ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 4, pp. 361-369. © Pleiades Publishing, Inc., 2006.
Published in Russian in Biokhimiya, 2006, Vol. 71, No. 4, pp. 454-463.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM05-239, February 12, 2006.

L-Methionine γ-Lyase from *Citrobacter freundii*: Cloning of the Gene and Kinetic Parameters of the Enzyme

I. V. Manukhov[1], D. V. Mamaeva[2], E. A. Morozova[2], S. M. Rastorguev[1], N. G. Faleev[3], T. V. Demidkina[2]*, and G. B. Zavilgelsky[1]*

[1]*State Research Institute of Genetics and Selection of Industrial Microorganisms, 1-yi Dorozhnyi Proezd 1, 117545 Moscow, Russia; fax: (495) 315-0501; E-mail: zavilgel@genetika.ru*
[2]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, 119991 Moscow, Russia; fax: (495) 135-1405; E-mail: tvd@eimb.ru*
[3]*Nesmeyanov Institute of Elementoorganic Compounds, Russian Academy of Sciences, ul. Vavilova 28, 119991 Moscow, Russia; fax: (495) 135-5085; E-mail: ngfal@ineos.ac.ru*

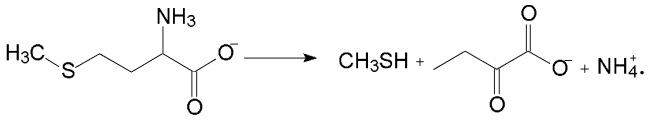
Received September 23, 2005
Revision received November 23, 2005

Abstract—It is shown for the first time for the *Enterobacteriaceae* family that a gene encoding L-methionine γ-lyase (MGL) is present in the genome of *Citrobacter freundii*. Homogeneous enzyme has been purified from *C. freundii* cells and its N-terminal sequence has been determined. The hybrid plasmid pUCmgl obtained from the *C. freundii* genomic library contains an EcoRI insert of about 3000 bp, which ensures the appearance of MGL activity when expressed in *Escherichia coli* TG1 cells. The nucleotide sequence of the EcoRI fragment contains two open reading frames. The first frame (the *megL* gene) encodes a protein of 398 amino acid residues that has sequence homology with MGLs from different sources. The second frame encodes a protein with sequence homology with proteins belonging to the family of permeases. To overexpress the *megL* gene it was cloned into pET-15b vector. Recombinant enzyme has been purified and its kinetic parameters have been determined. It is demonstrated that a presence of a hybrid plasmid pUCmgl, containing the *megL* gene in the *E. coli* K12 cells, leads to a decrease in efficiency of EcoKI-restriction. It seems likely that decomposition of L-methionine under the action of MGL leads to a decrease in the intracellular content of S-adenosylmethionine. Expression of the *megL* gene in the *C. freundii* genome occurs only upon induction by a significant amount of L-methionine.

DOI: 10.1134/S0006297906040031

Key words: *Enterobacteriaceae* family, *Citrobacter freundii*, pyridoxal 5′-phosphate-dependent L-methionine γ-lyase, gene, cloning, kinetic parameters, S-adenosylmethionine

L-Methionine γ-lyase (MGL, EC 4.4.1.11) is a pyridoxal 5′-phosphate-dependent enzyme catalyzing γ-elimination of L-methionine to produce methylmercaptan, ammonia, and α-ketobutyrate [1]:



The enzyme also catalyzes β-elimination reaction of L-cysteine and S-substituted L-cysteines as well as γ- and β-replacement reactions of L-methionine and L-cysteine and their analogs [1]. The latter reactions can be used for enzymatic synthesis of sulfur-containing amino acids. Thus, from L-methionine and alkyl- or arylthiols in the γ-substitution reaction, the respective analogs of L-methionine can be prepared [1]. When chiral alkylthiols are used, the γ-substitution of L-methionine is selective with respect to (S)-configuration of the thiol [2]. It was shown recently [3] that the phosphinic analog of L-methionine is a substrate of MGL, and this fact creates an opportunity for using the γ-substitution reaction for the synthesis of biologically active phosphinic analogs of L-methionine and L-homocysteine [4]. In β-substitution

Abbreviations: MGL) L-methionine γ-lyase; rMGL) recombinant MGL; NCBI) National Center for Biotechnology Information; bp) base pair; PCR) polymerase chain reaction.
* To whom correspondence should be addressed.

reactions, S-alkylcysteines can be synthesized from S-methyl-L-cysteine and alkylthiols [1]. MGL also catalyzes decomposition of L-selenomethionine, and the enzyme can be used for the enzymatic synthesis of seleno-substituted amino acids [5]. The ability of MGL to catalyze the isotopic exchange of α- and β-protons of L-methionine and S-methyl-L-cysteine can be used for preparation of α- and β-deuterated or tritiated amino acids [6]. The enzyme has been isolated from bacteria (*Pseudomonas putida*, *Aeromonas* sp., *Clostridium sporogenes*, *Phophyromonas gingivalis*, *Brevibacterium linens* BL2) and also from primitive protozoa (*Trichomonas vaginalis* [7] and *Entamoeba histolytica* [8]). However, steady-state kinetic parameters have been determined only for the enzymes from *P. putida* [9, 10] and *T. vaginalis* [7].

The gene encoding MGL has been found in many bacteria, Archaea, and primitive protozoa**.** However, no data concerning the presence of the gene encoding MGL in the *Enterobacteriaceae* family have been reported. Similar genes were not found in completely sequenced genomes of species of the *Enterobacteriaceae* family: *E. coli*, *Salmonella enterica* serovar Typhimurium, *Shigella flexneri*.

Earlier, it was shown by one of the authors [11] that MGL is synthesized in *Citrobacter intermedius* cells in the presence of L-methionine, which leads to a decrease in L-methionine concentration and appearance of α-ketobutyrate in the medium.

In the present work, we have cloned and sequenced a polynucleotide from the genome of *C. freundii* (*megL* gene) homologous to the genes encoding MGL. We also obtained homogeneous preparations of MGL from *C. freundii* cells, and recombinant enzyme expressed in *E. coli* cells, and determined steady-state parameters of the recombinant enzyme in reactions of γ- and β-elimination.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Citrobacter freundii* ATCC 21434 cells from the American Type Culture Collection were a kind gift of R. S. Phillips (University of Georgia, USA). *Escherichia coli* TG1 and BL21 (DE3) strains were used as host strains for cloning and expression, respectively. *Escherichia coli* K12 AB1157 cells were used for evaluation of activity of the restriction–modification system EcoKI, type I. Vectors pUC18, pUC19, and pET-15b Novagen (Germany) were used for cloning.

Chemicals. L-Methionine, DL-homocysteine, L-cysteine, S-Me-L-cysteine, S-Et-L-cysteine, L-vinylglycine, DL-homoserine, dinitrophenylhydrazine, phenylmethylsulfonyl fluoride (PMSF), NADH, lactate dehydrogenase from rabbit muscle, dithiothreitol (DTT), and pyridoxine hydrochloride were from Sigma (USA); L-methionine and S-Bzl-L-cysteine from Serva (Germany); pyridoxal 5′-phosphate from Merck (Germany); sodium L-lactate from Lancaster (UK); DEAE-cellulose from Whatman (UK); Sephacryl S-200 HR and Luria–Bertani medium (LB-medium) from Amersham Biosciences (Sweden); restriction enzymes, DNA ligase, and Klenow fragment of DNA polymerase from Fermentas (Lithuania).

Media and growth conditions. *Escherichia coli* cells were grown on LB and minimal M9 media. Antibiotics were added to media in concentrations as follows: ampicillin (100 μg/ml), tetracycline (10 μg/ml), and kanamycin (40 μg/ml). *Escherichia coli* cells were grown on agarized media at 37°C. Cultures grown overnight were rinsed into LB or M9 media and the following growth was conducted on a shaker at 30 or 37°C.

Citrobacter freundii cells were grown in medium containing (%, w/v): $(NH_4)_2SO_4$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.1; KH_2PO_4, 0.34; K_2HPO_4, 1.76; $FeSO_4 \cdot 7H_2O$, 0.002; pyridoxine hydrochloride, 0.01; sodium L-lactate, 0.5; DL-methionine, 0.4; pH 7.0. The cells were grown in 800 ml flasks containing 100 ml of the medium under shaking (190 rpm) for 28 h at 30°C. The cells were harvested by centrifugation (3000*g*) and washed two times with water. Optical density of suspensions of cells was determined using KFK-2MP colorimeter.

Isolation of chromosomal DNA. Chromosomal DNA of *C. freundii* was isolated using DNA-extraction kit from Promega (USA) and digested with restriction endonucleases EcoRI, BamHI, and XbaI.

Synthetic oligonucleotides and PCR amplification. For screening of the genomic DNA fragment containing the gene encoding MGL, the following oligonucleotides were used as primers: a complementary degenerative oligonucleotide N_{vir} (5′-ACITAYAARTTYAAYACICARATHGT-3′, 26 bp; Y – C, T; R – A, G; H – A, C, T; I – A, G, C, T) constructed on the basis of the N-terminal amino acid sequence of the fragment from *C. freundii*: MSDCRTYGFNTQIV; a degenerate reverse primer A_{vir} (5′-GGRTTIGCIGGIGTYTCRAARTA-3′) constructed on the basis of alignment of amino acid sequences of MGLs from several sources, and corresponding to the conservative region Y155-P163 (numeration for the amino acid sequence of *P. putida* MGL [12]). For screening of DNA fragment containing the entire gene *megL*, we used as primers the nucleotides N_{dir} 5′-GAGCCTTCTACAGGCGCGGGTAG-3′ and N_{rev} 5′-GATATACACTACTTTGGTTTCCGG-3′ complementary to the 5′-terminal part of the gene and to the nucleotides 442-465 in the nucleotide sequence of the 5′-terminus of the *megL* gene, which was determined at the previous stage. Oligonucleotide N_{super} 5′-GATATCCATGGCTGACTGTCGTACTTAC-3′, complementary to the 5′-terminal sequence of the gene *megL* and containing the restriction site NcoI (underlined), was used as primer for construction of the overproducing strain. Oligonucleo-

tide N_{super} and the standard reverse primer for pUC18 were used for PCR-amplification of the fragment of DNA containing the entire *megL* gene. This DNA fragment was cloned in pET-15b vector, strain BL21 (DE3).

Screening of the pUC-library of the chromosomal DNA of *C. freundii*. EcoRI-, BamHI-, and XbaI-fragments of chromosomal DNA were ligated into pUC18 vector. Ligation with EcoRI-, BamHI-, and XbaI-sites of pUC18 DNA was performed using T4 DNA-ligase. The ligating mixture was used to transform *E. coli* TG1 cells according to [13].

The DNA sequence was determined on both strands by the dideoxy chain termination method of Sanger [14].

Expression of the *megL* gene in *E. coli* BL21 (DE3) cells. Cells of *E. coli* BL21 (DE3) carrying the hybrid plasmid pETmgl with the *megL* gene under the control of T7 promoter were grown at 30°C in LB broth containing 100 μg/ml ampicillin until the culture medium reached 0.6 OD at 600 nm. The T7 RNA polymerase was then induced using 1 mM isopropyl-β-D-thiogalactopyranoside for 3 h. The cells were harvested by centrifugation (3000*g*, 30 min).

Determination of enzymatic activity of MGL during its purification and steady-state parameters of the γ- and β-elimination reactions. The unit of activity was defined as the amount of MGL that catalyzes the formation of 1.0 μmol/min of α-ketobutyrate at 30°C. The specific activity was expressed as units per mg of the protein. For measuring the activities during the purification procedure, we used reaction mixtures containing 40 mM L-methionine in 10 mM potassium phosphate, pH 8.0, 0.1 mM pyridoxal 5′-phosphate, 1 mM EDTA, and 5 mM DTT. For the determination of kinetic parameters of γ-elimination reaction, we used assay mixtures containing variable concentrations of substrates and all other components mentioned above. The rate of enzymatic reaction was determined by the rate of α-ketobutyrate formation using dinitrophenylhydrazine [15]. The reaction was initiated by addition of 6-8 μg of the enzyme. The reaction mixture was incubated for 15 min at 30°C, and the reaction was stopped by addition of trichloroacetic acid to a final concentration of 12.5%, w/v.

The steady-state parameters of β-elimination reactions were determined by the rate of pyruvate formation, using the coupled assay with lactate dehydrogenase, measuring the rate of the decrease in absorption of NADH at 340 nm ($\Delta\varepsilon = -6220\ M^{-1}\cdot cm^{-1}$) at 30°C. The reaction mixture contained 10 mM potassium phosphate (pH 8.0), 0.1 mM pyridoxal 5′-phosphate, 1 mM EDTA, 5 mM DTT, 0.2 mM NADH, 10 units of lactate dehydrogenase, and variable amounts of amino acid substrate in a total volume of 1 ml. The reaction was initiated by addition of the enzyme (6-7 μg).

Steady-state kinetic parameters were obtained by fitting the kinetic data to the Michaelis–Menten equation using the Enzfitter program. In calculations, we used a subunit molecular mass of 43 kD.

Determination of protein concentration. During the purification procedure, protein content was determined by the method of Lowry [16]. Concentrations of homogeneous preparations of MGL were calculated used a subunit molecular mass of 43 kD.

Purification of the enzyme from *C. freundii* cells. All operations during the purification of MGL were performed at 4°C. The buffers used throughout contained 0.1 mM pyridoxal 5′-phosphate, 1 mM EDTA, and 5 mM DTT. *Citrobacter freundii* cells were suspended in 10 mM potassium phosphate buffer (pH 8.0) containing 0.5 mM PMSF and treated by ultrasound for 4 min with cooling. The cell debris was removed by centrifugation (30 min, 6400*g*). A solution of 2% (w/v) protamine sulfate was added to the supernatant to a final concentration of 0.33% (w/v), and the precipitate was removed by centrifugation (6400*g*, 30 min). The supernatant was heated at 60°C for 5 min, then the solution was cooled and the precipitate was removed by centrifugation. The supernatant was applied to a DEAE-cellulose column (35 × 20 mm) equilibrated with 10 mM potassium phosphate buffer (pH 8.0). The column was washed with the same buffer containing 0.1 M KCl. The enzyme was eluted by the buffer supplemented with 0.5 M KCl, concentrated, and dialyzed against 10 mM potassium phosphate buffer (pH 8.0) using a Centricon-30 ultrafiltration unit (Amicon, USA). Then the enzyme was purified by gel filtration on a Sephacryl S-200 HR column (1.5 × 60 cm) equilibrated with 10 mM potassium phosphate buffer, pH 8.0. The yield of the enzyme was about 21% based on the total cell protein.

The purity of the enzyme preparations was monitored by electrophoresis in a gradient polyacrylamide gel concentration in the system of Laemmli [17].

N-Terminal amino acid analysis. Preparations of MGL obtained after Sephacryl S-200 column chromatography were subjected to electrophoresis in 7% polyacrylamide gel. The purified enzyme was obtained by elution of the single band, and its enzymatic activity was determined. The enzyme was electroblotted from the gel as described in [13] with a Protblott instrument (Applied Biosystem, USA) and the membrane was stained with Coomassie Brilliant Blue G-250. The colored spot was cut out, and the amino acid sequence was determined with a model 475A gas-phase Sequencer with on-line model 120A PTH Analyzer for identification of phenylthiohydantoines (both instruments from Applied Biosystem). As a result, the following N-terminal amino acid sequence was determined: MSDCRTYGFNTQIV.

Restriction coefficients were determined using an unmodified DNA phage λ.0 as a target and strains *E. coli* K-12 AB1157 r^+m^+ and *E. coli* TG1 r^-m^- as described in [18]. We used unmodified λ.0 phages and modified phages λ.k grown on *E. coli* TG1 and *E. coli* K12 AB1157, respectively.

Search for homological sequences from NCBI database was performed using program VECTOR NTI-6, AlignX, v.6.0.0.0.

RESULTS

Cloning of the *megL* gene of *C. freundii* encoding MGL. Using the N-terminal sequence of MGL and the consensus of amino acid sequences of MGLs in the region Y155-P163 (numeration for MGL of *P. putida* [12]), we composed degenerate primers N_{vir} and A_{vir} and used them for PCR-amplification of the 5′-region of *C. freundii* gene encoding MGL, of approximately 500 bp in size. Following the separation of DNA fragments in an agarose gel, a stripe of DNA of about 500 bp was isolated and these DNA fragments were used for cloning into pUC18 vector with blunt ends (single-strand fragments at the ends of the DNA molecule were blunted using Klenow fragment of DNA polymerase). The ligated DNA mixture was used for transformation of *E. coli* TG1 cells. Several clones containing the hybrid plasmids with DNA fragments of about 500 bp were obtained. DNA sequencing had shown that one of these fragments is homologous to the 5′-end of the *P. putida* gene encoding MGL. Using this sequence, we prepared the primers N_{dir} and N_{rev}.

In order to clone the full-sized gene coding MGL, we constructed a library on the basis of pUC18 vector containing EcoRI, BamHI, or XbaI fragments of *C. freundii* chromosomal DNA. To this end, colonies from Petri dishes containing about 1000 hybrid clones were transferred to a new dish with a replica. The primary dish was split into sectors of approximately 100 colonies each, which were rinsed with water and 5 μl of suspension was used as a matrix for subsequent PCR-amplification. The amplification was conducted using two primers N_{dir} and N_{rev}. Separation of the amplification products in an agarose gel revealed that colonies from two sectors only could give a PCR-product of about 500 bp. The same procedure (PCR-amplification with N_{dir} and N_{rev} primers followed by identification of 500 bp fragments in agarose gel) was applied to each of the 100 colonies from the two selected sectors. Finally, two clones were selected which carried hybrid plasmids with the same EcoRI fragment inserted into pUC18 vector, whose size was approximately 3000 bp (the hybrid plasmids were denoted as pUCmgl1 and pUCmgl2; taking into account that the inserted fragment is the same in both plasmids they further are termed as pUCmgl).

Nucleotide sequence of the EcoRI-fragment of *C. freundii*. The nucleotide sequence of DNA fragment of *C. freundii* of 3000 bp in size (NCBI number AY204910) contains two open reading frames. The first frame contains 1194 nucleotides and encodes a polypeptide of 398 amino acid residues. According to the analysis of protein sequences of NCBI database, this polypeptide was identified as MGL. The second frame, containing 1296 nucleotides, encodes a polypeptide of 432 amino acid residues. The analysis of protein sequences of the NCBI database revealed that this polypeptide belongs to the family of permeases, being homologous (about 90% identical amino acid residues) to permeases of *E. coli* and *S. enterica*.

Figure 1 shows the alignment of amino acid sequences of the MGLs. The amino acid residues, which, according to X-ray data, form active sites of MGLs of *C. freundii* [19], *T. vaginalis* [20], and *P. putida* [21] are marked with an asterisk. The lysine residue that binds the cofactor covalently occupies position 210. D185 forms a salt bridge with the nitrogen atom of the pyridine ring of pyridoxal 5′-phosphate. Residues R60, S207, T209, G88, and I89 participate in the binding of the phosphate "handle" of pyridoxal 5′-phosphate. Y113 is in stacking interaction with the cofactor. For MGL of *P. putida* the role of the homologous Y114 residue as a possible general acid catalyst during the elimination of the γ-substituent of the substrate was considered [10]. The guanidine group of R374 residue, according to X-ray data, should bind the carboxylic group of L-methionine, while side groups of the L61, I57, and F49 residues should form a hydrophobic pocket for binding of the substrate. In general, the content of identical amino acid residues is not high (less than 55%). In addition, the phylogenetic tree, built up in accordance with the level of homology (Fig. 2), does not correspond to the taxonomic positions of species, which indicates that spreading of the *megL* gene among species was mainly performed by "horizontal transfer" [22].

Synthesis of MGL in *C. freundii* cells and purification of the recombinant enzyme. Table 1 shows data on MGL activity in *C. freundii* cells determined in the process of their growth in medium containing sodium lactate without methionine, and in the presence of 2 mg/ml L-methionine. In the presence of L-methionine, a 5-fold enhancement of MGL synthesis was observed at the early exponential phase of growth, whereas at the end of the exponential phase the enhancement increased to 50-fold. Consequently, L-methionine is an inducer of MGL synthesis in the *C. freundii* cells.

To ensure the overproduction of MGL, the *megL* gene was recloned into pET-15b vector. To reach this end, after PCR-amplification of the fragment containing the *megL* gene in the pUCmgl plasmid, using a primer N_{super} and the universal reverse-primer pUC18, and subsequent isolation of the PCR product from the agarose gel, the fragment was inserted into pET-15b via NcoI and BamHI sites. As a result of transformation of BL21 (DE3) cells, we selected a clone containing a hybrid plasmid pETmgl. The superproducing strain constructed in this way did synthesize, in the presence of isopropyl-β-D-thiogalactopyranoside, up to 20% MGL based on total protein content. The recombinant MGL (rMGL) did not contain

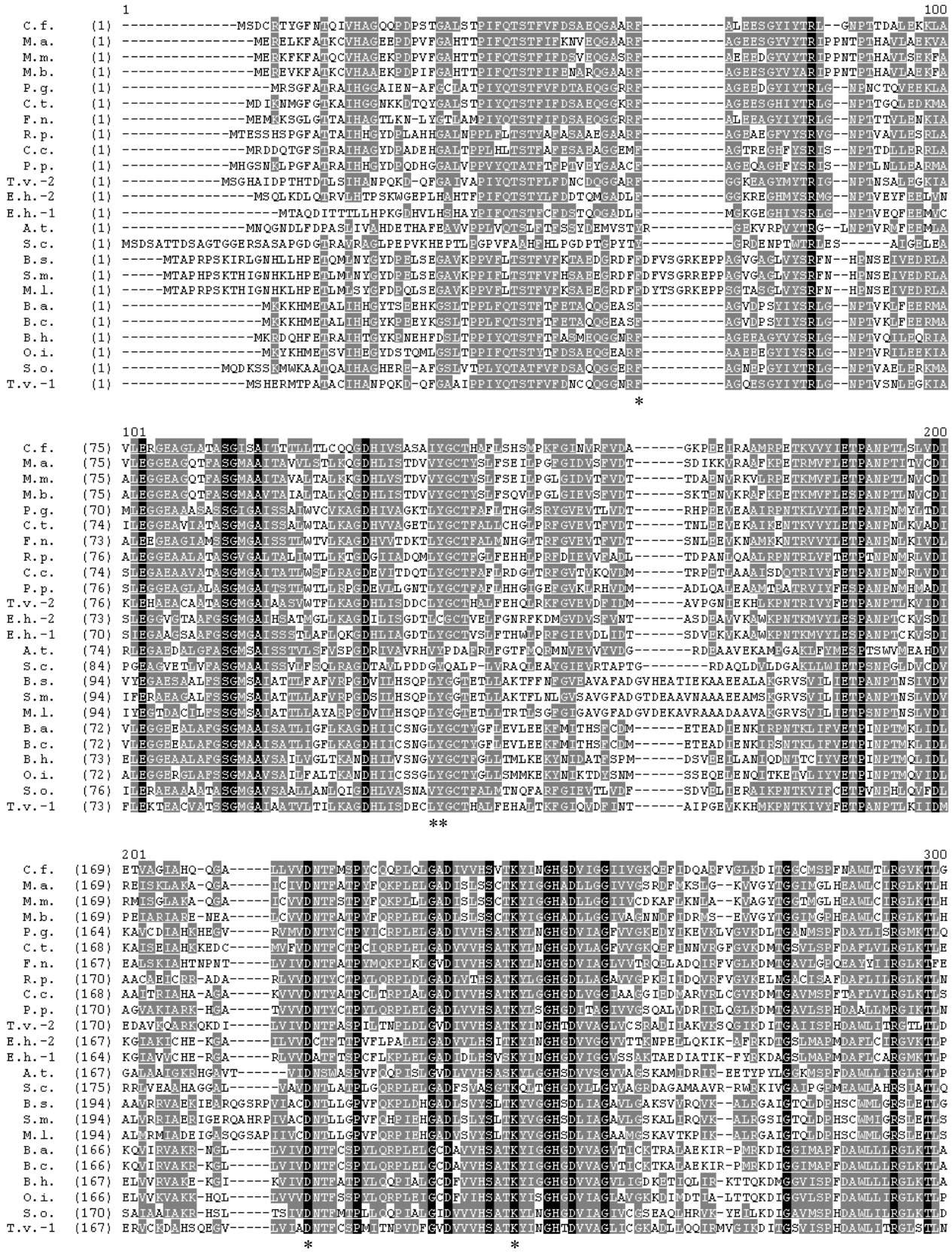


Fig. 1. Alignment of amino acid sequences of MGLs from 22 species.

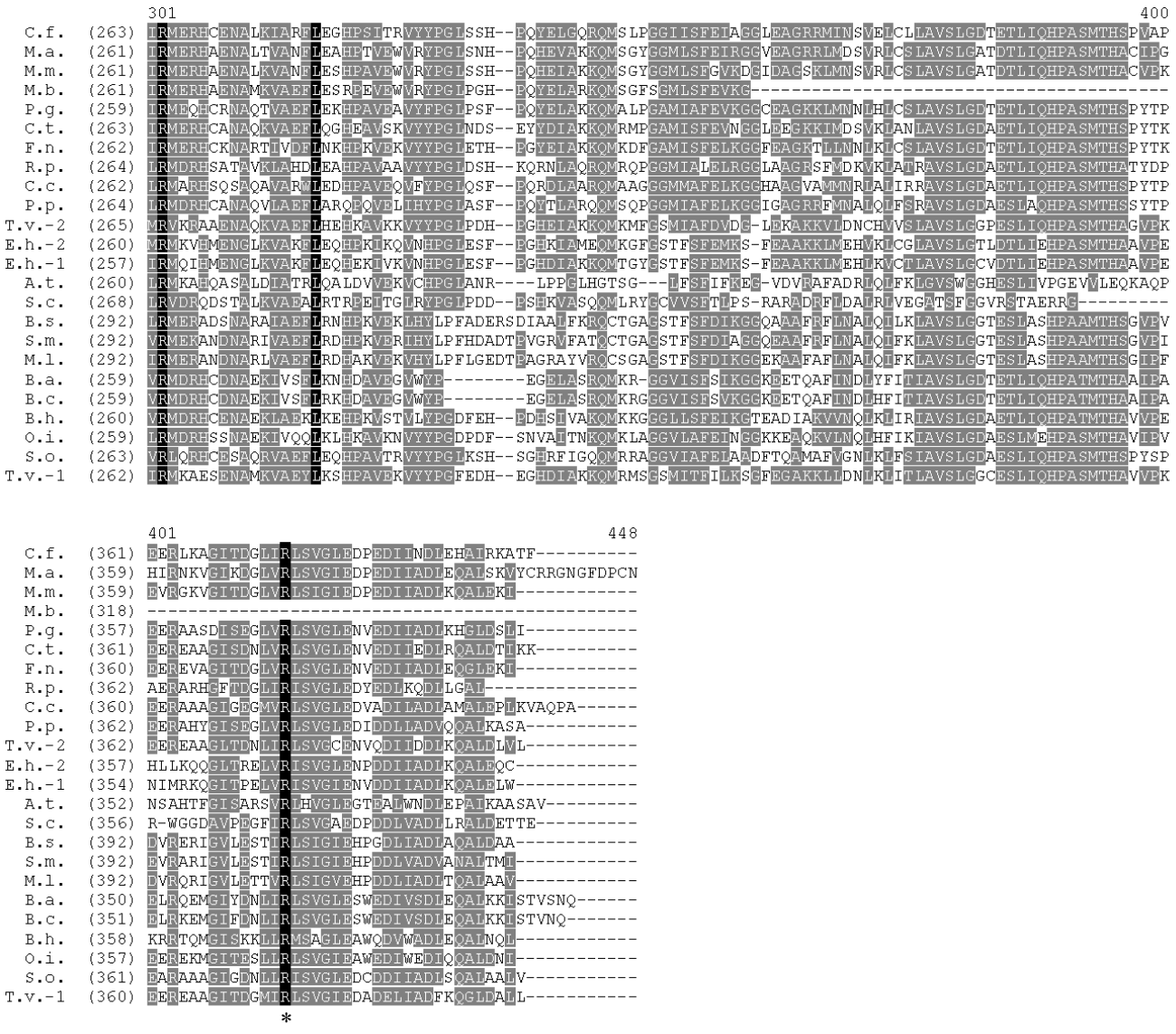


Fig. 1. (Contd.) Alignment of amino acid sequences of MGLs from 22 species. The alignment was carried out according to the Clustal method. In brackets, accession numbers for NCBI data bank are presented. A.t.) *Agrobacterium tumefaciens* (NP356816); B.h.) *Bacillus halodurans* (NP241665); B.s.) *Brucella suis* (NP698941); C.c.) *Caulobacter crescentus* (NP421962); F.a.) *Fusobacterium nucleatum* (BAC02724); M.l.) *Mesorhizobium loti* (AP105358); M.a.) *Methanosarcina acetivorans* (NP617435); O.i.) *Oceanobacillus iheyensis* (NP693968); P.p.) *Pseudomonas putida* (L43133); S.m.) *Sinorhizobium meliloti* (NP386384); T.v.) *Trichomonas vaginalis* (AA04125); C.f.) *Citrobacter freundii* (AAO46884); B.a.) *Bacillus anthracis* (AP658687); B.c.) *Bacillus cereus* (NP834360); C.t.) *Clostridium tetani* (NP783053); E.h.) *Entamoeba histolytica* (BAC75878); M.b.) *Methanosarcina barkeri* (ZP00075765); M.m.) *Methanosarcina mazei* (NP635109); Sh.o.) *Shewanella oneidensis* (NP717420); S.c.) *Streptomyces coelicolor* (NP628105; T37173).

His-Tag element because the respective site of DNA was deleted from pET-15b as a result of the use of restriction sites NcoI-BamHI for ligation.

The procedure of purification of MGL from *E. coli* cells containing hybrid plasmids with the *megL* gene was analogous to the procedure used for the isolation of MGL from *C. freundii* cells, only the stage of heat treatment was omitted. Finally, the preparation of MGL was obtained whose specific activity was 11.0 units/mg; the yield of the enzyme was about 36% of the total cell protein.

Kinetic parameters of MGL from *C. freundii*. The kinetic parameters of *C. freundii* rMGL and enzymes from *P. putida* and *T. vaginalis* are presented in Table 2. For the natural substrate, L-methionine, values of K_m for rMGL of *C. freundii* were similar to K_m values for rMGL of *P. putida* and rMGL1 of *T. vaginalis*, values of k_{cat} for the enzymes from *C. freundii* and *T. vaginalis* (rMGL1) were also practically equal. For the enzyme of *P. putida*, it is characteristic that k_{cat} values for the γ-elimination of L-methionine and for the reaction with L-vinylglycine are

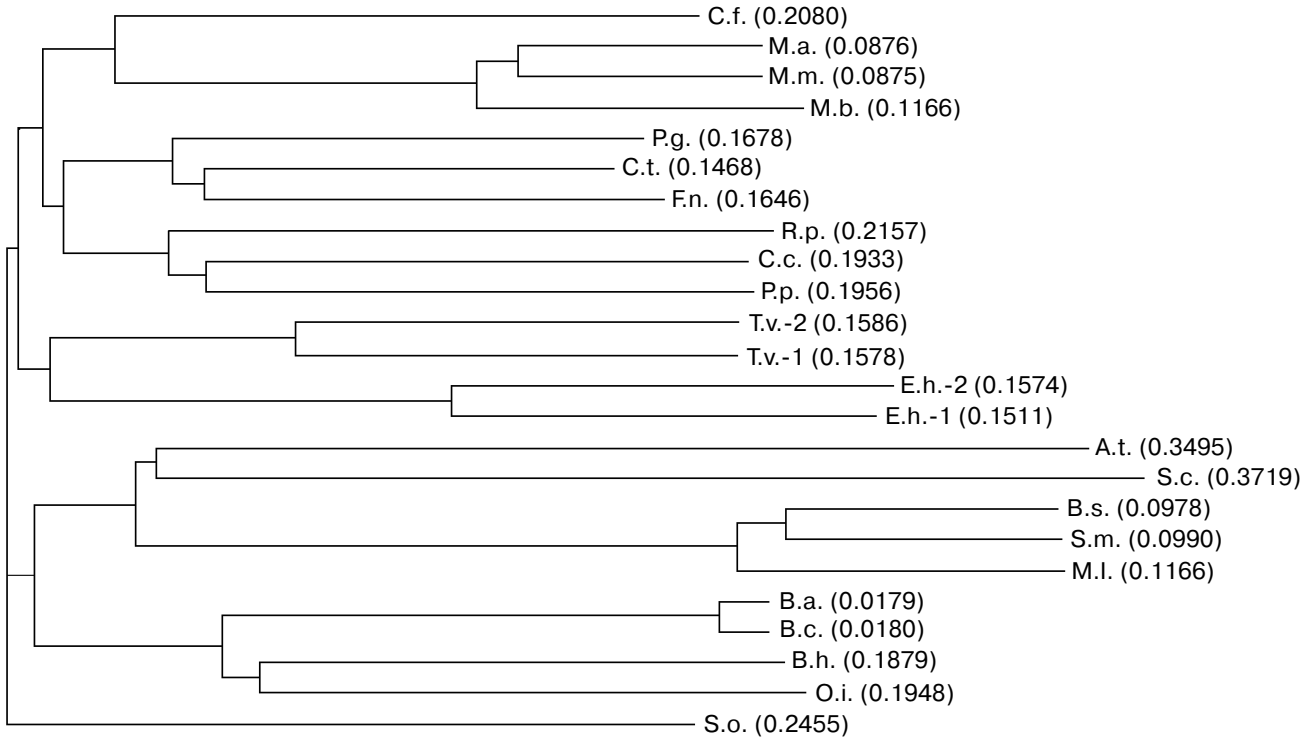


Fig. 2. Phylogenetic tree constructed according to the alignment of amino acids sequences shown in Fig. 1. Species are denoted as in Fig. 1.

considerably greater. The greatest difference in kinetic parameters for γ-elimination reaction is observed between rMGL of *C. freundii* and rMGL1 of *T. vaginalis* when L-homocysteine is the substrate. Values of k_{cat} for rMGL1 of *T. vaginalis* are considerably greater than respective values for rMGL of *C. freundii*, and the affinity to homocysteine for rMGL1 of *T. vaginalis* is considerably less that for rMGL of *C. freundii*.

Data concerning the enzymatic mechanisms of MGLs from various sources are at present very limited, so further studies are necessary to explain the observed differences in their specificities and catalytic efficiencies.

Antirestriction properties of MGL. We examined the influence of the cloned *megL* gene on the activity of a restriction–modification system of type I (EcoKI) in *E. coli* K12 AB1157 cells. It is obvious from the data presented in Table 3 that the presence of the hybrid plasmid in the bacteria leads to a considerable (about 20-fold) decrease in efficiency of EcoKI-restriction with the unmodified DNA of λ phage.

DISCUSSION

Until now, there were no data giving evidence for the existence of a gene encoding MGL in the completely sequenced genomes of the *Enterobacteriaceae* family: in *E. coli*, *S. enterica*, and *S. flexneri*. The results of the pres-

Table 1. Effect of L-methionine on synthesis of MGL in *C. freundii* cells

Medium	L* + M	L	L + M	L	L + M	L
A_{600}	0.5	0.5	1.0	1.0	1.7	1.7
Biomass, g	0.58	0.66	0.65	0.64	0.67	0.75
Total protein, mg	16.0	18.2	14.0	21.0	13.6	25.6
Total activity, units	6.3	1.4	12.6	1.26	5.28	0.21
Specific activity, units/mg	0.3	0.07	0.9	0.08	0.38	0.008

* L, medium with sodium L-lactate; L + M, medium with sodium L-lactate and 0.2% L-methionine.

Table 2. Kinetic parameters of β- and γ-elimination reactions catalyzed by MGLs from different species

Substrate	rMGL *C. freundii*		rMGL1* *T. vaginalis*		rMGL2* *T. vaginalis*		rMGL** *P. putida*	
	k_{cat}, sec^{-1}	K_m, mM	k_{cat}, sec^{-1}	K_m, mM	k_{cat}, sec^{-1}	K_m, mM	k_{cat}, sec^{-1}	K_m, mM
L-Met	6.2 ± 0.42	0.7 ± 0.11	7.43	0.65	0.48	10.6	48.6	0.90
L-Gly(vinyl)	1.9 ± 0.043	6.7 ± 0.66	—	—	—	—	44.4	7.22
DL-Hcy	8.51 ± 0.41	0.97 ± 0.15	264	12.2	94.8	37.7	—	—
DL-Hse	0.52 ± 0.017	56.5 ± 6.5	—	—	—	—		
S-Et-L-Hcy	6.78 ± 0.02	0.54 ± 0.01	—	—	—	—	33.4	0.27
L-Cys	2.33 ± 0.09	0.16 ± 0.02	4.3	8.5	1.72	22.3	—	—
S-Me-L-Cys	4.6 ± 0.29	0.71 ± 0.11	—	—	—	—	5.53	0.40
S-Et-L-Cys	5.03 ± 0.16	0.17 ± 0.02	—	—	—	—	5.79	0.48
S-Bzl-L-Cys	8.16 ± 0.23	0.18 ± 0.02	—	—	—	—	—	—

* Calculated from data of work [7] assuming molecular mass of 43 kD.
** Data from [10].

Table 3. Effect of *megL* gene on the restriction–modification system EcoKI in *E. coli* K12 AB1157r^+m^+ cells*

Plasmid	Restriction coefficient of phage λ.0 (K)**	Relief of EcoKI-restriction (R)***
pUC18	$2.0 \cdot 10^{-4}$	1
pUCmgl	$4.0 \cdot 10^{-3}$	20

* Data are means of five independent measurements.
** Restriction coefficient (K) is equal to ratio of the titer of phage λ.0 grown on AB1157 r^+m^+ cells to the titer of phage λ.0, grown on TG1 r^-m^- cells. Titers of phage λ.k on AB1157, AB1157 (pUCmgl), and TG1 were equal.
*** Relief of restriction R = K^+/K^- (K^- was determined for cells AB1157 without plasmid, K^+ for AB1157 cells with plasmid).

ent work compel us to revise these notions because an entire *megL* gene, encoding the active enzyme, was identified in the genome of *C. freundii* (Fig. 1), and fragments of the *megL* gene were found in the genomes of *S. enterica*, *S. flexneri*, *E. coli*, and *C. rodentium* [22].

It seems likely that destruction of *megL* gene in genomes of *S. enterica*, *E. coli*, *S. flexneri*, and *C. rodentium* and its stable existence in the genome of *C. freundii* might be associated with the influence of the *megL* gene on the intracellular content of S-adenosylmethionine. This consideration is substantiated by the reduction of *Eco*KI restriction in *E. coli* K12 bacteria containing a plasmid with the *megL* gene (Table 3). Moreover, it was demonstrated that there is a significant decrease in DNA methylation upon treatment of eukaryotic cells with MGL [23]. So far as S-adenosylmethionine is a principal donor of single carbon containing groups, the decrease in its intracellular content leads to loss of the viability of the cells [24].

Possibly, just the decrease in the intracellular pool of S-adenosylmethionine as a result of the MGL activity has led to destruction of *megL* gene in *E. coli*, *S. flexneri*, *C. rodentium*, and *S. enterica*. The reason for the retention of the active *megL* gene in *C. freundii* may be due to particularities of regulation of the expression of the gene, namely, to existence of a repression system to the effect that the *megL* gene opens only in the presence of comparatively large concentration of L-methionine (Table 1), and, consequently, its activity cannot considerably decrease the intracellular pool of S-adenosylmethionine.

This work was financially supported by the Russian Foundation for Basic Research (grant Nos. 05-04-48521, 03-04-48189, 05-04-49341, 04-04-49370, and MK-4575.2004.4), by Council of the Russian Federation President (grant for Outstanding Scientific Schools No. 1800.2003.4), and by the International Fogarty Foundation (grant No. 1 R03 TW006045-01A2).

REFERENCES

1. Tanaka, H., Esaki, N., and Soda, K. (1985) *Enzyme Microb. Technol.*, **7**, 530-537.
2. Faleev, N. G., Troitskaya, M. V., Paskonova, E. A., Saporovskaya, M. B., and Belikov, B. M. (1996) *Enz. Microb. Technol.*, **19**, 1-4.
3. Alferov, K. V., Faleev, N. G., Khurs, E. N., Zhukov, V. N., and Khomutov, R. M. (2002) *Mendeleev Comm.*, Nos. 1/2, 2-3.
4. Alferov, K. V., Zhukov, V. N., Faleev, N. G., Khurs, E. N., and Khomutov, R. M. (2003) *Mendeleev Comm.*, No. 3, 127-128.

5. Esaki, N., Tanaka, H., Uemura, S., Suzuki, T., and Soda, K. (1979) *Biochemistry*, **18**, 407-410.
6. Esaki, N., Sawada, S., Tanaka, H., and Soda, K. (1982) *Analyt. Biochem.*, **19**, 281-285.
7. McKie, A. E., Edlind, T., Walker, J., Mottram, J. M., and Coombs, G. H. (1998) *J. Biol. Chem.*, **273**, 5549-5556.
8. Tokoro, M., Asai, T., Kobayashi, S., Takeuchi, T., and Nozaki, T. (2003) *J. Biol. Chem.*, **278**, 42717-42727.
9. Esaki, N., Nakayama, T., Sawada, S., Tanaka, H., and Soda, K. (1985) *Biochemistry*, **24**, 3857-3862.
10. Inoe, H., Inagaki, K., Adachi, N., Tamura, T., Esaki, N., Soda, K., and Tanaka, H. (2000) *Biosci. Biotechnol. Biochem.*, **64**, 2336-2343.
11. Faleev, N. G., Troitskaya, M. V., Ivoilov, V. S., Karpova, V. V., and Belikov, V. M. (1994) *Prikl. Biokhim. Mikrobiol.*, **30**, 458-463.
12. Inoe, H., Inagaki, K., Sugimoto, M., Esaki, N., Soda, K., and Tanaka, H. (1995) *J. Biochem.*, **117**, 1120-1125.
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Second Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
14. Sanger, F., Nicklen, S., and Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
15. Friedemann, T. E., and Haugen, G. E. (1943) *J. Biol. Chem.*, **177**, 415-442.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
17. Laemmli, U. K. (1970) *Nature,* **227**, 680-685.
18. Rastorguev, S. M., and Zavilgelsky, G. B. (2003) *Genetika*, **39**, 218-224.
19. Mamaeva, D. V., Morozova, E. A., Nikulin, A. D., Revtovich, S. V., Nikonov, S. V., Garber, M. B., and Demidkina, T. V. (2005) *Acta Cryst.*, **F61**, 546-549.
20. Goodal, G., Mottram, J. C., Coombs, G. H., and Laptom, A. J. (2000) *PDB-Entry 1E5E.*
21. Motoshima, H., Inagaki, K., Kumasaka, T., Furuichi, M., Inoue, H., Tamura, T., Esaki, N., Soda, K., Tanaka, N., Yamamoto, M., and Tanaka, H. (2000) *J. Biochem.*, **128**, 349-354.
22. Manukhov, I. V., Mamaeva, D. V., Rastorguev, S. M., Faleev, N. G., Morozova, E. A., Demidkina, T. V., and Zavilgelsky, G. B. (2005) *J. Bacteriol.*, **187**, 3889-3893.
23. Machover, D., Zittoun, J., Saffroy, R., Broet, P., Giraudier, S., Magnaldo, T., Goldschmidt, E., Debuire, B., Orrico, M., Tan, Y., Mishal, Z., Chevallier, O., Tonetti, C., Joudult, H., Ulusakarya, A., Tanguy, M. L., Metzger, G., and Hoffman, R. M. (2002) *Cancer Res.*, **62**, 4685-4689.
24. Satishchandr, C., Taylor, J. C., and Markham, G. D. M. (1990) *J. Bacteriol.*, **172**, 4489-4496.