of IAA biosynthesis, indole-3-pyruvate decarboxylase, was obtained by homologous recombination with a mutant allele [11], to verify the hypothesis. The mutant synthesized 55% less IAA than AM1. Enzymological analysis demonstrated that methylotrophic growth of the *ipdC* mutant resulted in increased activity of most of the tested enzymes (except for MDH, formaldehyde dehydrogenase (FDH), and isocitrate dehydrogenase) in response to the addition of IAA (see the table).

To evaluate the effect of IAA upon the activity of the enzymes of the citric acid cycle (TCA cycle), as well as on the enzymes of C_1 metabolism under heterotrophic growth conditions, experiments were performed with the cells grown on succinate-containing media. Enzymes of C_1 metabolism were shown to be down-regulated; especially, formaldehyde and formate dehydrogenases. On the contrary, the activity of the TCA cycle enzymes isocitrate dehydrogenase, citrate synthase, and α-ketoglutarate dehydrogenase increased (see the table). In the case of heterotrophic growth of the mutant, enzyme activity was stimulated by IAA, in particular, levels of oxypyruvate reductase, citrate synthase, and isocitrate dehydrogenase were almost doubled. On the contrary, the effect of enzyme stimulation by exogenous IAA was less pronounced in the parent strain under methylotrophic and heterotrophic growth conditions. Therefore, the mutant with impaired IAA biosynthesis is a potent model for further investigation of the molecular mechanisms of the effect of this auxin on bacterial metabolism.

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