A Gene Encoding L-Methionine γ -Lyase Is Present in *Enterobacteriaceae* Family Genomes: Identification and Characterization of *Citrobacter freundii* L-Methionine γ -Lyase

Ilya V. Manukhov,¹ Daria V. Mamaeva,² Sergei M. Rastorguev,¹
 Nicolai G. Faleev,³ Elena A. Morozova,² Tatyana V. Demidkina,^{2†}
 and Gennadii B. Zavilgelsky^{1*†}

State Research Institute of Genetic and Selection of Industrial Microorganisms, 1st Dorozhnii pr. 1, Moscow 117545, Russia,¹ Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str., 32, Moscow 119991, Russia,² and Nesmeyanov Institute of Elementoorganic Compounds, Russian Academy of Sciences, Vavilov str., 28, Moscow 119991, Russia³

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Citrobacter freundii cells produce L-methionine γ -lyase when grown on a medium containing L-methionine. The nucleotide sequence of the hybrid plasmid with a *C. freundii* EcoRI insert of about 3.0 kbp contained two open reading frames, consisting of 1,194 nucleotides and 1,296 nucleotides, respectively. The first one (denoted *megL*) encoded L-methionine γ -lyase. The enzyme was overexpressed in *Escherichia coli* and purified. The second frame encoded a protein belonging to the family of permeases. Regions of high sequence identity with the 3'-terminal part of the *C. freundii megL* gene located in the same regions of *Salmonella enterica* serovar Typhimurium, *Shigella flexneri*, *E. coli*, and *Citrobacter rodentium* genomes were found.

L-Methionine γ -lyase (MGL; EC 4.4.1.11) is a pyridoxal 5' phosphate (pyridoxal-P)-dependent enzyme catalyzing the γ elimination of L-methionine to produce methanethiol, ammonia, and α -ketobutyrate (Fig. 1).

The enzyme also catalyzes the β elimination reaction of L-cysteine and S-substituted L-cysteines as well as the γ and β replacement reactions of L-methionine and L-cysteine and their analogues (11).

MGL has been isolated from *Pseudomonas putida* (8), from some other bacteria, and from the primitive protozoa *Entamoeba histolytica* (12) and *Trichomonas vaginalis* (6).

Citrobacter intermedius cells produce MGL when grown on a medium containing lactate and L-methionine (3). This finding was the only indication of the presence of MGL in the *Enterobacteriaceae* family. In the present work, we have purified MGL from *Citrobacter freundii* cells and have cloned and sequenced the 3.0-kb insert which contained two open reading frames (ORFs), the first one encoding MGL and the second one encoding a putative permease. For overproduction of the *C. freundii* MGL, the plasmid pET-mgl has been constructed and homogeneous recombinant MGL (rMGL) has been obtained. Kinetic parameters for γ and β elimination reactions catalyzed by both wild-type enzyme and rMGL have been determined.

In the genomes of *Escherichia coli*, *Shigella flexneri*, *Salmo-*

nella enterica serovar Typhimurium, and *Citrobacter rodentium*, we found the regions with high sequence identity to the 3'-terminal region of the *megL* gene located upstream of the gene encoding a putative permease, as that seen in *C. freundii*.

Purification of MGL from *C. freundii*. *C. freundii* cells (ATCC 29063) were grown in the presence of 0.4% DL-methionine as described in reference 3. The elaborated procedure for purification of MGL is presented in Table 1. All operations were performed at 4°C. One unit of activity was defined as the amount of enzyme which catalyzes the formation of 1.0 μ mol of α -ketobutyrate at 30°C per minute. The specific activity was expressed as units per mg of protein. Determination of α -ketobutyrate was performed with 2,4-dinitrophenylhydrazine (4). According to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the enzyme's preparations were 86% pure and the molecular mass of a subunit was estimated to be 43 to 45 kDa.

To determine the N-terminal sequence of the enzyme, it was additionally purified by nondenatured polyacrylamide gel electrophoresis. The enzyme was electroblotted from the gel onto

TABLE 1. Purification of MGL from 30 g of *C. freundii* cells

Step	Total protein (mg)	Total activity (U)	Sp act (U/mg of protein)	Activity yield (%)
Crude extract	414	189	0.46	100
Nucleic acids				
Precipitation	409	188	0.46	99
Heat treatment	129	184	1.43	97
DEAE-cellulose	27	122	4.5	65
Sephacryl S-200 HR	4	40	10.0	21

* Corresponding author. Mailing address: State Research Institute of Genetic and Selection of Industrial Microorganisms, 1st Dorozhnii pr. 1, Moscow 117545, Russia. Phone: (7095) 315 37 29. Fax: (7095) 315 05 01. E-mail: zavilgel@genetika.ru.

† T.V.D. and G.B.Z. contributed equally to this paper.

FIG. 1. MGL, a pyridoxal-P-dependent enzyme catalyzing the γ elimination of L-methionine to produce methanethiol, ammonia, and α -ketobutyrate.

a polyvinylidene fluoride membrane according to the method described in reference 9, and the sequence of amino acid residues obtained by automatic sequencing was MSDCRTYG FNTQIV.

Cloning of the *C. freundii* gene encoding MGL. To screen the *C. freundii* genomic DNA for the gene encoding MGL, we used degenerate primers N_{vir} and A_{vir} for PCR amplification of approximately 470 bp of the 5' tail fragment of the *megL* gene. Primer N_{vir} (5'-ACITAYAARTTYAAYACICARATHGT-3' [26 bp], where Y is C or T; R is A or G; H is A, C, or T; and I is A, G, C, or T) corresponds to the N-terminal amino acid sequence of MGL. Primer A_{vir} (5'-GGRTTIGCIGGIGTYTC RTTSTA-3') corresponds to the conservative amino acid sequence YFETPANP of *P. putida* MGL (5). Following separation of the DNA fragments in an agarose gel, a band of DNA of about 500 bp was isolated, ligated into pUC18 with blunt ends, and introduced into *E. coli* TG1 cells. *E. coli* cells were grown on Luria-Bertani medium in the presence of ampicillin. Several clones containing the plasmids with DNA fragments of about 500 bp were obtained. DNA sequencing showed that one of these fragments is homologous to the 5' end of the *P. putida* gene encoding MGL (5). Using this sequence, we prepared the primers N_{dir} (5'-GAGCCTTCTACAGGCGCGGGTAG-3') and N_{rev} (5'-GATATACACTACTTTGGTTTCCGG-3'). In order to clone the entire *megL* gene, we constructed a library in the pUC18 vector containing EcoRI fragments of *C. freundii* chromosomal DNA. Colonies from petri dishes containing about 1,000 clones per petri dish were replica plated. The primary dish was split into sectors of approximately 100 colonies each, which were suspended in water, and 5 μ l of this

suspension was used as template for PCR amplification. The amplification was conducted using two primers, N_{dir} and N_{rev} . Agarose gel electrophoresis revealed that colonies from two sectors gave 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 an agarose gel) was applied to each of the 100 colonies from the two selected sectors. Finally, two clones which carried plasmids with the same EcoRI fragment in pUC18 (of about 3.0 kbp) were selected.

Nucleotide sequence of the *C. freundii* 3.0-kbp fragment. The nucleotide sequence of the 3.0 kbp fragment was determined on both strands. It contained two ORFs: ORF1 of 1,194 nucleotides, starting with the ATG initiation codon and ending with the TGA terminator codon, at position 1445, and ORF2, starting with an ATG initiation codon, located at position 1563 of the 3' region of the ORF1. The region of 1,194 nucleotides encoded a protein of 398 amino acid residues. This protein was identified as MGL based on a computer search of GenBank. The gene encoding the enzyme was designated *megL*. The sequence of the ORF2 encoded a protein of 432 amino acid residues. A computer search of GenBank found that this protein belongs to a family of permeases. This gene was designated *aap*. The nucleotide sequences of the upstream region and the spacer between the *megL* and *aap* genes of *C. freundii* are shown in Fig. 2. In the sequence preceding the *megL* gene, the putative elements of the regulatory region are shown, namely the ribosome binding site and -10 and -35 sites of a putative promoter. Downstream of the *megL* gene is an inverted repeat sequence of 28 bp, and an initiation ATG codon

FIG. 2. The nucleotide sequences of the upstream region and the spacer between the *megL* and *aap* genes of *C. freundii*. Genes *megL* and *aap* are shown as boxes with initiation and termination codons. The putative ribosome binding site (rbs) and promoter sites (-10 and -35) are underlined. The inverted repeat sequence (28 bp) between *megL* and *aap* is marked with arrows.

TABLE 2. Kinetic parameters of β and γ elimination reactions for *C. freundii* MGLs^a

Substrate	<i>C. freundii</i> , wild-type MGL		<i>C. freundii</i> , rMGL	
	k_{cat} (s ⁻¹)	K_m (mM)	k_{cat} (s ⁻¹)	K_m (mM)
L-Methionine	6.5 $\pm$ 0.12	0.7 $\pm$ 0.05	6.2 $\pm$ 0.42	0.7 $\pm$ 0.11
DL-Homocysteine	5.1 $\pm$ 0.16	1.1 $\pm$ 0.13	8.51 $\pm$ 0.41	0.97 $\pm$ 0.15
L-Ethionine	6.2 $\pm$ 0.28	0.56 $\pm$ 0.09	6.78 $\pm$ 0.02	0.54 $\pm$ 0.01
L-Cysteine	2.24 $\pm$ 0.13	0.18 $\pm$ 0.02	2.33 $\pm$ 0.09	0.16 $\pm$ 0.02
S-Me-L-cysteine	5.97 $\pm$ 0.08	0.61 $\pm$ 0.02	4.6 $\pm$ 0.29	0.71 $\pm$ 0.11
S-Et-L-cysteine	6.8 $\pm$ 0.19	0.49 $\pm$ 0.02	5.03 $\pm$ 0.16	0.17 $\pm$ 0.02
S-Bzl-L-cysteine	10.9 $\pm$ 0.17	0.19 $\pm$ 0.01	8.16 $\pm$ 0.23	0.18 $\pm$ 0.02

^a Kinetic parameters of γ elimination reactions have been determined by 2,4-dinitrophenylhydrazine; kinetic parameters of β elimination reactions have been determined by coupled assay with L-lactate dehydrogenase and NADPH (7).

for ORF2 is located at 82 bp from the inverted repeat element. The sequence identity between MGLs from different species did not exceed 60%. Phylogenetic analysis of MGLs showed that there is no correlation between the amino acid sequence identity and the taxonomic position of species on the phylogenetic tree. One may assume that in these species genes coding MGL arose for the most part by horizontal transfer.

Production, purification, and kinetic parameters of MGL.

The DNA fragment containing the *megL* gene was amplified by PCR using primer N_{super} (5'-GATATCCATGGCTGACTGT CGTACTTAC-3'), complementary to the 5' terminus of the gene encoding MGL and containing the restriction site NcoI (underlined; the substituted nucleotide is shown in italics) and the reverse primer of pUC18. After isolation from agarose gel, the fragment was inserted into pET-15b via NcoI and BamHI sites. Following transformation of *E. coli* BL21 (DE3) cells, a clone containing the plasmid (named pET-mgl) was obtained. It produced MGL up to 20% of total cell protein. The purification procedure for rMGL was the same as for the wild-type enzyme except the heat treatment step was omitted. The enzyme with specific activity of 10 U/mg was obtained with a total yield of 36%. Steady-state kinetic parameters of the wild-type and recombinant enzyme are presented in Table 2. They were obtained by fitting the kinetic data to the Michaelis-Menten equation using EnzFitter (1).

Comparative analysis of nucleotide sequences of the *megL* gene in *C. freundii*, *E. coli*, *S. flexneri*, serovar Typhimurium, and *C. rodentium* genomes. Taking as a starting point in the genomes of *C. freundii*, *E. coli*, *S. flexneri*, *S. enterica* serovar Typhimurium, and *C. rodentium* the nucleotide sequence of the gene encoding putative permease (*aap* for *C. freundii*; *yhjV* for *E. coli*, *S. enterica* serovar Typhimurium, and *S. flexneri*), we carried out a comparative analysis of the sequences preceding this gene. The results of this analysis are presented in Fig. 3. It may be seen that in the genomes of serovar Typhimurium, *S. flexneri*, *E. coli*, and *C. rodentium*, the sequences homologous (especially in the serovar Typhimurium genome) to the sequence of the *megL* gene of *C. freundii* are located in the same regions. However, the sequence similarity is observed only for a region of 237 bp of the 3' terminus part of the *C. freundii megL* gene, which corresponds to 79 C-terminal amino acids of the MGL. As far as about 500 bp upstream of this site, any sequence similarity with the *C. freundii megL* gene is absent, although about 80% sequence similarity among serovar Typhimurium, *S. flexneri*, and *E. coli* sequences is observed.

To our knowledge, no data indicating the presence of the

gene coding MGL in genomes of *Enterobacteriaceae* family were known. The results presented here allow a revision of the notion of the absence of a gene coding MGL in genomes of the *Enterobacteriaceae*. It is noteworthy that the *megL* gene is located in the same environment in the genomes of *C. freundii*, serovar Typhimurium, *S. flexneri*, *E. coli*, and *C. rodentium*, and the breaking of the *megL* gene and deletion of its 5'-terminal sequence in genomes of *E. coli*, *S. flexneri*, *S. enterica* serovar Typhimurium, and *C. rodentium* took place at the same site (Fig. 3).

We may suppose that in the process of evolution, the following events have taken place (Fig. 4). First, the *megL* gene was inserted into the genome of an ancestor 1 as a result of the horizontal transfer (stage I). Next, a certain branch (*C. freundii*) continued to exist with the complete and active *megL* gene. At stage II, the *megL* gene was broken (its 5' fragment was deleted) in the genome of an ancestor 2 of *E. coli*, *S. flexneri*, and serovar Typhimurium. At stage II, the 5' fragment was deleted in the genome of an ancestor 2 of *E. coli*, *S. flexneri*, and serovar Typhimurium. At the stage III, a deletion of the 3' terminus of *megL* and the spacer in *E. coli* and *S. flexneri* genomes had taken place. It seems likely that a deletion of the *megL* gene and the spacer between the *aap* and *megL* genes in the *C. rodentium* genome is associated with a repeated horizontal transfer of this DNA fragment from the *E. coli* (or *S. flexneri*) genome. Probably, a definite role in these reconstructions was played by the inverted repeat element (28 bp) located immediately after the *megL*. It is known that inverted repeat sequences may determine the recombination's "hot spots" (2).

The comparative analysis of nucleotide sequences of completely sequenced genomes of *Erwinia carotovora*, *Klebsiella pneumoniae*, *Yersinia pestis*, and *Photobacterium luminescens* has revealed the absence of sequences highly similar to the sequences of *aap* and *megL*. This may be considered as evidence that the horizontal transfer and insertion of this cluster into the genome of ancestor 1 (Fig. 4) had taken place after the divergence of these species in the course of evolution. We may only speculate as to the reasons that have led to the destruction of the *megL* gene in *E. coli*, *S. flexneri*, serovar Typhimurium, and *C. rodentium* and ensured its stable existence in the genome of *C. freundii*. This may be associated with the influence of the *megL* gene on the intracellular content of S-adenosylmethionine (AdoMet). Being a donor of the methyl group, AdoMet belongs to a group of molecules participating in a number of important processes in cells, and any decrease in the levels of AdoMet should lead to a loss of the cell's viability (10). The reason for its retention in the genome of *C. freundii* may be

3'-fragment of *megL* gene

C.f. GAA ATC GCT GGC GGC CTC GAG GCT GGC ACA CGG ATC ATC AAT TCT
 S.t.deletion.....] CT- GAG GCT GGC AGT CaG ccG cTC AAT TCT
 E.c.deletion.....] CTC cAG aCT GGT AGT gaa ATG cgC AAT TCT
 S.f.deletion.....] CTC cAG aCT GGT AGT gaa ATG cgC AAT TCT
 C.r.deletion.....] caa tCG GCT GGC AGT Ctc tcc tgC gCT TCT

C.f. GTA GAA TTG TGC CTC CTC GCG GTC AGT CTC GGT GAT ACC GAA ACC
 S.t. GTA -AA TTa TGC CTC CTC GCG GcC AGT CcC GGT GAT ACC GAA ACC
 E.c. GTA GAA TTG cGC CTC CTC GCG acC tcc Cca Gtg aAT ctg ttg cCt
 S.f. GTA GAA TTG cGC CTC CTC GCG acC tcc Cca Gtg aAT ctg ttg cCt
 C.r. GTA GAA TTG cGt CcC CTC GCG GcC [.....deletion.....]

C.f. CTC ATT CAG CAC CCA GCG TCT ATG ACA CAT TCG CCC GTT GCG CCA
 S.t. CTC gTT CAG Cat CCT GCG TCT ATG ACA tAT TCG CCT GTT GCG CCA
 E.c. Cat tTT tta Cat aCt GCG TaT tcG ACt tcT cCa CCT GTT GCG CaA
 S.f. Cat tTT tta Cat aCt GCG TaT tcG ACt tcT cCa CCT GTT GCT CaA
 C.r.deletion.....deletion.....

C.f. GAG GAA CGG CTT AAA GCA GGT ATT ACC GAC GGG CTT ATC CGT CTT
 S.t. GAG GAA tGG CTa AAA GCA GGT ATT ACC GAC Gaa CTT ATC CGT CTT
 E.c. GAG aAA Ct[.....deletion.....] GGG tTT AtT CaT tTT
 S.f. GAG aAA Ct[.....deletion.....] GGG tTT AtT CaT tTT
 C.r.deletion.....deletion.....

C.f. TCT GTG GGT CTT GAA GAT CCA GAA GAT ATT ATT AAC GAC CTT GAA
 S.t. TCa GcG GGA CcT GAA GAT CCc GAc GAT ATT ATT cAt GAC CTT GAA
 E.c. T[.....deletion.....]gc GAg G[.....deletion.....]
 S.f. T[.....deletion.....]gc GAg G[.....deletion.....]
 C.r.deletion.....deletion.....

C.f. CAC GCC ATC AGA AAG GCA ACA TTC TGA
 S.t. CgC GCC AgC AGA AAa GCA gCA TTC TGA
 E.c.deletion.....
 S.f.deletion.....
 C.r.deletion.....

intergenic region

C.f. CCATTATGACCTGAACCGACGCGGATCGGTTTGTGAGAATCAAG
 S.t. CgATTgctcggcGAACCGctGtC-AcCGGTTtgccgcaTcGgGtATCAAG
 E.c.deletion.....]ACCGACTtCt [.....deletion.....
 S.f.deletion.....]ACCGACTtCt [.....deletion.....
 C.r.deletion.....deletion.....

C.f. GCACAACAGTCTGGTAATACGCGCAATCCTGd TTT CGG GGA GAG CGC
 S.t. GCACTAtAGcCTGGTtATAgCagcAtTcTtt aTg aGa Gag ctG CGt
 E.c.deletion.....
 S.f.deletion.....
 C.r.deletion.....] aTg acG ccg tgG aGg

C.f. GTA TTT TGG GCA GGG GGT TCT
 S.t. aTA TTT TGG GCA GGG GGC TCT
 E.c.] TTC TGG aCA GGA --c TtT
 S.f.] TTC TGG aCA GGA --c TtT
 C.r. cTA TTT ctG GCA GGG Gac Ttc

FIG. 3. Alignment of the *C. freundii*, serovar Typhimurium, *S. flexneri*, *E. coli*, and *C. rodentium* 3'-terminal fragments of the *megL*, spacer, and 5' fragment of *aap*. Nucleotide substitutions encountered in the DNA of serovar Typhimurium (S.t.), *S. flexneri* (S.f.), *E. coli* (E.c.), and *C. rodentium* (C.r.) are shown in lowercase letters. The spacer inverted repeat sequence (28 bp) is italicized. The TGA stop codon of *megL* and the ATG initiation codon of *aap* are italicized and underlined. GenBank nucleotide and genome accession number information is as follows: for *C. freundii*, AY204910; for serovar Typhimurium, NC_003197; for *E. coli* K12, NC_000913; for *S. flexneri*, NC_004337; for *C. rodentium*, http://www.sanger.ac.uk/Projects/C_rodentium; for *E. carotovora* subsp. *atroseptica* SCRI1043, NC_004547; for *P. luminescens*, NC_000913; for *K. pneumoniae* subsp. *pneumoniae* MGH 78578, NC_002941; and for *Y. pestis* KIM, NC_004088.

	aap gene																			
C.f.	ATG	CAG	GAC	GAC	ACA	TTA	CAA	CTA	AGC	AAC	AAT	ACA	GCA	ACC	ACC	GTA	CCA			
S.t.	ATG	CAG	GAC	GAC	ACA	TTA	CcA	tTA	Aat	AAC	AgT	Aac	GtA	ACg	ACC	acA	CCg			
E.c.	ATG	CAG	CAC	aAC	ACA	cTA	tcg	aaA	caC	Aat	[.....deletion.....]									
S.f.	ATG	CAG	CAC	aAC	ACA	cTA	acg	aaA	caC	Aat	[.....deletion.....]									
C.r.	ATG	CAG	CAC	GAC	ACA	ccA	Ctt	tct	caC	cAC	AAc	Agc	GCg	AgC	[...deletion...]					
C.f.	TTC	TCA	AAG	AAA	ATA	CCG	TTT	ACG	AAA	TAT	GAC	TTT	GGC	TGG	GTA	TTA	CTG			
S.t.	cTt	TCA	AcG	AgA	tTA	CCG	TTT	ACa	AAA	TAT	GAt	TTT	GGC	TGG	GTg	cTc	tTG			
E.c.	]	cAG	AAA	tTg	CCG	TTT	ACa	cgc	TAc	GAC	TTc	GGC	TGG	GTt	TTA	tTA				
S.f.	]	cAG	AAA	tTg	CCG	TTT	ACa	cgc	TAc	GAC	TTc	GGC	TGG	GTt	TTA	tTA				
C.r.	]	TCA	cgc	AgT	cTA	CCT	TTT	ACc	cgc	TAc	GAt	TTc	GGC	TGG	GTt	cTg	CTA			
C.f.	TGT	ATC	GGC	ATG	GCA	ATT	GGT	GCT	GGA	ACG	GTA	CTG	ATG	CCG	GTA	CAA	ATA			
S.t.	TGT	ATC	GGA	ATG	GCG	ATc	GGc	Gcc	GGT	ACc	GTc	tTa	ATG	CCa	GTg	CAA	ATc			
E.c.	TGc	ATa	GGC	ATG	GCG	ATT	GGT	Gcc	GGA	ACc	GTg	CTG	ATG	CCa	GTA	CAA	ATt			
S.f.	TGc	ATa	GGC	ATG	GCG	ATT	GGT	Gcc	GGA	ACc	GTg	CTG	ATG	CCa	GTA	CAA	ATt			
C.r.	TGc	ATt	GGC	ATG	GCG	ATc	GGc	Gca	GGT	ACg	GTc	CTG	ATG	CCg	GTA	CAA	ATt			

Fig. 3—Continued.

particularities of regulation of gene's expression, namely the existence of a repression system to the effect that the *megL* gene opens only in the presence of a comparatively large concentration of L-methionine, and, consequently, its activity cannot decrease the intracellular pool of AdoMet to a critical value.

Nucleotide sequence accession number. The nucleotide sequences reported in this paper have been submitted to GenBank with accession number AY204910.

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REFERENCES

1. Cleland, W. W. 1979. Statistical analysis of enzyme kinetic data. *Methods Enzymol.* **63**:103–138.
2. Cromie, G. A., C. B. Millar, K. H. Schmidt, and D. R. Leach. 2000. Palindromes as substrates for multiple pathways of recombination in *Escherichia coli*. *Genetics* **154**:513–522.
3. Faleev, N. G., M. V. Troitskaya, V. S. Ivoilov, V. V. Karpova, and V. M. Belikov. 1994. Methionine γ -lyase in *Citrobacter intermedium* cells. Synthesis of S-butyl-L-homocysteins and S-butyl-L-cysteine. *Prikl. Biokhim. Microbiol.* **30**:458–463. (In Russian.)
4. Friedemann, T. E., and G. E. Haugen. 1943. The determination of keto acids in blood and urine. *J. Biol. Chem.* **177**:415–442.
5. Inoue, H., K. Inagaki, M. Sugimoto, N. Esaki, K. Soda, and H. Tanaka. 1995. Structural analysis of the L-methionine γ -lyase gene from *Pseudomonas putida*. *J. Biochem.* **117**:1120–1125.
6. Lockwood, B. C., and G. H. Coombs. 1991. Purification and characterization of methionine γ -lyase from *Trichomonas vaginalis*. *Biochem. J.* **279**:675–682.
7. Morino, Y., and E. Snell. 1970. Tryptophanase (*Escherichia coli* B). *Methods Enzymol.* **17A**:439–446.
8. Nakayama, T., N. Esaki, K. Sugie, T. T. Beresov, H. Tanaka, and K. Soda. 1984. Purification of bacterial L-methionine γ -lyase. *Anal. Biochem.* **138**:421–424.
9. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
10. Satishchandr, C., J. C. Taylor, and G. D. M. Markham. 1990. Novel *Escherichia coli* K-12 mutants impaired S-adenosylmethionine synthesis. *J. Bacteriol.* **172**:4489–4496.
11. Tanaka, H., N. Esaki, and K. Soda. 1985. A versatile bacterial enzyme: L-methionine γ -lyase. *Enzyme Microb. Technol.* **7**:530–537.
12. Tokoro, M., T. Asai, S. Kobayashi, T. Takeuchi, and T. Nozaki. 2003. Identification and characterization of two isoenzymes of methionine γ -lyase from *Entamoeba histolytica*. *J. Biol. Chem.* **279**:42717–42727.

FIG. 4. Schematic representation of postulated events of the *megL* gene mutations in the process of evolution of *C. freundii*, *E. coli*, *S. flexneri* and serovar Typhimurium (*S. typhimurium*) genomes. *megL**, deletion of the *megL* 5' fragment; *megL***, deletions of the *megL* 5' fragment and a part of the 3' fragment.