ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 1, pp. 1–6. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © E.P. Ryzhkova, A.L. Brukhanov, 2009, published in Mikrobiologiya, 2009, Vol. 78, No. 1, pp. 5–11.

EXPERIMENTAL ARTICLES

Effect of a Corrinoid on *Methanosarcina barkeri* DNA Synthesis

E. P. Ryzhkova[1] and A. L. Brukhanov

Moscow State University, Department of Microbiology, Moscow, Russia

Received November 29, 2007

Abstract—*Methanosarcina barkeri* is capable of synthesizing large amounts of corrinoids, compounds of the vitamin B_{12} group, although not cobalamin. In the present work, exogenous cobalamin was demonstrated to upregulate DNA synthesis in *M. barkeri* cell suspensions incubated under air. The effect is similar to the one in *Propionibacterium freudenreichii* cells, though less pronounced. The growth of the archaeon under anaerobic conditions was shown to be suppressed by cobalamin and 5,6-dimethylbenzimidazole. The data obtained suggest the presence of a corrinoid-dependent ribonucleotide reductase in the archaeal cells which provides for deoxyribose precursors for DNA biosynthesis independently of the presence of molecular oxygen in the medium. Growth suppression under anoxic conditions by cobalamin and 5,6-dimethylbenzimidazole may be due to a decrease in the concentration of factor III, a polyfunctional corrinoid dominating in *M. barkeri* cells.

Key words: Methanosarcina barkeri, Propionibacterium freudenreichii, DNA biosynthesis, corrinoids, factor III, cobalamin, adenosylcobalamin, ribonucleotide reductase.

DOI: 10.1134/S0026261709010019

Methanosarcina barkeri is a biological agent of methanogenesis important for the cycle of carbon in nature. Both negative (methane explosions in mines) and positive implications of methanogenesis (sewage plants and biogas) are of importance. Thus, comprehensive study of the physiology and metabolism of the given archaebacterium is of importance.

Scientific literature lacks data on the effect of corrinoids (compounds of the vitamin B_{12} group) on the DNA biosynthesis in *Methanosarcina barkeri*. However, a number of prokaryotes are known to involve cobalamin (Cbl) into DNA synthesis via the functioning of an adenosylcobalamin (AdoCbl)-dependent ribonucleotide reductase (RNRase) [1–5]. The enzyme hasn't been identified in *M. barkeri.*

M. barkeri synthesizes large quantities of corrinoids. On a methanol-containing medium with high concentrations of cobalt salts (9.6 mg/l), 5700 μg/g ASB corrinoids were formed. Factor III (5-hydroxybenzimidazolylcobamide) makes up 19% among them, 80% are made of nucleotide-free (at the α-ligand of the cobalt atom) factor B [6]. Data on the composition for the corrinoids of *M. barkeri* are in accordance with those of other authors [7–9]. Moreover, factor B was shown to be present in an adenosylated form [8], and while the majority of factor III (80% to total corrinoids) is methylated, adenosylated and some unidentified derivatives were also revealed [10]. Cobalamin was not detected in *M. barkeri* cells in the cited works. However, one cannot exclude its presence in the methanosarcina biomass, since a protein complex of aquacobalamin (H_2O–Cbl) involved in methane biosynthesis in vitro was isolated from *M. barkeri* [11].

Methanogenic bacteria (*Methanobacterium thermoautotrophicum* and *M. barkeri*) are known to assimilate corrinoids and 5,6-dimethylbenzimidazole (5,6 DMB) in the course of growth. In this process, "complete" corrinoids such as Cbl, containing 5,6-DMB, are partially transformed into factor III. Exogenous complete corrinoids (factor III, Cbl, etc.) and 5,6-DMB suppress de novo biosynthesis of factor III [12]. The formation of true vitamin B_{12} (cobalamin) in the presence of exogenous 5,6-DMB (at 30% of the total corrinoids) was confirmed for *M. barkeri* and *M. vacuolata* [7].

Factor III is a biochemically polyfunctional corrinoid [5]. In the course of methanogenesis by *M. barkeri* grown on methanol-containing media it functions as a part of an active site of methyltransferase I (MTPase I) interacting with methanol, methyltransferase II (MTPase II), tetrahydromethanepterin, and CO dehydrogenase/acetyl-CoA synthase. Participation of *M. barkeri* corrinoids in processes other than methanogenesis (factor III), such as constructive metabolic reactions, for example, DNA and/or methionine biosynthesis, cannot be ruled out.

Does true vitamin B_{12} (cobalamin) participate in methane formation in *M. barkeri*? MTPase II is known to catalyze CH_3 group transfer from the methylated factor III present in the active site of MTPase I to coenzyme M (SH-CoM) [5]. In addition, in experiments in vitro free Cbl also was found to be an acceptor of the methyl group transferred from methanol by MTPase I. In turn, MTPase II, which catalyzes the methylation of

[1] Corresponding author; e-mail: epr322@mail.ru

SH-CoM, uses (alternatively) the free methyl-Cbl as a substrate [13]. In vivo functioning of this system is an open issue so far.

Ribonucleotide reductase (RNRase, 1.17.4.-) is a universal enzyme catalyzing the transformation of ribonucleotides into deoxyribonucleotides. This is the first specific and rate-limiting stage of the metabolic pathway for DNA synthesis as a whole. There are three classes of RNRases [1–3, 14, and 15]: "aerobic" Fe–O–Fe- or Mn-containing RNRase (class I), adenosylcobalamin (AdoCbl)-dependent RNRase inert to molecular oxygen (class II), and an "anaerobic" Fe–S-containing RNRase sensitive to molecular oxygen (class III). Importantly, RNRases of various classes may be present in the genomes and function in the cells of a single prokaryote species [15–20]. According to their physiology (strict anaerobes), methanosarcina may contain RNRases of classes II and/or III. Sometimes they are not detected biochemically as the gene expression is stipulated by specific physiological conditions. However, AdoCbl-dependent RNRase of an aerobic archaeon *Pyrococcus furiosus* was isolated and studied in the presence of air [21].

An indirect indication for the presence of a class II RNRase is a vitamin B_{12}-dependent pattern of DNA synthesis. The indicated approach to analyze the type of RNRase was applied towards *Brevibacterium ammoniagenes* [22]. Earlier we have observed a direct correlation between the effect of AdoCbl on DNA biosynthesis and the activity of AdoCbl-dependent RNRase in *Propionibacterium freudenreichii* ssp. *shermanii* VKM-103 [15].

The goal of the present work was to reveal the probable involvement of a corrinoid (exemplified by cobalamin) in DNA biosynthesis in *Methanosarcina barkeri* cells.

MATERIALS AND METHODS

The following bacteria were used in the work: *Methanosarcina barkeri* strain Fusaro (DSMZ 804, Deutche Sammlung von Mikroorganismen und Zellkultlren, Braunschweig, Germany) and *Propionibacterium freudenreichii* strain RVS-4-irf (VKPM V-9654, All-Russian Collection of Industrial Microorganisms, Moscow, Russia).

M. barkeri was cultured under anaerobic conditions in the dark (37°C, 72 h) on a synthetic medium prepared according to Karrash [23] with modifications concerning the cobalt salt concentration.

The concentrated (×5) salt solution contained the following (g/l): imidazole, 13.6; $NaH_2PO_4 \cdot H_2O$, 0.345; $Na_2HPO_4 \cdot 2H_2O$, 0.44 (pH 6.4, adjusted with 37% HCl); $CaCl_2 \cdot 2H_2O$, 1.24; $MgCl_2 \cdot 6H_2O$, 2.0; KCl, 2.0; NaCl, 10.0; NH_4Cl, 2.5; $FeCl_3 \cdot 6H_2O$, 0.015; and 0.5 ml of 0.2% resazurin solution.

The microelement solution contained the following (g/l): EDTA, 1.5 (pH 6.5–7.0); $MnSO_4 \cdot H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.15; $CoCl_2 \cdot 6H_2O$, 0.50; $ZnSO_4 \cdot 7H_2O$, 0.10; $CuSO_4 \cdot 6H_2O$, 0.01; $AlCl_3 \cdot 6H_2O$, 0.01; H_3BO_3, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $NiCl_2 \cdot 6H_2O$, 0.03; and $NaHSeO_3$, 0.015 (according to Wolf).

The vitamin solution contained the following (mg/l): biotin, 2.0; folic acid, 2.0; pyridoxine-HCl, 10.0; thiamine (HCl), 5.0; riboflavin, 5.0; nicotinic acid, 5.0; pantothenic acid, 5.0; vitamin B_{12}, 0.1; *p*-aminobenzoic acid, 5.0; and lipoic acid, 5.0.

$Na_2S \cdot 9H_2O$ solution, 5.6 mg per 100 ml of distilled water, was sterilized separately at 1 atm for 20 min. It was introduced (5 ml/l) into the complete medium which was sterilized at 0.5 atm for 20 min.

To prepare the complete medium, 10 ml of the microelement solution and 10 ml of the vitamin solution were added to 200 ml of the concentrated (×5) salt solution and filled with double distilled water up to 1 l (final pH 7.0). Then, methanol (12 ml/l) was added, as well as L-cysteine-HCl as a reducing agent (140 mg/l).

P. freudenreichii was cultured under conditions of free oxygen access in 70%-filled Erlenmeyer's flasks for 72 h at 30°C on a semisynthetic medium containing the following (g/l distilled water): glucose, 20.0; tryptone (Biokar Diagnostics, France), 0.5; $(NH_4)_2SO_4$ – 3.0; KH_2PO_4 –1.5; $MgSO_4 \cdot 7H_2O$, 0.25; (mg/l) $CoCl_2 \cdot 6H_2O$ (in some variants, the cobalt salt wasn't added), 1.0; NaCl, 5.0; $MnSO_4$, 5.0 or $MnCl_2$, 4.0; $ZnSO_4 \cdot 7H_2O$, 0.01; $FeCl_3 \cdot 6H_2O$, 0.005; and the following vitamins (μg/l): calcium pantothenate, 1000.0; thiamine, 200.0; and biotin, 1.0; pH 6.8–7.0. Glucose and salt solutions were sterilized separately for 30 min at 0.5 atm. Vitamins were sterilized at 0.5 atm for 15 min.

At a cobalt salt concentration of 1.0 mg/l, the cells of propionic acid bacteria (PAB) contained about 1000 μg/g corrinoids ASB (full Cor^+ variant). The residual corrinoid content in the cells grown on a cobalt-free medium was ~10 μg/g ASB (corrinoid deficient variant, Cor^-). After 72 h of cultivation, PAB variants grown on the indicated medium reached the concentration of 9.0 and 8.0 optical density units (D. U.) at A_{540}.

Archaeal cell suspensions (72 h of anaerobic cultivation) were prepared under free air access to the incubation solution consisting of a salt base of the growth medium supplemented with methanol (5 ml/l), cysteine (140 mg/l), and sodium sulfide (5 ml/l of the above solution). Cell concentration in the suspension was 4–5 D. U. (A_{580}). Cell suspensions of *P. freudenreichii* (after 48–50 h of cultivation) were also prepared in the air in the salt solution of the growth medium supplemented with 0.5% glucose. Cell concentration in the suspension was 16–17 D. U. (A_{540}).

Total DNA synthesis in the cells was registered as follows: labeled [8-^{14}C]adenine (PA Izotop, Saint-Petersburg, Russia) along with the unlabeled were added to the cell suspensions in the cold. The radioactive adenine concentration was 74 kBq/ml (specific radioactivity of 10.2 GBq/g). The unlabeled adenine concentration was 5 μg/ml. Then, cobalamin deriva-

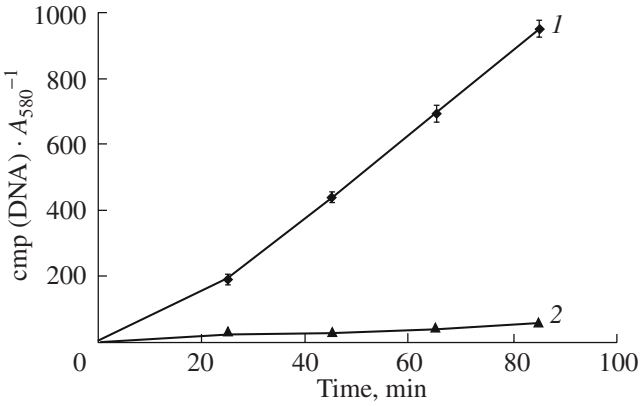

Fig. 1. Incorporation of labeled adenine into DNA in *M. barkeri* cell suspension in the air in the absence of inhibitor and (*1*), in the presence of mytomycin C (*2*).

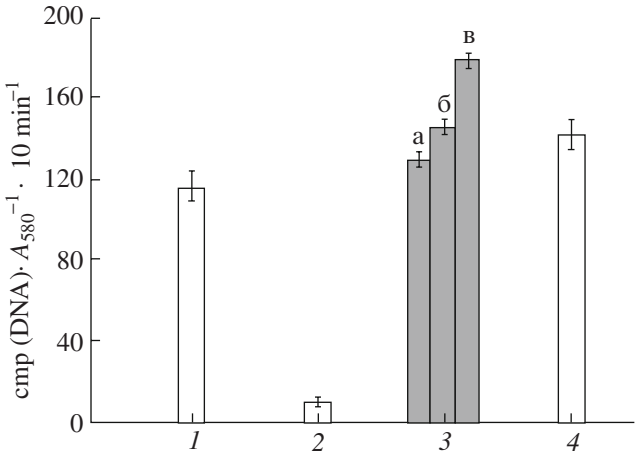

Fig. 2. DNA biosynthesis intensification in *M. barkeri* cell suspension upon exogenous cobalamin introduction in the air (according to the rate of labeled adenine incorporation into DNA): no cobalamin (control) (*1*); with the addition of mytomycin C (*2*); with the addition of AdoCbl (μg/ml): a, 1.0; b, 2.5; and c, 5.0 (*3*); and with the addition of OH-Cbl, 7.0 μg/ml (4). Preincubation with adenine and the additives was performed at 37°C for 15 min; the rate of inclusion was calculated from the linear regions of the curves (over the 30–60 min incubation interval, see Fig. 1).

tives in various concentrations or mitomycin C (4–5 μg/ml) were added. Cobalamin derivatives were kindly provided by Prof. V. Ya. Bykhovskii (Laboratory of Vitamins, Bach Institute of Biochemistry, Russian Academy of Sciences) and mitomycin C was obtained from Calbiochem, United Kingdom. Preincubation of the suspensions was carried out at 37°C for 10–15 min. The suspensions were then incubated at 37°C, with aliquots collected at regular time intervals.

For quantitative isolation of DNA, the aliquots were treated as follows: the alkali-stable (18 h hydrolysis in 0.5 N NaOH at 37°C), acid-insoluble (1 N $HClO_4$ in the cold) DNA fraction was separated from RNA. The DNA precipitate was washed with cold 0.3 N $HClO_4$ containing unlabeled adenine, then 70% ethanol, and then dried. An automatic counter (LKB, Sweden) was used to measure the activity of the washed and dried DNA precipitate. DNA formation rate (intensity) was calculated as the acquired DNA radioactivity per 1 D. U. (A_{580}) of cell suspension after 10 min incubation.

All experiments were carried out at least in triplicate. The data were statistically treated using Sigma Plot and Sigma Stat programs (Systat software).

RESULTS AND DISCUSSION

To register DNA biosynthesis, a 72-h cell suspension of *M. barkeri* was incubated in the presence of labeled and unlabeled adenine in the air. After the separation of the alkali-stable, acid-insoluble DNA fraction from RNA, increase in DNA radioactivity was measured. To estimate the incorporation of exogenous adenine into DNA (in the process of biosynthesis), incorporation of the label in the presence of an inhibitor, mitomycin C, was measured. The experimental data (Fig. 1) proved the possibility for monitoring DNA biosynthesis in the archaeon by the rate of incorporation of exogenous labeled adenine into the molecule. Upon treatment with the antibiotic, DNA radioactivity after 85 min of incubation was less than 10%, which is probably due to residual adenine sorption. The data also show that *M. barkeri* enzymes involved in the synthesis of DNA precursors (deoxyribonucleotides) and in replication proper are active in cells in the presence of molecular oxygen.

Cobalamin derivatives (AdoCbl or hydroxycobalamin, OH-Cbl) were then introduced in varying concentrations into the suspension already containing radioactive adenine. A stimulating effect of exogenous cobalamin on DNA biosynthesis was observed in *M. barkeri* cell suspensions in air (Fig. 2). Corrinoid uptake by archaeal cells has been proved previously for *M. thermoautotrophicum* [7, 12].

The stimulatory effect of a corrinoid AdoCbl towards prokaryotic DNA biosynthesis was also revealed in *P. freudenreichii*. The data presented in Fig. 3 confirm that DNA biosynthesis in this bacterium was dependent upon the endogenous corrinoid levels and intensified significantly upon treatment with exogenous AdoCbl. The bacteria possess a class II AdoCbl-containing ribonucleotide reductase [15]. *M. barkeri* cell suspension responded similarly to cobalamin introduction in the air (see Fig. 2). Apparently, the difference in the absolute values of DNA incorporation in methanosarcina and in the propionic acid bacterium is due to the differences in the patterns and rates of growth and also the peculiarities in DNA anabolism. Since a possibility exists that cobalamin is transformed into factor III in archaeal cells, a fairly short incubation period is required to trace the specific interactions

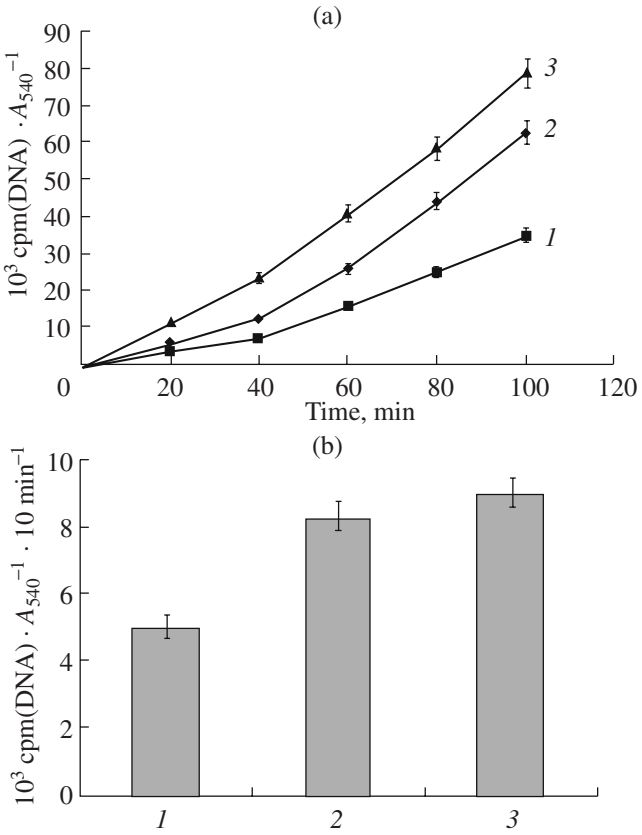

Fig. 3. DNA biosynthesis intensification in corrinoid-deficient (Cor^{-}) *P. freudenreichii* cells under exogenous adenosylcobalamin effect (according to the labeled adenine incorporation into DNA, Fig. 3a, and to the rate of incorporation, Fig. 3b): *1*, corrinoid-deficient PAB cells, ~10 μg/g DW (Cor^{-} variant); *2*, completely corrinoid-positive PAB cells, 1000 μg/g DW (Cor^{+} variant); and *3*, variant Cor^{-} + AdoCbl. Preincubation with AdoCbl (3.0 mg/l) was performed at 37°C for 10 min; the rate of inclusion was calculated from the linear regions of the curves (over the 50–80 min incubation interval.

between cobalamin and the corrinoid-dependent enzymes of DNA biosynthesis. Therefore, in experiments with *M. barkeri* cells the time of incubation was tens of minutes which is certainly less than the period of cell doubling (see Figs. 1 and 2 and table).

In experiments with the growing culture (strictly anaerobic growth of the archaeon), addition of cobalamin (AdoCbl) at inoculation (10–20 vol %), the stimulatory effect of the corrinoid on DNA biosynthesis was observed, although the growth rate of the bacteria decreased. Introduction of 5,6-DMB into the medium suppressed *M. barkeri* growth and DNA biosynthesis (see Table).

Thus, we have discovered controversial effects of exogenous cobalamin on *M. barkeri* metabolism. The negative effect of cobalamin and 5,6-DMB on *M. barkeri* growth is probably associated with a decrease in the level of endogenous factor III, a corrinoid important for the energy balance of a methanogenic archaeon. The positive effect of exogenous adenosylated cobalamin (AdoCbl) on DNA biosynthesis is probably due to its artificial (nonspecific) inclusion into the active site of the corrinoid-dependent RNRase (class II). The previously published data on Cbl interacting with the essential enzymes of methanogenesis and acetyl-CoA synthesis in *M. barkeri* in vitro support this idea [12, 24–26]. The instability of the synthesis rate and content of DNA in prokaryote cells is known and is caused by the lack of strict coordination between DNA replication and cell division [15].

The data obtained is implicit evidence for the presence of a class II corrinoid-dependent RNRase in methanosarcina cells. The enzyme is known to be inert towards molecular oxygen in contrast to the Fe–S-containing sensitive RNRase of class III. The enzyme functions through free-radical conversions initiated by the corrinoid in its active site [1–5, 15]. According to the published data, *M. barkeri* doesn't synthesize cobalamin [6, 7, 10]. On one hand, since DNA biosynthesis in cell suspensions was performed in the air, one cannot exclude the class II corrinoid-dependent RNRase to be the only type of the enzyme present in *M. barkeri* cells. Under physiological conditions, the RNRase of class II probably functions using an adenosylated derivative of factor III. A corrinoid-dependent RNRase of class II, obligately requiring AdoCbl in vitro, was detected

DNA synthesis in *M. barkeri* cells during anaerobic growth

Culture variants	Biomass accumulation after 72 h, A_{580}	DNA radioactivity in 72-h cultures by [8-^{14}C]-adenine, cpm/ml	DNA specific radioactivity in 72-h cultures, cpm/A_{580}
Control (C)	0.93	2121	2280
C + AdoCbl, μg/ml			
1.0	0.89	2406	2703
2.0	0.80		
4.0	0.71	2442	3439
Control (C)	0.96	2871	2990
C + 5,6-DMB, μg/ml			
5.0	0.92	1758	1910
10.0	0.91	1715	1885
20.0	0.90	1200	1333

(according to biochemical methods, structural data, and methods of molecular genetics) in an archaeon *Pyrococcus furiosus* [21]; however, the nature of the native corrinoid as part of a holoenzyme wasn't established.

On the other hand, functioning of a class III anaerobic RNRase is also possible in *M. barkeri* under anaerobic conditions, since we now know [15–20] that a single organism may possess RNRases of different classes acting under the relevant physiological conditions. While the strictly anaerobic *M. barkeri* normally uses a class III "anaerobic" Fe–S-containing RNRase (*nrdDG*), the corrinoid-dependent class II RNRase (*nrdJ*), probably synthesized during anaerobic growth as a constitutive duplicating (alternative) enzyme, may be an example of the organism adaptation to temporarily aerobic conditions. There are data available on methanosarcinas' ability to retain viability upon contact with oxygen [27] and, moreover, to proliferate in the presence of certain concentrations of the products of incomplete oxygen reduction [28, 29]. In the situation described, "anaerobic" RNRase would be inactive, while the corrinoid-dependent one would retain its activity.

REFERENCES

1. Blakley, R.L, *Cobalamin-Dependent Ribonucleotide Reductases, in B_{12} (two volums)*, Dolphin, D., Ed., New York: Wiley, 1982.
2. Harder, J., Ribonucleotide Reductases and Their Occurrence in Microorganisms: a Link to the RNA/DNA Transition, *FEMS Microbiol. Rev.*, 1993, vol. 12, pp. 273–292.
3. Reichard, P., From RNA To DNA, Why So Many Ribonucleotide Reductases?, *Science*, 1993, vol. 260, no. 5115, pp. 1773–1777.
4. Poole, A.M., Logan, D.T., and Sjöberg, B.-M., The Evolution of the Ribonucleotide Reductases: Much Ado About Oxygen, *J. Mol. Evol.*, 2002, vol. 55, pp. 180–196.
5. Ryzhkova (Jordan) E.P. Multiple Functions of Corrinoids in Prokaryote Biology, *Prikl. biokhim. Mikrobiol.*, 2003, vol. 39, no. 2, pp. 133–159 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 39, no. 2, pp 115–139].
6. Mazumdar Tapan Kumar, Nichio Naomishi, Fukuzaki Satoshi, Nagai Shiro. Production of Extracellular Vitamin B_{12} Compounds from Methanol by *Methanosarcina barkeri, Appl. Microbiol. Biotechnol.*, 1987, vol. 26, no.6, pp. 511–516.
7. Bykhovskii, V.Ya., Zaitseva, N.I., Zhilina, T.N., Nozhevnikova, A.R., and Zavarzin, G.A., Biosynthesis of Vitamin B_{12} and Its Analogues by Methanogenic Bacteria and Acetogenic Methylotrophs, *Dokl. Akad. Nauk SSSR*, 1987, vol. 294, no. 3, pp. 719–722.
8. Bykhovskii, V.Ya., Zaitseva, N.I., and Eliseev, A.A., Application of Anaerobic Processes for Biosynthesis of Vitamin B_{12} and Its Analogues, *Prikl. Biokhim. Mikrobiol.,* 1991, vol. 27, no. 4, pp. 467–481.
9. Eisenreich, W. and Bacher, A., Biosynthesis of 5-Hydroxybenzimidazolylcobamide (Factor III) in *Methanobacterium thermoautotrophicum, J. Biol. Chem.*, 1991, vol. 266, no. 35, pp. 23840–23849.
10. Whitman, W.B. and Wolfe, R.S., Purification and Analysis of Cobamides of *Methanobacterium bryantii* by High-Performance Liquid Chromatography, *Anal. Biochem.*, 1984, vol. 137, no. 1, pp. 261–265.
11. Wood, J.M., Moura, I., Moura, J.J., Santos, M.H., Xavier, A.V., LeGall, J., and Scandellari, M., Role of Vitamin B_{12} in Methyl Transfer for Methane Biosynthe-

sis by *Methanosarcina barkeri, Science*, 1982, vol. 216, no. 4543, pp. 303–306.

12. Stupperich, E., Steiner, I., and Eisinger, J., Substitution of Coα-(5-Hydroxybenzimidazolyl)Cobamide (Factor III) by Vitamin B_{12} in *Methanobacterium thermoautotrophicum, J. Bacteriol.*, 1987, vol. 169, no. 7, pp. 3076–3081.
13. Eliseev, A.A., Pusheva, M.A., Zavarzin, G.A., Stupperich, E., and Bykhovskii V.Ya. Regulation of the Enzymes of Vitamin B_{12} Biosynthesis and Metabolism in Microorganisms by Growth Substrates, *Dokl. AN SSSR*, 1993, vol. 331, no. 1, pp. 116–118.
14. Sze, I., McFarlan, S., Spormann, A., and Hogenkamp, H.P.C., A Possible New Class of Ribonucleotide Reductase from *Methanobacterium thermoautotrophicum, Biochem. Biophys. Res. Commun.*, 1992, vol. 184, no. 2, pp. 1101–1107.
15. Ryzhkova, E.P., Cobalt and Corrinoids in *Propionibacterium freudenreichii* Biology, *Extended Abstract of Doctoral (Biol.) Dissertation*, Moscow State Univ., Moscow, 2003.
16. Fontecave, M., Eliasson, R., and Reichard, P., Oxygen-Sensitive Ribonucleoside Triphosphate Reductase from *Escherichia coli* requires S-adenosylmethionine, *Proc. Natl. Acad. Sci. USA*, 1989, vol. 86, pp. 2147–2151.
17. Jordan, A., Torrents, E., Sola, I., Hellmann, U., Gibert, I., and Reichard, P., Ribonucleotide Reduction in *Pseudomonas* Species - Simultaneous Presence of Active Enzymes from Different Classes, *J. Bacteriol.*, 1999, vol. 181, no. 13, pp. 3974–3980.
18. Shearer, N., Hinsley, A.P., Van Spanning, R.J., and Spiro, S., Anaerobic Growth of *Paracoccus denitrificans* Requires Cobalamin: Characterization of *cobK* and *cobJ* Genes, *J. Bacteriol.*, 1999, vol. 181, no. 22, pp. 6907–6913.
19. Borovok, I., Kreisberg-Zakarin, R., Yanko, M, Schreiber, R., Myslovati, M., Aslund, F., Holmgren, A., Cohen, G., and Aharonowitz, Y., *Streptomyces* spp. Contain Class Ia and Class II Ribonucleotide Reductases: Expression Analysis of the Genes in Vegetative Growth, *Microbiology (UK)*, 2002, vol. 148, no. 2, pp. 391–404.
20. Danilova, I.V., Doronina, N.V., Trotsenko, Yu.A., and Netrusov, A.I., Ryzhkova (Iordan) E.P. The Aeration-Dependent Effect of Vitamin B_{12} on DNA Biosynthesis in *Methylobacterium dichloromethanicum, Mikrobiologiya*, 2004, vol. 73, no. 2, pp. 169–174 [*Microbiology* (Engl. Transl.), vol. 73, no. 2, pp. 134–138].
21. Riera, J., Robb, T., Weiss, R., and Fontecave, M. Ribonucleotide Reductase in the Archaeon *Pyrococcus furiosus*: a Critical Enzyme in the Evolution of DNA Genomes?, *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, no. 1, pp. 475–478.
22. Auling, G., Thaler, M., and Dieckmann, H., Parameters of Unbalanced Growth and Reversible Inhibition of DNA-synthesis in *Brevibacterium ammoniagenes* ATCC-6872 Induced by Depletion of Mn^{2+}, *Arch. Microbiol.*, 1980, vol. 127, no. 1, pp. 105–114.
23. Karrasch, M., Bott, M., and Thauer, R.K., Carbonic Anhydrase Activity in Acetate Grown *Methanosarcina barkeri, Arch. Microbiol.*, 1989, vol. 151, no. 2, pp. 137–142.
24. Weiss, D.S., Gartner, P., and Thauer, R.K., The Energetics and Sodium-Ion Dependence of N5-Methyltetrahydromethanopterin: Coenzyme M Methyltransferase Studied with Cob(I)Alamin as Methyl Acceptor and Methylcob(III)Alamin as Methyl Donor, *Eur. J. Biochem.*, 1994, vol. 226, pp. 799–809.
25. Maupin-Furlow, J. and Ferry, J.G., Characterization of the *cdhD* and *cdhE* Genes Encoding Subunits of the Corrinoid/Iron-Sulfur Enzyme of the CO Dehydrogenase Complex from *Methanosarcina thermophila, J. Bacteriol.*, 1996, vol. 178, no. 2, pp. 340–346.
26. Hippler, B. and Thauer, R.K. The Energy Conserving Methyltetrahydromethanopterin:Coenzyme M Methyltransferase Complex from Methanogenic Archaea: Function of the subunit MtrH, *FEBS Lett.*, 1999, vol. 449, no. 2–3, pp. 165–168.
21. Zhilina, T.N., Dying off of a Methanosarcina on air, *Mikrobiologiya*, 1972, vol. 41, no. 6, pp. 1105–1106.
28. Brioukhanov, A., Netrusov, A., Sordel, M., Thauer, R.K., and Shima, S., Protection of *Methanosarcina barkeri* against Oxidative Stress: Identification and Characterization of an Iron Superoxide Dismutase, *Arch. Microbiol.*, 2000, vol. 174, no. 3, pp. 213–216.
29. Brioukhanov, A.L., Netrusov, A.I., and Eggen, R.I.L., The Catalase and Superoxide Dismutase Genes Are Transcriptionally Up-Regulated upon Oxidative Stress in the Strictly Anaerobic Archaeon *Methanosarcina barkeri, Microbiology* (UK), 2006, vol. 152, no. 6, pp. 1671–1677.