ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 3, pp. 267–272. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © E.N. Detkova, V.V. Kevbrin, 2009, published in Mikrobiologiya, 2009, Vol. 78, No. 3, pp. 304–309.

EXPERIMENTAL ARTICLES

Cellobiose Catabolism in the Haloalkaliphilic Hydrolytic Bacterium *Alkaliflexus imshenetskii*

E. N. Detkova[1] and V. V. Kevbrin

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received March 19, 2008

Abstract—Cellobiose metabolism was studied in *Alkaliflexus imshenetskii*, a haloalkaliphilic hydrolytic bacterium capable of utilizing certain polymers of plant origin, as well as mono- and disaccharides. The major products of cellobiose fermentation by the bacterium were succinate and acetate, and formate was a minor product. Cellobiose could be split into glucose molecules by both β-glucosidase (hydrolytic pathway) and phosphorylase (phosphorolytic pathway); the activity of the former enzyme was two orders of magnitude higher (3600 nmol/(min mg) versus 36 nmol/(min mg)). In cell extracts of the bacterium, high activities of the Embden–Meyerhof–Parnas pathway enzymes—hexokinase, glucose-phosphate isomerase, and phosphofructokinase—were revealed, as well as the activities of glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and key enzymes of the Entner–Doudoroff pathway—6-phospho-gluconate dehydratase and 2-keto-3-deoxy-6-phospho-gluconate aldolase. Neither the activity of the key enzyme of the hexose-monophosphate pathway, 6-phospho-gluconate dehydrogenase, nor the activities of the key enzymes of the modified Entner–Doudoroff pathway, glucose dehydrogenase and 2-keto-3-deoxy-gluconate kinase, were revealed.

Key words: alkaliphiles, hydrolytic bacteria, cellobiose, β-glucosidase, phosphorylase, Embden–Meyerhof–Parnas pathway.

DOI: 10.1134/S0026261709030023

Cellulose is a widespread natural substrate utilized by diverse prokaryotes and fungi; its degradation is catalyzed by a multicomponent enzymatic complex, which in total is called cellulase. The main cellulase components are as follows [1]:

(1) endoglucanase (1,4-β-D-glucan-4-glucan hydrolase, EC 3.2.1.4), which cleaves β-1,4 bonds within cellulose macromolecule with the formation of oligosaccharides of varying length;

(2) cellobiohydrolase, or exoglucanase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91), which splits the cellobiose disaccharide from the cellulose chain;

(3) β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21), which hydrolyzes cellobiose with the formation of glucose.

In laboratory cultures, as a rule, microorganisms produce these enzymes only if cellulose is the only substrate available. The synthesis of the cellulase enzymes is suppressed both by alternative substrates and by cellobiose, which is the product of cellulose hydrolysis. Thus, cellobiose is an important regulatory intermediate. The most pronounced is the inhibitory effect exerted by cellobiose on cellobiohydrolase. Given that the growth of all currently known cellulolytic microorganisms on cellulose is not accompanied by cellobiose accumulation, they should possess enzymes for cellobiose degradation.

In prokaryotes, the biochemical pathways of cellulose hydrolysis have been thoroughly studied in aerobic and anaerobic neutrophilic bacteria, whereas alkaline cellulases are more or less studied in aerobic alkaliphiles only [2]. For the alkaliphilic anaerobic cellulolytic bacterium *Clostridium alkalicellulosi*, the presence of cellobiohydrolase and cellulosome proteins has been demonstrated [3]. It is worth noting that this cellulolytic bacterium shows equally good growth on cellulose and cellobiose [4], which suggests an efficient mechanism of cellobiose hydrolysis and utilization.

In most of the cellulolytic microorganisms studied, cellobiose is split via the hydrolytic pathway into two glucose molecules by cellobiase (β-glucosidase) [5]. However, in some bacteria, such as *Cellvibrio gilvus*, *Ruminococcus flavefaciens*, *R. albus*, and *Clostridium thermocellum*, cellobiose is converted to glucose-1-phosphate and glucose by cellobiose phosphorylase; i.e., phosphorolysis, or phosphorolytic cleavage, occurs [6–8]. It is commonly accepted that cellulolytic microorganisms possessing phosphorylase activity prefer cellobiose to glucose as the carbon and energy source.

Further degradation of glucose proceeds via the well-known biochemical pathways: the Embden–Meyerhof–Parnas (fructose-bisphosphate) pathway, hexose-monophosphate pathway, or Entner–Doudoroff

[1] Corresponding author; e-mail: detkovkate@rambler.ru

(2-keto-3-deoxy-6-phospho-gluconate) pathway. As far as alkaliphilic microorganisms are concerned, it was earlier shown that the alkaliphilic saccharolytic anaerobic bacteria *Halonatronum saccharophilum*, *Amphibacillus fermentum*, and *Amphibacillus tropicus*, catabolize glucose mainly via the fructose-bisphosphate pathway, although *H. saccharophilum* also employs the hexose-monophosphate pathway of glucose catabolism, and *A. tropicus* employs the Entner–Doudoroff pathway [9].

The hydrolytic alkaliphilic anaerobic bacterium *Alkaliflexus imshenetskii*, capable of utilizing mono-, di-, and polysaccharides, was earlier isolated in our laboratory from cyanobacterial mats of the lagoons of the alkaline lake Verkhnee Beloe (Buryat Republic, Russian Federation). Among disaccharides, cellobiose is the best energy substrate for this bacterium. The bacterium grows at a salinity from 0.8 to 53 g/l Na^+ (with an optimum at 20 g/l) and at pH from 7.2 to 10.2 with an optimum at pH 8.5 [10].

The aim of the present work was to investigate cellobiose catabolism in *Alkaliflexus imshenetskii*.

MATERIALS AND METHODS

The microorganism and its cultivation conditions. This work used the type strain *Alkaliflexus imshenetskii* Z-7010^T (=DSM 15055^T), obtained from T.N. Zhilina.

The bacterium was grown under strictly anaerobic conditions at 37°C on a mineral medium of pH 9.3 composed of (g/l): KH_2PO_4, 0.2; $MgCl_2 \cdot 6H_2O$, 0.05; NH_4Cl, 0.2; Na_2CO_3, 7.4; $NaHCO_3$, 18.5; yeast extract, 0.2; Lippert trace element solution [11], 1 ml; Wolin vitamin solution [12], 2 ml; 0.04% resazurin, 2 ml; $Na_2S \cdot 9H_2O$, 0.5. Cellobiose (0.3%) was added as the carbon and energy source.

All components of the medium, except cellobiose, yeast extract, sodium sulfide, and sodium bicarbonate, were dissolved in distilled water and boiled to remove oxygen. The solution was cooled in a flow of nitrogen gas for 15 min. The cooled medium was supplemented with yeast extract, sodium sulfide, and sodium bicarbonate. The reduced medium was dispensed into Hungate tubes or flasks under a nitrogen gas flow and sterilized for 30 min at 121°C. Cellobiose was sterilized separately as a concentrated aqueous solution which was then introduced into the medium with a syringe. For inoculation, mid-log-phase cultures were used in a dose of 1–3% (v/v).

Growth determination. The culture growth was determined from the increase in the optical density measured at 600 nm on a KFK-3 spectrophotometer (Russia) in 1-cm cuvettes. The protein content was determined by the method of Lowry et al. [13].

Analytical methods. Sugars and their fermentation products were determined by HPLC on a Staier chromatograph (Akvilon, Russia) equipped with an Aminex HPX-87H column (BioRad, United States) and a refractometric detector. Elution was performed with 5 mM H_2SO_4 at room temperature. Samples of microbial suspension taken at various growth stages were freed from cells and insoluble medium components by centrifugation for 3 min at 13500 *g*. The supernatant was supplemented with a 0.1 M solution of $CuSO_4$ in 5 M H_2SO_4 for removal of dissolved sulfide and simultaneous acidification of the medium to pH 2. After 1- to 2-h incubation for maturation of the CuS precipitate, the suspension was centrifuged under the same conditions and the supernatant was introduced into the chromatograph.

Obtaining of cell-free extracts. Cells were harvested by centrifugation at 9500 *g* for 15 min, resuspended in a buffer solution (prepared using oxygen-free water and stored under nitrogen), and disrupted in a CPX130PB ultrasonic disintegrator (Cole-Parmer, United States) at 0.4 mA in two 0.5-min sessions. Disrupted cells were centrifuged for 40 min at 4000 *g* to remove cell debris. The supernatant was used to determine the activity of enzymes.

Methods of determination of enzyme activity. Activity of enzymes was determined by monitoring the oxidation or reduction of NAD(H) or NADP(H) on a Specord (Germany) spectrophotometer at 340 nm. The activity of enzymes was expressed in nmol substrate/(min mg protein). In all cases, the volume of the reaction mixture was 1.05 ml, pH 7.5; the compositions of the reaction mixtures for particular enzymes were as follows.

β-Glucosidase: 20 mM cellobiose, 3 mM $MgSO_4 \cdot 7H_2O$, 1 mM ATP, 1 mM NADP, 5 U/ml hexokinase, 5 U/ml glucose-6-phosphate dehydrogenase, 50 mM Tris–HCl buffer [8].

Phosphorylase: 20 mM cellobiose, 33 mM Na–K-phosphate, 3 mM $MgSO_4 \cdot 7H_2O$, 1 mM NADP, 8 U/ml phosphoglucomutase, 5 U/ml glucose-6-phosphate dehydrogenase, 50 mM Tris–HCl buffer [8].

Hexokinase: 10 mM D-glucose, 15 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM ATP, 0.6 mM NADP, 2 U/ml glucose-6-phosphate dehydrogenase, 50 mM Tris–HCl buffer [14].

Glucose-phosphate isomerase: 1 mM EDTA, 4 mM fructose-6-phosphate, 0.6 mM NADP, 1 U/ml glucose-6-phosphate dehydrogenase, 50 mM Tris–HCl buffer [15].

Glucose-6-phosphate dehydrogenase: 15 mM $MgSO_4 \cdot 7H_2O$, 0.6 mM NADP, 1 mM glucose-6-phosphate, 50 mM Tris–HCl buffer.

6-Phosphogluconate dehydrogenase: 15 mM $MgSO_4 \cdot 7H_2O$, 0.6 mM NADP, 1 mM 6-phosphogluconate, 50 mM Tris–HCl buffer.

6-Phosphogluconate dehydratase and 2-keto-3-deoxy-6-phospho-gluconate aldolase: 15 mM $MgSO_4 \cdot 7H_2O$, 0.25 mM NADH, 2 mM EDTA, 1 mM 6-phosphogluconate, 5 U/ml lactate dehydrogenase, 50 mM Tris–HCl buffer.

Phosphofructokinase: 0.1 mM EDTA, 1 mM dithiothreitol, 6 mM $Mg(CH_3COO)_2$, 50 mM KCl, 4 mM

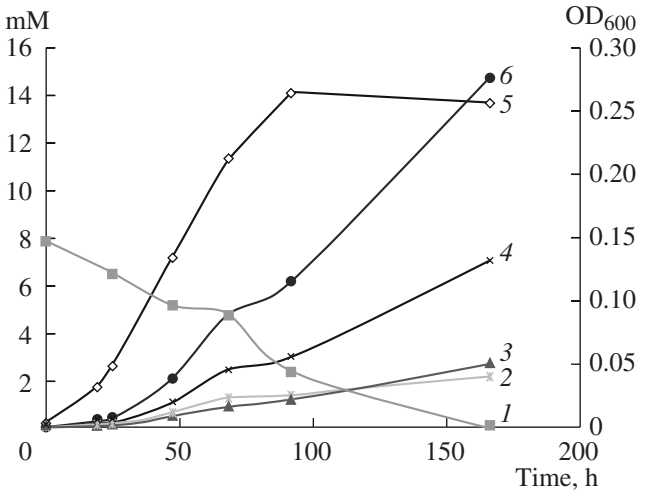

Fig. 1. *A. imshenetskii* Z-7010 growth and product formation on medium with cellobiose: (*1*) cellobiose; (*2*) formate; (*3*) pyruvate; (*4*) acetate; (*5*) OD_{600}; (*6*) succinate.

$(NH_4)_2SO_4$, 0.2 mM NADH, 1 mM fructose-6-phosphate, 1 mM ATP, 0.3 mM phosphoenolpyruvate, 2 U/ml pyruvate kinase, 2 U/ml lactate dehydrogenase, 50 mM Tris–HCl buffer [16].

Glyceraldehyde-3-phosphate dehydrogenase: 15 mM $MgSO_4 \cdot 7H_2O$, 5 mM EDTA, 2 mM cysteine, 2.5 mM ATP, 0.25 mM NADH, 10 mM 3-phosphoglyceric acid, 50 mM Tris–HCl buffer.

Glucose dehydrogenase: 1 mM NADP, 10 mM glucose, 50 mM Tris–HCl buffer [17].

2-Keto-3-deoxy-gluconate kinase: 10 mM $MgSO_4 \cdot 7H_2O$, 2 mM ATP, 0.3 mM NADH, 10 mM gluconate, 7 U/ml lactate dehydrogenase, 50 mM Tris–HCl buffer [17].

RESULTS AND DISCUSSION

Growth of *Alkaliflexus imshenetskii* on medium with cellobiose. The standard medium for cultivation of this bacterium contained 8 mM cellobiose. Figure 1 shows the curves of biomass accumulation, substrate consumption, and product formation by the alkaliphile *Alkaliflexus imshenetskii* Z-7010 grown on cellobiose. As seen from the figure, succinate and acetate were the major fermentation products, and formate was a minor product. Pyruvate and fumarate (not shown in the figure) were detected in trace amounts; probably, they were intermediate products. The succinate/substrate and succinate/acetate ratios were calculated to be 0.77 and 2.06, respectively, in the exponential phase, and 1.85 and 2.05 in the stationary phase. The specific growth rate was 0.022 h^{-1}. Thus, two succinate molecules and one acetate molecule were formed from one cellobiose molecule. The substrate was utilized completely. In the description of the organism as a new taxon, propionate is stated to be one of major products of cellobiose fermentation [10]. We failed to detect propionate during growth on cellobiose and detected it in trace amounts during growth on other carbohydrates. The reasons of this metabolic variability remain unclear.

Cellobiose fermentation was accompanied by acidification of the medium, the pH value decreasing from 9.3 to 8.5.

Enzymes involved in cellobiose cleavage by *A. imshenetskii.* To find out which of the two possible pathways—the hydrolytic or the phosphorolytic one—is employed in the metabolism of *A. imshenetskii* we determined the activities of β-glucosidase and phosphorylase (table). The activity of β-glucosidase was two orders of magnitude higher than the phosphorylase activity. Thus, cellobiose utilization by the bacterium proceeds via both the hydrolytic and phosphorolytic pathways; however, the contribution of the former pathway is much greater. Prevalence of the hydrolytic pathway was also noted in a study of cellobiose cleavage by the cells of the non-cellulolytic bacterium *Prevotella ruminicola* [18]. On the contrary, *Ruminococcus albus*, a rumen bacterium possessing cellulolytic activity, utilizes cellobiose primarily via the phosphorolytic pathway [8]. Most likely, prevalence of a particular pathway is related to the ability of a microorganism to utilize cellulose as one of its substrates. Cellulolytic microorganisms primarily employ the phosphorolytic pathway for utilization of the cellobiose obtained as a result of cellulose hydrolysis by exo- and endocellulases. On the contrary, microorganisms incapable of growth on cellulose primarily employ the hydrolytic pathway of cellobiose cleavage.

Enzymes of carbohydrate metabolism. In the trophic system of the anaerobic alkaliphilic community, *A. imshenetskii* is a primary anaerobe which interacts both with bacteria of the first hydrolytic phase (e.g., *Clostridium alkalicellulosi* [4]) and with secondary

Activity of carbohydrate metabolism enzymes in saccharolytic anaerobes (nmol/(min mg protein))

Microorganism				*A. imshenetskii*	*H. saccharophilum* [9]	*A. fermentum* [9]	*A. tropicus* [9]
Enzymes involved in cellobiose cleavage							
β-glucosidase				3600	ND	ND	ND
phosphorylase				36	ND	ND	ND
Enzymes of carbohydrate metabolism	FBP	HMP	KDPG				
hexokinase	+	+	+	303.8	354.0	900.0	871.0
glucose-phosphate isomerase	+	–	–	256.0	ND	ND	ND
phosphofructokinase	+	–	–	201.6	24.0	14.6	10.8
glucose-6-phosphate dehydrogenase	–	+	+	69.5	23.0	31.4	178.5
6-phospho-gluconate dehydrogenase	–	+	–	0	1.6	23.2	0
6-phospho-gluconate dehydratase + 2-keto-3-deoxy-6-phospho-gluconate aldolase	–	–	+	6.0	0	55.7	17.0
glyceraldehyde-3-phosphate dehydrogenase	+	–	+	47.0	536.5	221.0	209.0

Note: FBP, fructose-bisphosphate pathway; HMP, the hexose-monophosphate pathway; KDPG, 2-keto-3-deoxy-6-phospho-gluconate pathway. "ND" stands for "not determined."

anaerobes, which utilize the metabolic products of hydrolytic bacteria. Although for cell–cell trophic interactions only the presence or absence of particular intermediates in the cell environment is important, it was expedient to investigate the possible intracellular pathways of carbohydrate fermentation by *A. imshenetskii* for fuller characterization of the microorganism involved in the trophic system.

Therefore, we studied the activity of the key enzymes of carbohydrate metabolism in *A. imshenetskii.* We revealed high activities of hexokinase, glucose-phosphate isomerase, and phosphofructokinase, which are the key enzymes of the fructose-bisphosphate pathway (FBP) (table). We also found activities of glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and key enzymes of the Entner–Doudoroff pathway—6-phospho-gluconate dehydratase and 2-keto-3-deoxy-6-phospho-gluconate aldolase. The activity of 6-phosphogluconate dehydrogenase, which is the key enzyme of the hexose-monophosphate pathway (HMP), was not detected; thus, this pathway of sugar metabolism cannot be operative in *A. imshenetskii.* Neither did we find activities of glucose dehydrogenase and 2-keto-3-deoxy-gluconate kinase, the key enzymes of the modified Entner–Doudoroff pathway. The pathways of cellobiose catabolism in *A. imshenetskii* and their interrelations are shown in Fig. 2.

Earlier, the carbohydrate metabolism of the saccharolytic alkaliphilic anaerobes *Halonatronum saccharophilum*, *Amphibacillus fermentum*, and *A. tropicus* was studied [9]. In the trophic system of the anaerobic alkaliphilic community, these microorganisms, like *A. imshenetskii*, are primary anaerobes and utilize certain mono-, di-, and polysaccharides as the source of carbon and energy [19, 20]. *H. saccharophilum* was shown to ferment glucose via the FBP and HMP pathways (table). The Entner–Doudoroff pathway is not operative in this haloanaerobe since its key enzymes are lacking. *A. tropicus* metabolizes glucose via the FBP and Entner–Doudoroff pathway. *A. fermentum* exhibits activity of the key enzymes of the three major pathways of glucose metabolism. It is probably due to this fact that *A. fermentum* can ferment a wide range of organic substrates [20].

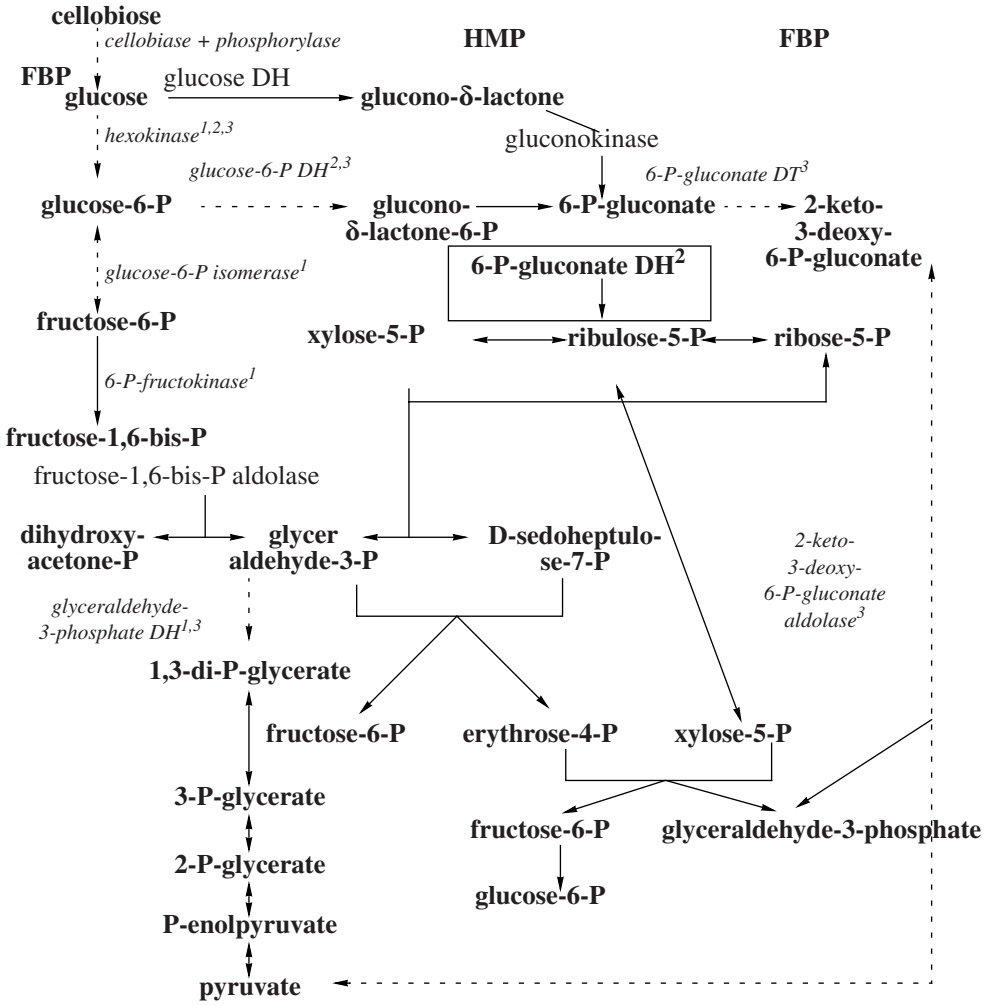

Fig. 2. Presumable pathways of cellobiose catabolism in *A. imshenetskii.* Interrelations of the three main glucose catabolism pathways are depicted according to Doelle [21]. Italicized are names of enzymes whose activities were found in *A. imshenetskii*; the reactions catalyzed by these enzymes are shown by dotted lines; the frame encloses an enzyme the activity of which was shown to be absent. Affiliations of enzymes with particular pathways are shown by uppercase numerals: *1*, fructose-bisphosphate pathway (FBP); *2*, hexose-monophosphate pathway (HMP); *3*, Entner–Doudoroff pathway (ED). DH means dehydrogenase, and DT means dehydratase. P should be read as "phosphate" or "phospho."

In *A. imshenetskii*, like in the above-discussed bacteria, activities of enzymes of several glucose catabolism pathways were revealed. In all of the saccharolytic alkaliphilic anaerobes studied in this respect, the highest was the activity of the enzymes of the Embden–Meyerhof–Parnas pathway (table).

ACKNOWLEDGMENTS

We are grateful to G.A. Zavarzin for helpful advice and valuable discussion.

This work was supported by the grant from the President of the Russian Federation "Leading Scientific Schools" NSh-1221.2008.4 and by the program of the Presidium of the Russian Academy of Sciences "Molecular and Cell Biology."

REFERENCES

1. Wood, T.M., Fungal Cellulases, *Biochem. Soc. Trans.*, 1992, vol. 20, pp. 46–53.
2. Lynd, L.R., Weimer, P.J., van Zyl, W.H., and Pretorius, I.S., Microbial Cellulose Utilization: Fundamentals and Biotechnology, *Microbiol. Mol. Biol. Rev.*, 2002, vol. 66, pp. 506–577.
3. Zvereva, E.A., Fedorova, T.V., Kevbrin, V.V., Zhilina, T.N., and Rabinovich, M.L., Cellulase Activity of a Haloalkaliphilic Anaerobic Bacterium, Strain Z-7026, *Extremophiles*, 2006, vol. 10, pp. 53–60.
4. Zhilina, T.N., Kevbrin, V.V., Tourova, T.P., Lysenko, A.M., Kostrikina, N.A., and Zavarzin, G.A., *Clostridium alkalicellum* sp. nov., an Obligately Alkaliphilic Cellulolytic Bacterium from a Soda Lake in the Baikal Region, *Mikrobiologiya*, 2005, vol. 74, pp. 642–653 [*Microbiology* (Engl. Transl.), vol. 74, no. 5, pp. 557–566].

5. Hallwell, G., Microbial β-Glucanases, *Prog. Ind. Microbiol.*, 1979, vol. 15, pp. 1–60.
6. Alexander, J.K., Cellobiose Phosphorylase from *Clostridium thermocellum, Methods Enzymol.*, Ginsburg, V., Ed., N.Y.: Academic Press, 1972, vol. 28, pp. 944–948.
7. Ng, T.K. and Zeikus, J.G., Differential Metabolism of Cellobiose and Glucose by *Clostridium thermocellum* and *Clostridium thermohydrosulfuricum, J. Bacteriol.*, 1982, vol. 150, pp. 1391–1399.
8. Lou, J., Dawson, K.A., and Strobel, H.J., Cellobiose and Cellodextrin Metabolism by the Ruminal Bacterium *Ruminococcus albus, Curr. Microbiol.,* 1997, vol. 35, pp. 221–227.
9. Garnova, E.S. and Krasil'nikova, E.N., Carbohydrate Metabolism of the Saccharolytic Alkaliphilic Anaerobes *Halonatronum saccharophilum, Amphibacillus fermentum*, and *Amphibacillus tropicus, Mikrobiologiya*, 2003, vol. 72, pp. 627–632 [*Microbiology* (Engl. Transl.), vol. 72, no. 5, pp. 558–563].
10. Zhilina, T.N., Appel, R., Probian, C., Brossa, E.L., Harder, J., Widdel, F., and Zavarzin, G.A., *Alkaliflexus imshenetskii* gen. nov. sp. nov., a New Alkaliphilic Gliding Carbohydrate-fermenting Bacterium with Propionate Formation from a Soda Lake, *Arch. Microbiol.,* 2004, vol. 182, pp. 244–253.
11. Pfennig, N. and Lippert, K.D., Über das Vitamin B_{12}-Bedtirfhis phototropher Schwefelbakterien, *Arch. Mikrobiol.*, 1966, vol. 55, pp. 245–246.
12. Wolin, E.A., Wolin, M.J., and Wolfe, R.S., Formation of Methane by Bacterial Extracts, *J. Biol. Chem.*, 1963, vol. 238, pp. 2882–2886.
13. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Rendall, R.J., Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.*, 1951, vol. 193, pp. 265–275.
14. Barnard, E.A., Hexokinases from Yeast, *Methods Enzymol.*, Wood, W.A., Ed., N.Y.: Academic Press, 1975, vol. 42C, pp. 6–20.
15. Gracy, R.W., Glucosephosphate Isomerase from Catfish Muscle and Liver and from Mammalian Tissues, *Methods Enzymol.*, 1982, vol. 89, pp. 550–558.
16. Kemp, R.G., Phosphofructokinase from Rabbit Skeletal Muscle, *Methods Enzymol.*, Wood, W.A., Ed., N.Y.: Academic Press, 1975, vol. 42C, pp. 71–77.
17. Johnsen, U., Selig, M., Xavier K.B., Santos, H., and Schönheit, P., Different Glycolytic Pathways for Glucose and Fructose in the Halophilic Archaeon *Halococcus saccharolyticus, Arch. Microbiol.*, 2001, vol. 175, pp. 52–61.
18. Lou, J., Dawson, K.A., and Strobel, H.J., Role of Phosphorolytic Cleavage in Cellobiose and Cellodextrin Metabolism by the Ruminal Bacterium *Prevotella ruminicola, Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 1770–1773.
19. Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A., and Zavarzin, G.A., *Halonatronum saccharophilum* gen. nov. sp. nov.: A New Haloalkaliphilic Bacterium of the Order *Haloanaerobiales* from Lake Magadi, *Mikrobiologiya*, 2001, vol. 70, pp. 77–85 [*Microbiology* (Engl. Transl.), vol. 70, no. 1, pp. 64–72].
20. Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A., and Zavarzin, G.A., *Amphibacillus fermentum* sp. nov. and *Amphibacillus tropicus* sp. nov., New Alkaliphilic, Facultatively Anaerobic, Saccharolytic Bacilli from Lake Magadi *Mikrobiologiya*, 2001, vol. 70, pp. 825–837 [*Microbiology* (Engl. Transl.), vol. 70, no. 6, pp. 711–722].
21. Doelle, H.W., *Bacterial Metabolism*, New York: Academic Press, 1975.