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SHORT COMMUNICATIONS

Sucrose as a Factor of Thermal Adaptation of the Thermophilic Methanotroph *Methylocaldum szegediense* O-12

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Methane-utilizing bacteria (methanotrophs) are known to include thermotolerant and moderately thermophilic members of the genera *Methylococcus, Methylocaldum*, and *Methylothermus* [1–5]. However, the mechanisms of their adaptation to elevated temperatures have not been studied, and this fact determined the goal of the present work: the search for potential thermoprotectants in methanotrophs.

The moderately thermophilic methanotroph *Methylocaldum szegediense* O-12, isolated from silage samples, was cultured at 42°C or 55°C; the thermotolerant *Methylococcus capsulatus* Bath was cultured at 37°C; and the mesophilic *Methylocystis echinoides* 2 was cultured at 28°C under the atmosphere of methane and oxygen (1 : 1) on P medium [6]. Low-molecular organic compounds were extracted from the cells with 80% ethanol [7]; the alcohol–water-soluble fraction was analyzed by thin-layer chromatography (TLC) and ^{1}H-NMR [8]. The content of sucrose was determined with anthrone reagent in modification [9]. Cell-free extracts were obtained and the activities of hydroxypyruvate reductase, hexulose phosphate synthase, formate dehydrogenase, and ribulose bisphosphate carboxylase were determined as described in [6]. The activity of sucrose phosphate synthase in cell-free extracts was determined by the formation of uridine diphosphate (UDP) with coupling enzymes (pyruvate kinase and lactate dehydrogenase); the activity of sucrose phosphorylase was estimated by the orthophosphate-dependent production of fructose from sucrose with the coupling enzymes (hexokinase, isomerase, and glucose-6-phosphate dehydrogenase) [10]. The thermostabilizing effect of sucrose was studied by exposing cell-free extracts of *Md. szegediense* O-12, *Mc. capsulatus* Bath, and *Ms. echinoides* 2, or commercial preparations of lactate dehydrogenase from rabbit muscle (Serva) to temperatures of 50 or 60°C for 10 or 20 min, respectively. Residual enzyme activities were determined at 30°C and expressed as a percentage of activity without heating.

Enzymological analysis of cell-free extracts showed that the optimal temperatures for formate dehydrogenase, hexulose phosphate synthase, and hydroxypyruvate reductase activities in the thermophilic *Md. szegediense* O-12 were higher (50, 60, and 65°C, respectively) than in the thermotolerant *Mc. capsulatus* Bath (40–50°C) or the mesophilic *Ms. echinoides* 2 (40°C) Strans (Fig. 1). This is evidence of adaptive changes in the properties of the enzymes of C_1 metabolism in thermophilic and thermotolerant methanotrophs.

Sucrose was found in the cells of *Md. szegediense* O-12 by the methods of ^{1}H-NMR and TLC (Fig. 2). The ability to accumulate sucrose in *Md. szegediense* O-12 correlates with the higher halotolerance of the strain (up to 3% NaCl) [6], which indirectly indicates the role of sucrose as an osmoprotectant. However, the content of sucrose increased both at elevated salinity of the medium and at elevated cultivation temperature, reaching maximal values (1.2%) in the cells growing at 57°C in the presence of 1% NaCl (Table 1). On the contrary, mesophilic *Ms. echinoides* 2 and thermotolerant *Mc. capsulatus* Bath did not accumulate sucrose at suboptimal growth temperatures (37–45°C).

Sucrose phosphate synthase activity was revealed in the extracts of *Md. szegediense* O-12 cells grown at 45°C or 57°C, evidencing that sucrose is synthesized in this methanotroph from UDP-glucose and fructose-6-phosphate, the primary metabolites of the ribulose monophosphate pathway of assimilation of C_1 compounds (Table 1). The presence of minor sucrose phosphorylase activity (~5 nmol min^{-1} mg^{-1} protein) in the extracts denotes the ability of this methanotroph to decompose sucrose to glucose-1-phosphate and fructose. It is noteworthy that the activity of sucrose phosphate synthase in the cells grown at 55°C was higher as compared with those grown at 42°C, but did not depend on the presence of NaCl in the medium. This finding supports the suggestion that sucrose accumulation in *Md. szegediense* O-12 is associated primarily with ther-

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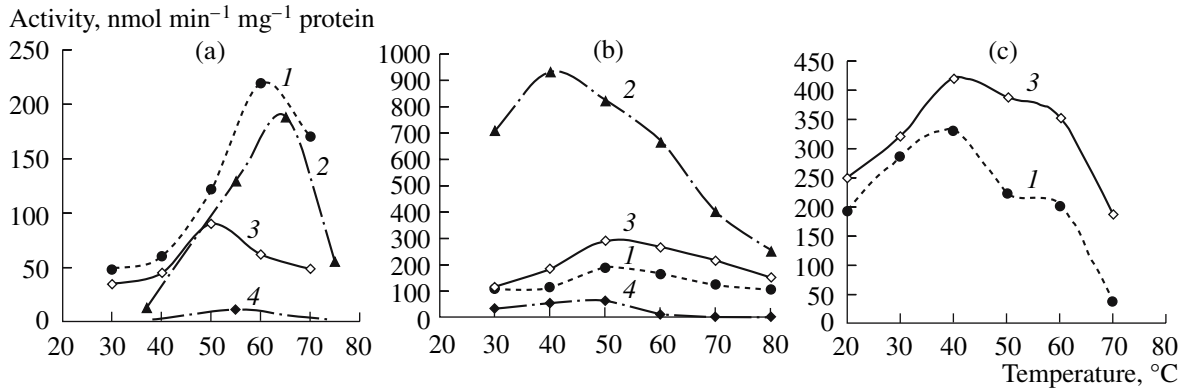

Fig. 1. The effect of measurement temperature on the key enzyme activities in cell-free extracts of *Methylocaldum szegediense* O-12 (a); *Methylococcus capsulatus* Bath; (b); and *Methylocystis echinoides* (c). *1*, hydroxypyruvate reductase; *2*, hexulose phosphate synthase; *3*, formate dehydrogenase; *4*, ribulose bis-phosphate carboxylase.

mal adaptation; the previously demonstrated stimulation of growth of thermophilic methanotrophs of the genera *Methylocaldum* and *Methylothermus* by adding NaCl to the medium [5, 6] seems to result from the necessity to equilibrate the osmotic pressure when this disaccharide accumulates in the cytoplasm.

The addition of 40–400 mM sucrose to the lactate dehydrogenase preparation increased the residual enzyme activity after 10 min heating at 50°C as compared with heating in the absence of sucrose (Table 2). Cell-free extracts of *Md. szegediense* O-12 also had a thermostabilizing effect on lactate dehydrogenase. Moreover, sucrose in a concentration of 10 or 20 mM stabilized formate dehydrogenase in cell-free extracts of *Md. szegediense* O-12. After 20 min of incubation at 60°C in the presence of 20 mM sucrose, 50% of the enzymatic activity was retained, as compared with the control without additions (10% of residual activity) (Fig. 3). Hence, sucrose may be considered as a thermoprotectant stabilizing the enzymes of C_1 metabolism in *Md. szegediense* O-12 and lactate dehydrogenase under the influence of higher temperatures.

Thus, for the first time we have revealed that the thermal adaptation of *Md. szegediense* O-12 occurs at

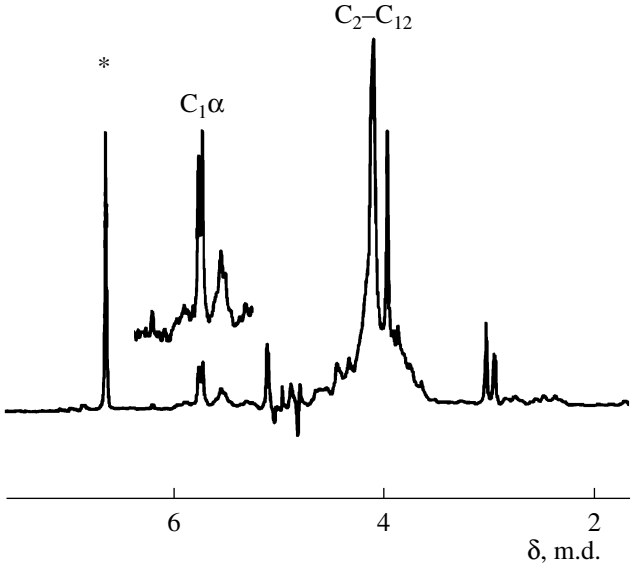

Fig. 2. The ^{1}H-NMR spectrum of the alcohol–water-soluble fraction of *Methylocaldum szegediense* O-12 cells grown at 55°C. The peaks correspond to proton signals at the respective sucrose carbon atoms ($C_{\alpha 1}$ and C_2–C_{12}). are proton signals of maleic acid as internal standard.

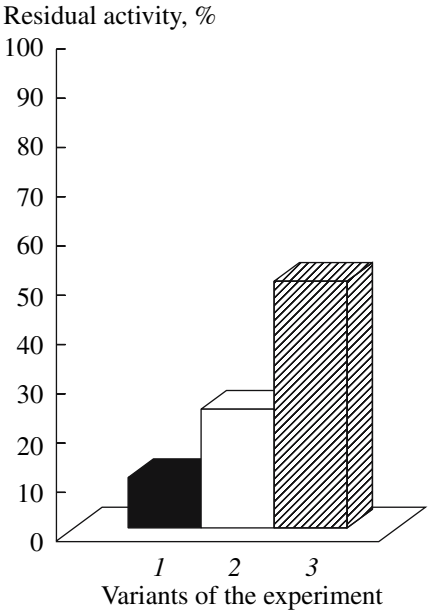

Fig. 3. The effect of sucrose on the thermal stability of formate dehydrogenase in cell-free extracts of *Methylocaldum szegediense* O-12. Residual enzyme activity after heating at 60°C in the absence (*1*) or presence of 10 mM (*2*) and 20 mM (*3*) of sucrose is expressed as a percentage of the control (without heating).

Table 1. Sucrose content in *Md. szegediense* O-12 cells and the activity of sucrose phosphate synthase under different cultivation conditions

Growth conditions	Sucrose content (μg/100 mg dry cells)	Sucrose phosphate synthase, nmol/(min mg protein)
45°C without NaCl	50–150	11.3
45°C + 1% NaCl	440	8.2
57°C without NaCl	250	20
57°C + 0.5% NaCl	555	20
57°C + 1% NaCl	1230	15.5

Table 2. The activity of lactate dehydrogenase (% of the initial value) after 10 min of incubation at 50°C in the presence of sucrose or extracts of *Md. szegediense* O-12 cells grown at 37°C or 57°C

Supplement	% of activity
Without supplements	38.0
Cell extract, 37°C	64.6
Cell extract, 57°C	69.7
Alcohol–water-soluble cell fraction	46.0
Sucrose, 40 mM	52.3
Sucrose, 400 mM	82.7

* Sucrose concentration was 10 mM.

the level of changes in enzyme properties as well as through accumulation of sucrose, which stabilizes proteins at elevated temperatures. Apparently, thermotolerant and thermophilic methanotrophs are of great interest as new model organisms for further study of the molecular mechanisms of thermal adaptation and as potential products of thermoenzymes and thermoprotectants from available renewable carbon sources (methane and methanol).

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