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SHORT COMMUNICATIONS

Energy Supply Processes in Moderately Thermophilic Bacteria of the Genus *Sulfobacillus*

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Bacteria of the genus *Sulfobacillus*, family *Alicyclobacillaceae* are aerobic acidophilic chemolithotrophs capable of utilizing mineral and organic compounds as energy sources and electron donors [1].

All sulfobacilli survive an unlimited number of transfers only under mixotrophic conditions [2]. We have previously demonstrated for the thermotolerant bacterium *Sulfobacillus thermotolerans* strain Kr1^T that a low ATP pool in autotrophically and heterotrophically grown cells was among the possible explanations for limited lithotrophic and organotrophic growth [3].

The goal of the present work was to investigate ATP pools in the moderately thermophilic species *S. sibiricus* N1^T [4] and *S. thermosulfidooxidans* 1269^T [5] and to elucidate the reasons for growth cessation under auto- and heterotrophic conditions. An obligate autotroph *Acidithiobacillus* (*At.*) *ferrooxidans* TFBk and an obligate heterotroph *Alicyclobacillus* (*Alb.*) *acidocaldarius* 27009^T were used for comparison.

S. sibiricus N1^T (VKM B-2380^T = DSM 17363^T) and *S. thermosulfidooxidans* 1269^T (VKM B-1269^T = DSM 9293^T) were cultivated on a modified 9K Silver and Lundgren liquid mineral medium [6] at 55 and 50°C, respectively. For autotrophic cultivation, the medium was supplemented with $FeSO_4 \cdot 7H_2O$ (2.0–2.5 g/l as Fe^{2+}) and 1–2 mM $Na_2S_2O_3$ (for strain 1269). For mixotrophic growth, a mixture of glucose (0.02%) and yeast extract (0.02%) was used as a substrate together with ferrous iron (and thiosulfate in the case of strain 1269) in the concentrations specified above. Media with glucose (0.02%) and yeast extract (0.02%) were used for organotrophic growth. The inocula were obtained under optimal conditions of mixotrophic growth. *At. ferrooxidans* TFBk [7] was grown at 28°C on 9K medium with $FeSO_4 \cdot 7H_2O$ to the final concentration of 7.0 g/l (as Fe^{2+}). *Alb. acidocaldarius* ATCC 27009^T = DSM 446^T was grown at 55°C on BAM medium [8] with glucose (0.5%) and yeast extract (0.2%) as energy sources. For acidithiobacillus, sulfobacilli, and alicyclobacillus, pH was adjusted to 1.8, 2.0, and 4.0, respectively, with 10 N H_2SO_4. The cultures were grown in Erlenmeyer flasks with shaking (180 rpm). The parameters of bacterial growth and energy substrate utilization were determined as described in [3]. The ATP pool was measured in the growth dynamics by a bioluminescence method [9] with modifications [3]. All experiments were carried out with at least five trials; typical experimental results are presented in the work. The results obtained for *S. sibiricus* N1 are shown in the figures.

In both strains of moderately thermophilic sulfobacilli, a similar relationship was revealed between the growth parameters and the intracellular ATP content. Strain *S. sibiricus* N1 was shown to grow better than *S. thermosulfidooxidans* 1269 under heterotrophic conditions. The highest biomass values for strains N1 and 1269 were 13.5 (Fig. 1, curve *1*) and 12.6 μg protein/ml, respectively (table). Glucose utilization by strain 1269 commenced only after 6 h of incubation, probably due to deficiency in the transport system [10] and/or insufficient activity of the enzymes required for glucose oxidation and assimilation under unfavorable conditions [11]. *S. sibiricus* N1 utilized this substrate throughout the growth cycle (Fig. 1, curve *2*). High activities of the following enzymes were revealed in *S. sibiricus* N1 cell-free extracts: 6-phosphogluconate dehydrogenase, the key enzyme of the oxidative pentose phosphate pathway (OPPP), and glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase, the enzymes of the glycolytic pathway [12]. In organotrophically-grown *S. thermosulfidooxidans* 1269, no activity of OPPP was detected; the enzymatic activity suggested sugar catabolism via two pathways, glycolysis and the Entner–Doudoroff pathway [11]. For both sulfobacilli, intracellular ATP content was shown to change in the course of growth (Fig. 1, curve *3*). Under organoheterotrophic growth conditions, the highest ATP content in strain N1 was ~1.5 times higher than in strain 1269 (0.7 and 0.5 nmol/mg protein, respectively; see table). In obligately heterotrophic *Alb. acidocaldar-*

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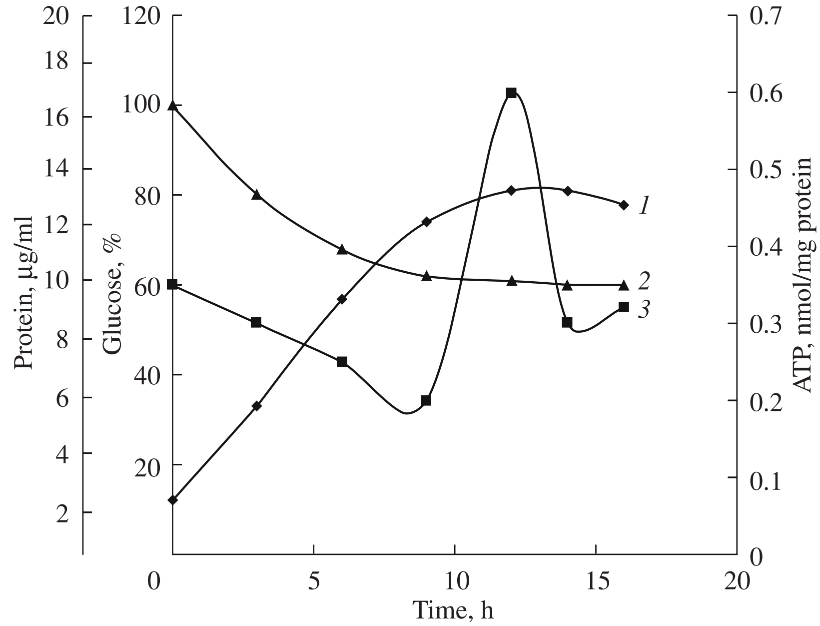

Fig. 1. Organotrophic growth of *S. sibiricus* N1 in the medium with glucose and yeast extract: *1*, growth, protein, µg/ml; *2*, glucose utilization, %; *3*, ATP, nmol/mg protein.

ius 27009, the highest intracellular ATP content (12.8 nmol/mg protein) was ~20 times higher than in organotrophic sulfobacilli; this fact may explain weak organotrophic growth of the latter (table).

When *S. sibiricus* N1 and *S. thermosulfidooxidans* 1269 were transferred from mixotrophic conditions to autotrophic ones, iron oxidation was observed throughout the experiment (Fig. 2, curve *2*). The cultures experienced stress; the lag phase was prolonged (6 h), and the ATP content decreased, probably due to the increased energy required for the maintenance of vital activity [13], for the functioning of the Calvin cycle [11], and for other processes. Strain 1269 grew more actively than strain N1, probably due to its ability to oxidize reduced sulfur compounds formed from thiosulfate and to its higher ribulose bisphosphate carboxy-

Growth parameters and ATP content in the cells of the cultures under study

Cultivation conditions; energy substrates	Protein, µg/ml	ATP, nmol/mg protein	Oxidized Fe^{2+}, %	Utilized glucose, %
S. sibiricus N1				
Mixotrophic, Fe^{2+}, glucose, yeast extract	20.0	2.4	100	45
Heterotrophic, glucose, yeast extract	13.5	0.7	–	40
Autotrophic, Fe^{2+}	3.6	0.2	100	–
S. thermosulfidooxidans 1269				
Mixotrophic, Fe^{2+}, $S_2O_3^{2-}$, glucose, yeast extract	22.5	4.4	100	35
Heterotrophic, glucose, yeast extract	12.6	0.5	–	20
Autotrophic, Fe^{2+}, $S_2O_3^{2-}$	6.3	0.1	100	–
At. ferrooxidans TFBk				
Autotrophic, Fe^{2+}	16.2	17.0	100	–
Al. acidocaldarius 27009				
Heterotrophic, glucose, yeast extract	92.0	12.8	–	60

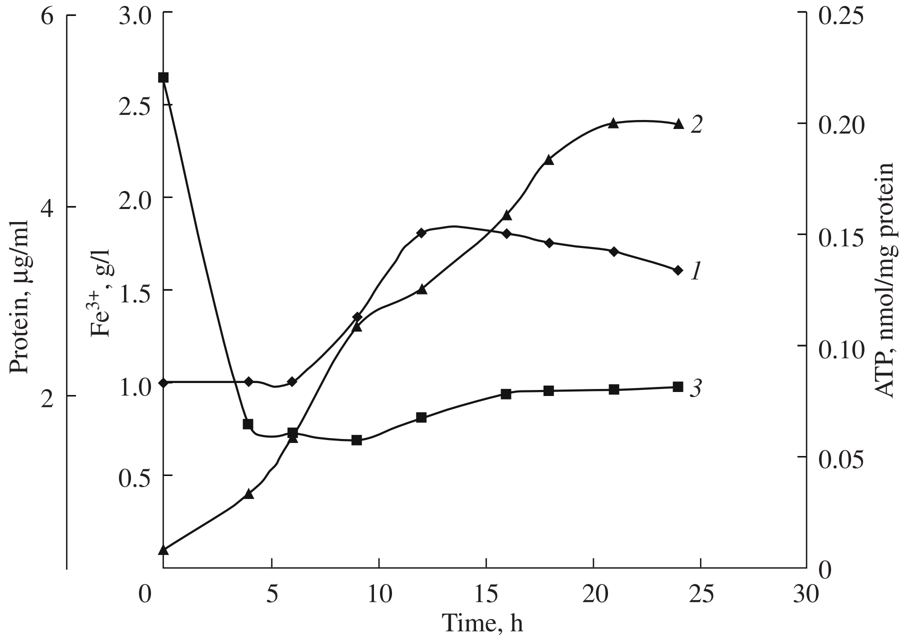

Fig. 2. Lithotrophic growth of *S. sibiricus* N1 in the medium with ferrous iron: *1*, growth, protein, μg/ml; *2*, Fe^{2+} oxidation, g/l; *3*, ATP, nmol/mg protein.

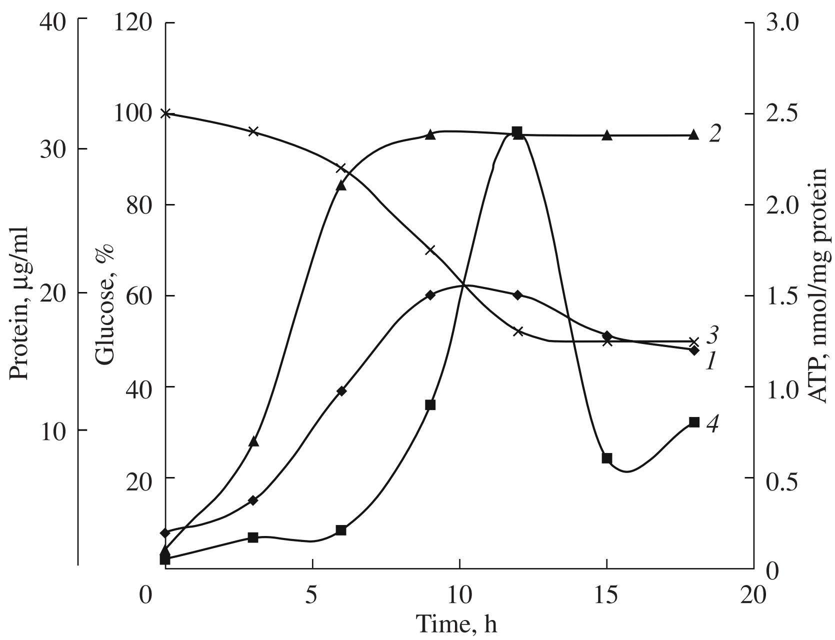

Fig. 1. Mixotrophic growth of *S. sibiricus* N1 in the medium with ferrous iron, glucose, and yeast extract: *1*, growth, protein, μg/ml; *2*, Fe^{2+} oxidation, g/l; *3*, glucose utilization, %; *4*, ATP, nmol/mg protein.

lase activity [14]. ATP content in the sulfobacilli cells grown under autotrophic conditions did not exceed 0.2 nmol/mg protein (Fig. 2, curve *3*); it was thus an order of magnitude lower than under optimal mixotrophic conditions (Fig. 3, curve *3*) and two orders of magnitude lower than in obligately autotrophic *At. ferrooxidans* cells (table). The cultures therefore experienced energy limitation.

Under optimal mixotrophic conditions, the highest biomass yield (as protein) was observed, 20–22.5 μg/ml; this was possibly the result of oxidation of all the iron introduced and of higher glucose utilization compared to organotrophic growth (45 and 35% of the total amount for strains N1 and 1269, respectively) (Fig. 3, curves *1–3*; table). Activity of the enzymes of all the three major pathways was shown for strain 1269 glucose metabolism; in strain N1, the enzymes of two pathways were revealed, the glycolytic and the oxidative pentose phosphate ones [11, 12]. Under mixotrophic conditions, the ATP content in sulfobacilli reached its maximum, 2.4–4.4 nmol/mg protein (Fig. 3, curve *4*; table). The table presents the data on biomass, iron oxidation, glucose utilization, and intracellular ATP content under different growth conditions.

Thus, the results of our investigation on energy supply (as ATP) for growth of moderately thermophilic sulfobacilli confirm the previous conclusion for thermotolerant mixotrophs [3] that a low level of intracellular ATP is among the possible reasons for cessation of organo- and lithotrophic growth after several transfers. The patterns of the changes in the energetic characteristics were found to be similar in thermotolerant and moderately thermophilic sulfobacilli.

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