Cloning and Nucleotide Sequences of the Homoserine Dehydrogenase Genes (*hom*) and the Threonine Synthase Genes (*thrC*) of the Gram-Negative Obligate Methylotroph *Methylobacillus glycogenes*

HIROAKI MOTOYAMA,¹* KAZUTOSHI MAKI,¹ HIDEHARU ANAZAWA,¹ SYUICHI ISHINO,² AND SADAO TESHIBA¹

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd., Tokyo 194,¹ and Technical Research Laboratories, Kyowa Hakko Kogyo Co. Ltd., Yamaguchi 747,² Japan

Received 21 June 1993/Accepted 31 October 1993

We have cloned the homoserine dehydrogenase genes (hom) from the gram-negative obligate methylotrophs Methylobacillus glycogenes ATCC 21276 and ATCC 21371 by complementation of an Escherichia coli homoserine dehydrogenase-deficient mutant. The 4.15-kb DNA fragment cloned from M. glycogenes ATCC 21371 also complemented an E. coli threonine synthase-deficient mutant, suggesting the DNA fragment contained the thrC gene in addition to the hom gene. The homoserine dehydrogenases expressed in the E. coli recombinants were hardly inhibited by L-threonine, L-phenylalanine, or L-methionine. However, they became sensitive to the amino acids after storage at 4°C for 4 days as in M. glycogenes. The structures of the homoserine dehydrogenases overexpressed in E. coli were thought to be different from those in M. glycogenes, probably in subunit numbers of the enzyme, and were thought to have converted to the correct structures during the storage. The nucleotide sequences of the hom and thrC genes were determined. The hom genes of M. glycogenes ATCC 21276 and ATCC 21371 encode peptides with M_r s of 48,225 and 44,815, respectively. The thrC genes were located 50 bp downstream of the hom genes. The thrC gene of ATCC 21371 encodes a peptide with an M_r of 52,111, and the gene product of ATCC 21276 was truncated. Northern (RNA) blot analysis suggests that the hom and thrC genes are organized in an operon. Significant homology between the predicted amino acid sequences of the hom and thrce genes from other microorganisms was found.

Methanol is a good carbon source to use in amino acid fermentation because of its high purity and low price. With the use of methanol, raw material costs could be reduced greatly, and the purification of the product and the treatment of wastewater could be simplified. Despite the importance of methanol as a carbon source, reports on the breeding of amino acid producers from methylotrophs have been limited because isolation of mutants from methylotrophs is quite difficult for unknown reasons.

Recently we succeeded in the isolation of L-glutamic acidhyperproducing mutants from the gram-negative obligate methylotrophs *Methylobacillus glycogenes* ATCC 21276 and ATCC 21371 and the derivation of L-threonine- and L-lysineproducing mutants from them (15). The L-threonine and L-lysine biosynthetic enzymes of the L-threonine- and L-lysine-producing mutants were characterized, and several regulatory enzymes were found to be desensitized to feedback inhibition by L-threonine and/or L-lysine (16).

Interestingly, some of the L-threonine and L-lysine biosynthetic enzymes of *M. glycogenes* were also sensitive to aromatic amino acids synthesized by a pathway different from that of the L-aspartate family amino acids. Homoserine dehydrogenase (HD), a major regulatory enzyme of L-threonine biosynthesis, is as sensitive to L-phenylalanine as it is to L-threonine. An interrelationship between the biosyn-

* Corresponding author. Mailing address: Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd., 3-6-6 Asahi-machi, Machida-shi, Tokyo 194, Japan. Phone: 81 (427) 25-2555. Fax: 81 (427) 26-8330. thetic pathway of the L-aspartate family amino acids and that of the aromatic amino acids has been suggested (16).

In many microorganisms, HD is regulated by feedback inhibition or feedback repression, and its gene forms an operon with other L-threonine biosynthetic genes. In Escherichia coli, HD I is fused with aspartate kinase I (AK I) and is inhibited by L-threonine. The thrA gene encoding the fused protein AK I-HD I is organized in an operon, thrABC, with the *thrB* gene encoding homoserine kinase and the *thrC* gene encoding threonine synthase. The thrABC operon is regulated by an attenuation mechanism sensitive to L-threonine and L-isoleucine (6). In Bacillus subtilis, HD is a separate protein from AK and is inhibited by L-threonine (18) and the hom gene is organized in an operon, hom-thrCthrB (17). The hom gene of Corynebacterium glutamicum forms an operon with thrB in the order hom-thrB (20) and is repressed by L-methionine (7). In Pseudomonas aeruginosa (5) and Methylobacillus flagellatum (13), HD is inhibited by L-threonine and the hom gene forms an operon, hom-thrC.

It is worthwhile to clone and investigate the M. glycogenes hom genes, whose products have unique properties including strong inhibition by L-phenylalanine. In this paper, we describe cloning of the hom genes and thrC genes found downstream of the hom genes from M. glycogenes ATCC 21276 and ATCC 21371 and some enzymatic properties of the HDs expressed in E. coli recombinants. The nucleotide sequences of the hom and thrC genes were determined, and the deduced amino acid sequences were compared with those of counterparts from other microorganisms.

Strain or plasmid	Genotype or relevant characteristic(s)	Phenotype ^a	Reference
Strains M glycogenes			
ATCC 21276		Wild type	
ATCC 21371		Wild type	
- ··			
E. coli	(1, 41017,, 41) (1005, 1, -0.1004,, 1001, (1, 1, 1, -1.41,, -T1, () =)	AV- UD- UV- TO-	21
GI 5 C'C 100	$thrA1017$ metLM1005 lysC1004 pro-1001 tht-1 relA1 sp011 (λ)	AK HD HK 15	21
	thrA1015 metLM1005 tysC1004 tht-1 retA1 sp011 (Λ)	HD HW-	21
Hir 3000 YA 73	thrB1000 thi-1 relA1 spo11 (λ)	HK	21
Gif 41	thrC thi-1 relA1 spo11 (λ)	15	21
Plasmids			
pBR322	Vector	Tc ^r Amp ^r	19
pUC19	Vector	Amp ^r	24
pIHD-1	Derivative of pBR322 carrying 3.1-kb PstI fragment cloned from M. glycogenes ATCC 21276	Tc ^r HD ⁺	This study
pIK-1	Derivative of pUC19 carrying 2.1-kb SmaI fragment of pIHD-1	Amp ^r HD ⁺	This study
pIK-2	Derivative of pUC19 carrying 2.1-kb Smal fragment of pIHD-1 (the insert	Amp ^r HD ⁺	This study
	was ligated to pUC19 in the opposite direction from pIK-1)		This studes
pIKD-21	Deletion derivative of pIK-2 overexpressing HD	Amp ⁻ HD	This study
pIHD-5	Derivative of pUC19 carrying 1.2-kb Smal-EcoRV fragment of pIHD-1	Amp.	This study
pIHD-6	Derivative of pUC19 carrying 0.7-kb Smal-EcoRV fragment of pIHD-1	Amp.	This study
pTHD-1	<i>M</i> glycogenes ATCC 21371	IC HD IS	This study
nTHD-2	Derivative of pBR322 carrying 3.2-kb <i>Eco</i> RI- <i>Pst</i> I fragment of pTHD-1	Tc ^r HD ⁺	This study
nTK-1	Derivative of pUC19 carrying 2.1-kb Smal fragment of pTHD-1	Amp ^r HD ⁺	This study
nTK-2	Derivative of pUC19 carrying 2.1-kb Smal fragment of pTHD-1 (the insert	Amp ^r HD ⁺	This study
P	was ligated to pUC19 in the opposite direction from pTK-2)	r	,
pTHD-5	Derivative of pUC19 carrying 1.2-kb Smal-EcoRV fragment of pTHD-1	Amp ^r HD ⁺	This study
pTTS-1	Derivative of pUC19 carrying 2.3-kb <i>Eco</i> RV- <i>Pst</i> I fragment of pTHD-1	Amp ^r TS ⁺	This study
pTTS-2	Derivative of pUC19 carrying 2.3-kb <i>Eco</i> RV- <i>Pst</i> I fragment of pTHD-1	Amp ^r TS ⁺	This study
r	(the insert was ligated to pUC19 in the opposite direction from pTTS-1)	r	-

TABLE 1. Bacterial strains and plasmids used in this study

^a Abbreviations: Amp^r, ampicillin resistance; HK, homoserine kinase; Tc^r, tetracycline resistance; TS, threonine synthase.

MATERIALS AND METHODS

Bacteria and plasmids. The bacteria and plasmids used in this study are listed in Table 1.

Media and culture methods. M. glycogenes ATCC 21276 and ATCC 21371 were cultivated in shake flasks as described in our previous study (16). E. coli cells were cultivated as described previously (12). One hundred milligrams of ampicillin per liter or 10 mg of tetracycline per liter was used to supplement liquid or agar media to cultivate E. coli strains containing plasmids. M9S1 medium (M9 medium [12] supplemented with 50 mg [each] of 18 amino acids [except L-threonine and L-methionine] and with 50 mg of 2,6-diaminopimelic acid per liter) was used for the complementation tests of the E. coli mutants Gif 102, Hfr 3000 YA 73, and Gif 41. M9S5 medium (M9 medium supplemented with 50 mg [each] of 20 amino acids per liter) was used for the complementation test of E. coli GT 5.

DNA manipulations. DNA restriction enzyme digestion, separation of DNA fragments by gel electrophoresis, DNA ligation, and transformation of E. *coli* strains were performed by standard methods as described previously (12). Restriction enzymes and DNA ligase were supplied by Takara Shuzo Co. Ltd. (Kyoto, Japan). Southern hybridization was done with a DNA labeling and detection kit (Boehringer Mannheim) according to the method recommended by the supplier.

Construction of gene libraries. Chromosomal DNAs of *M. glycogenes* were prepared by the method described by Marmur (14). The chromosomal DNAs of *M. glycogenes* ATCC 21276 and ATCC 21371 were partially digested with

PstI and separated by agarose gel electrophoresis. The 3- to 6-kb DNA fragments from each strain were purified from the gel, ligated into the *PstI* site of pBR322 to construct gene libraries, and used to transform *E. coli* Gif 102.

Subcloning of plasmids. The 2.1-kb SmaI fragment of pIHD-1 was inserted into the SmaI site of pUC19 to form pIK-1 and pIK-2 in both orientations. pIKD-21, a plasmid with overexpression of HD, is a deletion derivative of pIK-2. pIHD-5 and pIHD-6 were constructed by ligating the 1.2-kb SmaI-EcoRV and 0.7-kb SmaI-EcoRV fragments of pIHD-1 into the SmaI site of pUC19, respectively. pTHD-2 was constructed by the self-ligation of the 6.8-kb EcoRI fragment of pTHD-1. The 2.1-kb SmaI fragment of pTHD-1 was inserted into the SmaI site of pUC19 to form pTK-1 and pTK-2 in both orientations. pTHD-5 was constructed by inserting the 1.2-kb SmaI-EcoRV fragment of pTHD-1 into the SmaI site of pUC19. The 2.3-kb EcoRV-PstI fragment of pTHD-1 was treated with a blunting kit (Takara Shuzo Co. Ltd.) to create blunt ends and was inserted into the SmaI site of pUC19 to construct pTTS-1 and pTTS-2 in both orientations.

DNA sequence analysis. Both strands of DNA were sequenced by the dideoxy method with double-stranded DNA (4). The deletion derivatives of the inserts were obtained by treating pIK-1, pIK-2, pTK-1, pTK-2, pTTS-1, and pTTS-2 with a deletion kit (Takara Shuzo Co. Ltd.). DNA sequences were analyzed with GENIUS software (Mitsui Knowledge Industry Co. Ltd., Tokyo). Amino acid sequences were analyzed with PRINAS software (Mitsui Knowledge Indus-



FIG. 1. Physical maps and subcloning of the plasmids harboring the hom and thrC genes of M. glycogenes ATCC 21276 (A) and ATCC 21371 (B). The positions of the ORFs determined by sequencing are shown by arrows. P_{lac} denotes the position of the *lac* promoter of pUC19. The complementation activities conferred by each plasmid in E. coli Gif 102 (hom) or Gif 41 (thrC) are indicated by + or -. Abbreviations: EI, EcoRI; EV, EcoRV; C, ClaI; H, HincII; K, KpnI; N, NcoI; P, PstI; S, SmaI; Sp, SphI; N.T., not tested.

try Co. Ltd.). Homology analyses of amino acid sequences were done by a method described previously (11).

Northern blot analysis. Northern (RNA) blot analysis was performed as described previously (1). DNA probes were prepared by PCR with the Gene Amp kit (Perkin-Elmer Cetus) as follows. A 555-bp DNA fragment internal to the hom gene and a 541-bp DNA fragment internal to the thrC gene of M. glycogenes ATCC 21371 were amplified by PCR oligonucleotides 5'-AACCCATCAATGTTG with the GCCTG-3' (5' primer) and 5'-CCAGGCGTTGAGCTTC CTTA-3' (3' primer) for hom and 5'-ATCGCCTGCACTGTC TTTCT-3' (5' primer) and 5'-TTGCAGGCTGAACATCTG TG-3' (3' primer) for thrC, respectively, and pTHD-1 was used as a template. PCR was done with a thermal program: 30 cycles at 94°C for 1.5 min, 45°C for 2 min, and 72°C for 3 min and 1 cycle at 72°C for 4 min. The DNA fragments obtained were labeled with $[\alpha^{-32}P]dCTP$ (Amersham) with the Bca BEST labeling kit (Takara Shuzo Co. Ltd.) and used as probes.

Enzymatic methods. Preparation of cell extracts and measurements of HD activities were performed as described previously (16). One unit of HD was defined as the amount of enzyme which oxidized 1 μ mol of NADH in 1 min. A 4.5% polyacrylamide gel was used to separate proteins. Sodium

dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done according to the method of Laemmli (9). Protein concentrations were determined with a Bio-Rad protein assay kit with bovine gamma globulin as the standard protein.

Purification of HD from *E. coli* recombinants. The cell extracts from *E. coli* Gif 102/pIKD-21 or Gif 102/pTK-2 were applied to a DEAE-Sepharose CL-6B column (2.5 by 40 cm) (Pharmacia) preequilibrated with MET buffer (20 mM Tris-HCl [pH 7.0], 2 mM MgCl₂, 2 mM EDTA, 20 mM 2-mer-captoethanol, 0.02 mM phenylmethylsulfonyl fluoride), washed extensively with MET buffer, and eluted with a linear 0.1 to 0.4 M KCl gradient. The active fractions collected were applied to a hydroxyapatite column (1.5 by 20 cm) (Seikagaku Kogyo, Tokyo, Japan) preequilibrated with MEP buffer (10 mM potassium phosphate buffer [pH 7.0], 2 mM MgCl₂, 2 mM EDTA, 20 mM 2-mercaptoethanol, 0.02 mM phenylmethylsulfonyl fluoride), washed extensively with MEP buffer, and eluted with a linear gradient of 10 to 70 mM potassium phosphate.

Immunochemical methods. Preparation of the antiserum against the HD purified from *E. coli* Gif 102/pIKD-21 and Western blotting (immunoblotting) were done according to the method described previously (8).

 TABLE 2. Inhibitory effects of amino acids on HD activities in

 E. coli Gif 102 containing M. glycogenes hom genes

Strain	Amino acid	Relative activity (%) ^a	
Strain 3if 102/pIHD-1 <i>A. glycogenes</i> ATCC 21276 3if 102/pTHD-1 <i>A. glycogenes</i> ATCC 21371	addition Before A storage sto		After storage
Gif 102/pIHD-1	None	100	100
1	10 mM Thr	88	29
	10 mM Phe	93	6
	10 mM Met	108	72
M. glycogenes ATCC 21276	None	100	ND
	1 mM Thr	4	ND
	1 mM Phe	4	ND
	1 mM Met	102	ND
	10 mM Met	33	ND
Gif 102/pTHD-1	None	100	100
•	30 mM Thr	58	33
	10 mM Phe	62	25
	10 mM Met	97	65
M. glycogenes ATCC 21371	None	100	ND
	1 mM Thr	6	ND
	1 mM Phe	3	ND
	1 mM Met	106	ND
	10 mM Met	19	ND

^a Inhibitory effects of amino acids on the HD activities in the cell extracts of the *E. coli* recombinants were examined soon after the extracts were prepared (before storage) and after storage at 4°C for 4 days (after storage). ND, not determined.

Nucleotide sequence accession number. The nucleotide sequences of the 2.1-kb *SmaI* fragment encoding the HD and the N-terminal sequence of the threonine synthase of *M. glycogenes* ATCC 21276 and the 3.4-kb *SmaI-PstI* fragment encoding the HD, threonine synthase, and N-terminal sequence of thymidylate synthase of *M. glycogenes* ATCC 21371 will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data libraries under the accession no. D14070 and D14071, respectively.

RESULTS AND DISCUSSION

Cloning of the hom and thrC genes from M. glycogenes ATCC 21276 and ATCC 21371. Several genes of gramnegative methylotrophs have been cloned by heterologous complementation of E. coli mutants (10), so we tried to clone the hom genes of M. glycogenes ATCC 21276 and ATCC 21371 by complementation of an E. coli HD-deficient mutant. Gene libraries of M. glycogenes ATCC 21276 and ATCC 21371 were constructed as described in Materials and Methods, and an E. coli HD-deficient mutant, Gif 102, was transformed. The transformed cells were plated onto M9S1 medium with L-threonine and L-methionine omitted, and plasmids from the colonies that appeared on the medium were analyzed.

A plasmid containing a 3.1-kb PstI fragment, designated pIHD-1, and a plasmid containing a 4.15-kb PstI fragment, designated pTHD-1, were obtained from *M. glycogenes* ATCC 21276 and ATCC 21371, respectively (Fig. 1). The specific activities of the HD in *E. coli* Gif 102 carrying pIHD-1 and pTHD-1 were 0.078 and 0.06 U/mg of protein, respectively, whereas no activity was detected in *E. coli* Gif 102 containing pBR322. The chromosomal DNAs of the methylotrophs were digested completely with PstI, separated by agarose gel electrophoresis, and hybridized with probes prepared from the cloned *PstI* fragments. Only the expected 3.1- and 4.15-kb bands were detected in the *PstI*.

1 CCCGGGCAACTGCGATCCAGACCCCCGTGGGGCTACCAGAGTATAGACGACCTGCTGGGCCTAGCCCCTTGTCCAGCGCGCGGCCGACCAG 101 ADTGATACCGACTTACCTGGTGGGCTGGCCTGGCGCGGGCCGGTGTACCTGTCCGGCGGCCCCCATCAGGATATACCCATCAGACCGCCTG 201 GCCGGGACGGTAGGCCGCCTGGGCTGGCCGGCGCTCAACGGGGGGGCGGCGGACCTTCATCGCGGGGGGGCGGACCTTCATCCGGTGGTGGACGGATG 201 CCGGAGACCTCACCGAAGCCTTGCCCGGCTTCCTGGAAACTACCGCAGGAGGACGCACCTCACGGTGGACGGAAGCTGCGGA
ORF1->> M K P I N V 401 <u>Agaca</u> gecaageaageagegagegagegagegagegegtettgatacagestgaagetaagegaagetaactggaagetageageageageageageageageageageageageagea
G L L G I G T V G G G T Y T V L T R N Q E G N R A P C R P P N C H H 501 geotheticgeategetactedegegegegecatataccettataccetateaceaaccageaageaategegegegecectecegecegececategeate
A R R D R N L E L A R K V T G G Q I D V T D D A F A V V V R P R H 601 Accecegtocccaaccecaatettgaattgeoccccaagetaacgegageccaattgacetcaccaatecaattgeocgatecattgeocgtegtogtageaccaac
R H R G R I D R R I H H R A R T G Y E G D R E R Q A R V A A N K A 701 TCGACATCGTGGTGGATTGATCGGCGGATACACCGACGTGGGCAAGCAGGCAG
L M L A W Q R D F R R V A Q Q K G V I V A F E A A V A G G I P I I K 801 CTGATGCTTGCATGGCAACGAGATTTTGGCGCGTTGCAGCGGGCGG
A V A A G A G R Q P S S R V L R A I I N G T T N F I L S E M R E K 901 AGECCETGECGECAGECETEGECGECCAACCETCATEGEAGAATEGECEGAAAT
G L A F A D V L K E A Q P R L C E A T R P S T S R A W T A H K L M 1001 GEGECTEGECTTECEGAGETETCAAGGAGEGECAAGECTAAGECTATEGEGAGEGEGACGEGETEGAGEGEGEGEGEGAGAGECTEATE
I L A A I G F G I P N Q F D K A Y V E G I S K L D A L D I R Y A E E 1101 ATCCTGECCGCGATCGGCATCCGGATGCCGGTGCGACGACGGCGTGGACGACGCATGCAGGCGCATGCGGAGG
L G Y R Q A A W H A P S A P A R G V E L A R A P D P D P R E T P D 1201 AACTGGGCTACCGTCAAGCTGCCCGGCAGCAGCGGCAGGGGGGTGGAACTGCGCGGAGCCGGAACGCCCGAAAAGGCCCGA
R Q C Q R R H N A V L V S D A C R S H L V Y R P D A C R B H A S A 1301 TCGCCAATGTCAACGGCGCATAATGCCGATGCGGATGCGATGCGATGCGATGCGATGCGATGCGACGCGAGGCGATGCGATGCGATGCGATGCGGGGAGGCGGATGCGGATGCGGGGGGGG
V V A D I V D G T R T A Y H R R A P A V P H L L S S P T S S W T C R 1401 GTGGTTECGGATATCGTCGACGGTACCGCCGACCACCGACGACGGGCGGCCGCCGCCGCCTGCTGCCGCCGGCCTGCC
FAIGEVSSAYYLRLRAVDKPGRDGHVTRILATG
S L H R C D D S E G N R R Q A E R R R S G R H H H P D H V T V E K 1601 CAGTITICATICATICATICAGAAGECAACCECAGECAGECGEGGGGEGEGGAAGATCAGEGCCGACATCATCATCCTGACCATGTCACEGGTCGAGAG
N N D D A I V A I E A L P A I S G S V T R L R M E E L S R + 1701 AACATGGATGACGCGATTGTCCCCCATCGAGGCATACTTGACGCAA
ORF2->>> NKKYVSTRGQSAGGGATAGG <u>CCTGCTGGA</u> TCAGTGFGGGAAATACGTTTCCACCGCGGGGGATCGCCCCCCACTGTCGTTTACCGAAATCGTGCT
G G L G P D G G L Y L P E Q Y P Q F S Q D E L N A N R G N N Y R D 1901 TGGCGGCCTGGGGCCCGACGCGGCCTCTACCTGCCCGACGAATACCGCAATGCGAGGACGAACTGAATGCGATGCGGCCATGAATTACCGGCAC
L A F A I L S R L I D D I P A D D L R A I V D K T Y R A D V Y A Y A 2001 CTGGCGTTCGCTATCCTCTCGCCCGACGACGATATTCCCCGCTGACGACGCCGTCGTCGACGAGACGTATCGCCGCGGAGGGTGTGTGT
^{R P} 2101 0505000566 EIG 2 Nucleotide sequence of the 2 1-kb Smal fragment of M

FIG. 2. Nucleotide sequence of the 2.1-kb *SmaI* fragment of *M. glycogenes* ATCC 21276. A putative promoter sequence is underlined. A stem-loop structure is indicated by arrows.

digested cDNAs of ATCC 21276 and ATCC 21371, respectively, suggesting the absence of any other homologous DNA in the chromosomal DNAs (data not shown). The 3.1-kb fragment of pIHD-1 and the 4.15-kb fragment of pTHD-1 were hybridized with one another, indicating that the DNA sequences of the fragments were homologous.

To examine whether pIHD-1 and pTHD-1 contain other L-threonine biosynthetic genes, pIHD-1 and pTHD-1 were used to transform other *E. coli* L-threonine auxotrophic mutants, GT 5 (*thrA metLM lysC*), Hfr 3000 YA 73 (*thrB*), and Gif 41 (*thrC*), and the complementation of the L-threonine auxotrophy by the plasmids was tested. Although pIHD-1 could not complement any other L-threonine-auxotrophs, pTHD-1 complemented the L-threonine auxotrophy of Gif 41, suggesting that pTHD-1 contains the *thrC* gene in addition to the *hom* gene.

Subcloned fragments from pIHD-1 and pTHD-1 were tested for the complementation of Gif 102 and Gif 41 to localize the *hom* and *thrC* genes (Fig. 1). Both of the *hom* genes of pIHD-1 and pTHD-1 were localized in the 2.1-kb *SmaI* fragments, and the *thrC* gene of pIHD-1 was localized in the 2.3-kb *EcoRV-PstI* fragment, respectively.

Properties of the HD activities produced in *E. coli.* The molecular masses of the HDs of *E. coli* Gif 102 containing pIKD-21 and pTK-2, purified with DEAE-Sepharose CL-6B and hydroxyapatite columns, were estimated by SDS-PAGE

to be 50 and 48 kDa (data not shown), close to the values deduced from the nucleotide sequences described below.

The HDs of *M. glycogenes* ATCC 21276 and ATCC 21371 are inhibited strongly by L-threonine or L-phenylalanine and are inhibited weakly by L-methionine (Table 2). The HDs in the cell extracts of Gif 102 harboring pIHD-1 or pTHD-1 were hardly inhibited by these amino acids (Table 2). However, they became sensitive to the amino acids after storage at 4° C for 4 days.

The HDs in the cell extracts of the *E. coli* recombinants before and after storage at 4°C for 4 days were separated by SDS-PAGE, and the molecular masses were compared by Western blotting with the antiserum against the purified HD prepared from Gif 102/pIKD-21. The molecular masses of the enzymes after storage were the same as those before storage (data not shown), indicating that the HDs in *E. coli* recombinants were neither digested nor greatly modified during the storage, although the possibility of covalent modification with a small molecule such as phosphate cannot be ruled out.

Turano et al. (23) reported that the carrot HD was interconvertible between an L-threonine-nonsensitive homodimer and an L-threonine-sensitive homotrimer. We speculate that the L-threonine-nonsensitive HDs of the *E. coli* recombinants are different from the L-threonine-sensitive ones of *M. glycogenes* in the subunit numbers of the enzyme, as observed in the carrot. Since the HDs were overproduced 10- to 20-fold in *E. coli* compared with in *M. glycogenes*, it might take a long time to form the correct structures susceptible to inhibition by the amino acids in *E. coli*.

Nucleotide sequences of the 2.1-kb SmaI fragment of pIHD-1 and the 3.4-kb SmaI-PstI fragment of pTHD-1. The 2.1-kb SmaI fragment of pIHD-1 was sequenced on both strands, and two open reading frames (ORFs) were found (Fig. 2). ORF1 encodes a predicted peptide with an M_r of 48,225 initiated at the ATG codon (nucleotides 483 to 485) and terminated at the TAA (nucleotides 1788 to 1790). ORF2 encodes a truncated peptide of 89 amino acid residues initiated at the ATG codon (nucleotides 1842 to 1844). The amino acid sequence deduced from ORF2 is quite homologous to the N-terminal region of the predicted peptide of the threonine synthase of M. glycogenes ATCC 21371, thought to be the N-terminal domain of the thrC gene of M. glycogenes ATCC 21276. A putative promoter sequence, 5'-AAAGACA-3' (-35) 5'-CAGAAC-3' (-10), homologous to a proposed consensus promoter sequence of gram-negative methylotrophs, 5'-AAAGACA-3' (-35) 5'-TAGAAA-3' (-10) (10), was found 80 to 50 bp upstream of the initiation codon of the hom gene (underlined in Fig. 2). A promoterlike sequence was not found upstream of the *thrC* gene. Similarly, the nucleotide sequence of the 3.4-kb SmaI-PstI

Similarly, the nucleotide sequence of the 3.4-kb SmaI-PstI fragment of pTHD-1 was determined, and three ORFs were found (Fig. 3). ORF1 encodes a deduced peptide with an M_r of 44,815 initiated at the ATG codon (nucleotides 491 to 493) and terminated at the TAA (nucleotides 1727 to 1729). ORF2 encodes a predicted peptide with an M_r of 52,111 initiated at the ATG codon (nucleotides 1779 to 1781) and terminated at the TGA (nucleotides 3204 to 3206). ORF3 encodes a truncated peptide of 64 amino acid residues initiated at the ATG codon (nucleotides 3219 to 3221).

ORF1 and ORF2 are suggested to be the coding sequences of the *hom* and *thrC* genes of *M. glycogenes* ATCC 21371, respectively, by the complementation analysis results shown in Fig. 1. The deduced amino acid sequence of ORF3 is homologous to those of thymidylate synthase genes (*thyA*) from other microorganisms, assumed to be the N-terminal

1 CCCGGGCAATTGGCTATCCAGACCGCGCTTGGTGGTTACCAGAGCATCGATGACCTGGTGGCACCGACTGGGCGCTTGTGCAAGCAGCGCGACCTTGCCT 00871->> И К Р I 401 ассессиладалатасаласаладоссасастастастастастастастостостстаталасталалесталаласталалесса A I T R V A D R N L E L A R Q V T G G K I D V T D D A F A I V S D 501 CGCCATTACCCGTGTTGCCGATCGTAATCTGGAGCTGCTCGCCACGTGCTGGGAAAAATTGATGTCACCGATGATGCTTTTGCCATCGTGTCTGAT P A I D I V V E L I G G Y T V A R E L V L K A I E N G K H V V T A N 701 CCGCAATTGATATTGTTGTGAACTGATCGGTGCCGCACCGTGGCCGCGTGACTGGTGCGAAGCCCATTGAGAATGGCAAGCACGTGGTCACGGCCA KALIAC MANKFLPLRRKKASSSLLKLPLUV SP 801 ATAAGGCCTTGATTGCCTGCGAGGAAATTTTGCCGCTGCGGCGGAGAAAAAGGCGTCATCGTCGCTTTGCAGCTCCCGTTGCTGGTGGTATCCCC L F K A V R E G L A A N R I E W I A G I I N G T T N F I L S E N R 901 ATTATICAAGGCCGTACGTGAGGCCTGGCGGCCAATGGTATGAGTGGATTGCGGCATCAATGAGCACGACGACTATTTCATTGTCTGGGAATGGGT E K G L A F A D V L K E A Q R L G Y A E A D P T F D V E G I D A A H 1001 GAMAAGGETCTGGCGTTTGCTGATGTGCTTAAGGAAGCTCAACGCCTGGGTTATGCCGAGCCCGACCTTCGATGTCGAAGGCATTGATGCTGCGC K L N I L A A N L V L F V H S L C R G I T K L D A V D I T K R T D 1101 ACAAGCTCATGATCCTTGCTGCGATGCTTTTGCTATGTCGAGCCATCACGAAGCTGGATGCCGTGGATATCACCAAGCGTACCGA K G V E L R V H P T L I P E K R L I C Q C E W R N E C C A G Q G R 1201 TAAGGGCGTGGACTTGGCTGTGCGCCAACCTTGATCGCGGAAAGCGCCTGATTGGCAATGGCGCAATGGCGCGAATGGCGGACTGGCGCGACGGCGAA C C W P T L Y Y G A G A G A E P T A S A V A D L V D G T D R G I S C 1301 TGCTGTTGGCCTACTTGATTATGGTGCCGGTGCCGGTGCCGACGTGCCGACGTGCCGACGGTGCCGACCGTGGCACCAGCT PHLAFQPDRLVDLPILPIGEISSAYYLRLRAVD 1401 GTCCACACCTGGCTTTCCACCGCAGACCGCCTGGTAGACTTGGCCAGACTTAGCAGTGCGTATTAGCGCGGCGCGCGGGCAGGGGA A D I I I L T H V T V E K N M D D A I A A I E A L P A I S G K V T R 1601 GCCGACATCATCATCTCACCCACGTCACAGTTGAGAAAAAACATGGATGATGCCACTGCGCCATTGAGGCACTACCTGCCATTTCCGGCAAGGTCACGC L R N E E L S R • ORF2->> N K Y I S T R G 1701 GTTTGCGCATGGAAGAACTAAGCCGATAACTTGAGAAAGGGT<u>TGCAAGCGAGGCC</u>TATT<u>GCCTCCTGCA</u>TCAATGAAATACATTTCCACCCGGG Q S P A L S F S E I L L G G L A P D G G L Y L P E Q Y P Q F S A D 1801 GCCAATCGCCTGCACTGTCTTCTCTGAAATTCTCCTTGGCGGCTTGGCGCCTGATGGCGGCTTGATTTCCCCGAGCAATACCCGCAGTTTAGCGCGCTG A L S A N R G N N Y R D L A F T I L S R L I D D I P A D D L R I I 1901 CSCACTEACCECCATEGACTACCCCGCATGAACTACCCCCGCGTCGACGACGACGTCGCGCTCACC V D K T Y R A D V Y A Y A R P G Q D A E D I T P T Y K L E D D L Y L 2001 GTCGACAAGACCTATOGCCGGGGTGTATATGCCTATGCCCGGGGCCAGGATGCCGAAGACATTACGCCGACCTATAAGCTGGAGGACGACGTCTACC LS LS N G P T LA F K D N A N Q L L G N L F E Y V L A Q K G E T 2101 TECTTECCATEGECEAACCETECCAACCETECCAACCETECCAACCETETEGAATACETETEGECEAAACEGECEAAAC T N I L G A T S G D T G S A A E Y A N R G K Q G V K V F N L S P H 2201 GACTAATATTCTCGGCGCACCTCCGGCGATACCGGTTCTGGCGGGGAAACCACGGGGCAAGGTGTTCATGCTCTCGCCGGA Q K M S R F Q T A Q M F S L Q D D N I F N I A V K G V F D D C Q D I 2301 CAGAAGATGAGCGGTTTCCAGAGCGGCAGAGTGTTCAGGCTGCAGGAGCAACAATGTTCTTCAATATGGGGGTGAAGGGCGGTTTTGAGGACTGCGAGGAGA Y F K G Y F A V T A D N A Q Q V S F A V P S G N F G N V C A G H I 2501 TTACTTCAAGGGTTATTTCGCCGTCACTGCCGCAAATGCCCAGGCCATGTCGCCGCCAACTTCGGCCAACTTCGGCCAACTTCGGCCAATGTCGGCCATGTC A R N N G L P I A K L V V A T N E N D V L D E F F K T G V Y R P R G GCCCCCATGATGGGCTTGCCCAACTGGTGGTAGCGACCAACGACAACGACGTCGTGGTGATGAGTTCTTCAAAACTGGCGTCTACCGTCCGCGCG 2601 K V R E L W G K V D A G G S F D L N D G G W F A K V A D Y G F V S 2801 CAAGGTGCGCGAGCTATGEGEGAAGGTGGATGCGGGCGCGCGCTGGTTGCCAAGGTAGCGGATTACGCCTTCGTTGCCA G S S N H A N R N Q T I K A T H E R Y G V T I D T H T A D G L K V A 2901 ggcagcagcaaccatgccaaccecatgcagccatcaaggcgacgcatgaggcgtacggtgtcaccattgatacccacgccgacggtctcaaggtgg E R P H S L E G L E S L P Q R F E V M E A D A A V I K Q F I V E H 3101 CEAGCGTCCGCACAGCCTGGAAGCCTGGAAGCCCTGGAAGCCGTGGAGGCCGGATGCAGCCGTGTCAAACAGTTCATTGTTGAGGAT I • ORF3->>> N K V Y H D L N R H V L E H G H K K E D R T G T G T L S 3201 ATTTGATCGGGAGATGTCATGAAGGTCTATCACGACTTGATGCGCCACGTACGGGCACGCAGGAAGACGCCGTACCGGGCACCGTACCGGCACCTTAT V F G Y Q N R F D L A E G F P L L T T K K V H L K S I I H E L L W 3301 CAGTGTTGGCTACCAGATGCGTTTGGCCGAGGGCTTCCCGCTACTACCAGCAGGGGGCACCTGAAATCCATCATCATGAGTGCTTTG F L Q 3401 GTTCCTGCAG

FIG. 3. Nucleotide sequence of the 3.4-kb *SmaI-PstI* fragment of *M. glycogenes* ATCC 21371. A stem-loop structure is indicated by arrows.



FIG. 4. Putative secondary structures of mRNA in the intergenic regions of the *hom* and *thrC* genes from *M. glycogenes* ATCC 21276 (A) and ATCC 21371 (B). The stabilities of the stem-loop structures of ATCC 21276 and ATCC 21371 are calculated as ΔGs of -17.8 and -19.6 kcal (1 cal = 4.184 J), respectively, according to the method of Tinoco et al. (22).

sequence of the *thyA* gene of the strain (data not shown). A promoterlike sequence was not found in the upstream regions of the *hom*, *thrC*, and *thyA* genes.

The formation of stem-loop structures is possible in the mRNAs between the hom and thrC genes of ATCC 21276 and ATCC 21371 ($\Delta G = -17.8$ and -19.6 kcal [1 cal = 4.184 J], respectively) (Fig. 4). They may play roles in regulating the expression of the hom and thrC genes or stabilizing the transcripts. Similar structures of mRNA were reported in the intergenic regions between the hom and thrC genes from M. flagellatum (13), between the same genes from P. aeruginosa (5), and between the arcA/arcB and arcB/arcC genes of P. aeruginosa (3). The structure between the arcA/arcB and arcB/arcC genes of mRNA against 3'-exonuclease degradation.

Northern blot analysis. Northern blots of mRNAs were made to examine whether the *hom* and *thrC* genes of *M. glycogenes* are organized in an operon (Fig. 5). RNAs extracted from *M. glycogenes* ATCC 21276 and ATCC 21371 were separated by 1.2% agarose gel electrophoresis and hybridized with probes prepared from a 555-bp fragment internal to the *hom* gene (bp 495 to 1049) or a 541-bp fragment internal to the *thrC* gene (bp 1805 to 2345) of *M. glycogenes* ATCC 21371. The probes could hybridize not



FIG. 5. Northern blots of mRNA transcribed from the *hom-thrC* region. The RNAs extracted from *M. glycogenes* ATCC 21276 (lanes 1, 2, 5, and 6) and ATCC 21371 (lanes 3, 4, 7, and 8) were hybridized with probes prepared from a 555-bp DNA fragment internal to the *thrC* gene in lanes 1 to 4 and a 540-bp DNA fragment internal to the *hom* gene in lanes 5 to 8, respectively. The amounts of the RNAs used were 5 (lanes 3, 5, and 7), 10 (lanes 1, 4, 6, and 8), and 22.5 μ g (lane 2). The positions of size markers are indicated on the right side.

only the RNA from ATCC 21371 but also the RNA from ATCC 21276, although the intensities of the bands in the latter samples were weaker than those in the former ones, and the sizes of the bands detected by each probe were similar in the two samples.

The approximately 2.4-kb mRNAs were hybridized with both probes, suggesting that the *hom* and *thrC* genes are organized in an operon, as in *M. flagellatum* (13) and *P. aeruginosa* (5). Shorter bands (ca. 1.4 kb) detected by each probe were thought to be digested or processed products of the 2.4-kb mRNAs, but another explanation cannot be excluded. That is, the transcription of the *hom* genes could terminate at the intergenic regions of the *hom* and *thrC* genes, and the transcription of the *thrC* genes could initiate in the intergenic region, although promoterlike sequences were not found upstream of the *thrC* genes. Further studies are necessary to give a sufficient explanation of the results.

Comparison of the predicted amino acid sequences of the *hom* genes. The predicted amino sequences of the *hom* genes of *M. glycogenes* were compared with those of other microorganisms (Fig. 6). Those of the two methylotrophs were highly homologous; that is, 47.4% (206 of 435 amino acid residues) of the amino acid sequence of *M. glycogenes* ATCC 21276 is identical to that from ATCC 21371. Comparison of the sequence of ATCC 21371 with those of *P. aeruginosa*, *B. subtilis*, *C. glutamicum*, *E. coli* HD I, and *E. coli* HD II revealed identities of 49.0% (215 of 439 amino acid residues), 31.9% (138 of 433 amino acid residues), 31.5% (140 of 445 amino acid residues), 18.4% (66 of 358 amino acid residues), and 17.3% (62 of 358 amino acid residues), respectively.

E. coli HD I and HD II are less homologous to the *M. glycogenes hom* gene products than other HDs are. *E. coli* HD I and HD II are bifunctional proteins of AK-HD, and others are monofunctional HDs. This bifunctional HD, only found in members of the family *Enterobacteriaceae*, is thought to have evolved in a way different from those of other monofunctional HDs (18). The structural and evolutionary differences explain the differences in homology. The high degree of homology between the *M. glycogenes hom* gene product and other monofunctional *hom* gene products suggests that they have a common ancestor.

Several well-conserved domains were found in the amino acid sequences that were compared. The amino acid sequence motif at positions 10 to 15, G-X-G-X-X-G, sur-

		:::**	
M.G. IHD	(1)	MKPINVGLLGIGTVGGGTVTVLTRNGEGN-RAPCRPPNCHHARROR	(45)
M.G. THD	(1)	MKPINVGLLGIGTVGGGTYTVLTRNCEEIARRAGRPIAITRVA-DR	(45)
P.A.	(6)	VKPVKVGICGLGPSVAVPSMYSNATPRRLPAVPGRGIEVAQIA-AR	(50)
B. S.	(1)	MKA IRVGLLGLGTVGSGVVKI I QDHQDKLMHQVGCPVT I KKVLVK	(45)
C.G.	(16)	GSAVG I ALLEF GTVG TEVMRLMTEYGDELAHR I GGPLEVRG I AVS	(60)
E.C.HD I	(463)	DOV IEVEV I GVGGVGGALLEQEKROOSWLKNKH-I DLRVCGVANSKALLTNVHGLNLENW	(521)
E.C.HDII	(452)	EKRIGLVEFCKONICSRWELLFARECSTESARTGFEFVLAGVVDSRRSELSYDGEDASRA	(511)
		:	
M.G. IHD	(46)	NLELARKYTGGOIDYTDDAFAVYVRPR-HRHR-GRIDRRIHHRARTGYEGDRERQARVAA	(103)
M.G. THD	(46)	NLELAROVTGGK I DVTDDAFA I VSDPA I DI VV-ELIGGY TVARELVLKA I EN-GKHVVTA	(103)
P.A.	(51)	RPN-PKCDTGAT-PITADIFDVACNPEIDVVV-ELIGGPPWPIELVLKAIEN-GKHVVTA	(106)
B.S.	(46)	DLEKKREVDLPKEVLTTEVYDVIDDPDVDVVI-EVIGGVEQTKQYLVDALRSK-KHWYTA	(118)
C.G.	(61)	DISKPREGVAPE-LLTEDAFALIEREDVDIVY-EVIGGIEYPREVYLAALKA-GKSYVTA	(117)
E.C.HD I	(522)	QEELMQAKEPFN-LGRL IRLVKEYHLLNPV I WNCTSSQAVADQYADFLRE-GF#WWEP	(577)
E.C.HDII	(512)	LAFFNDEAVEAVEQDEESLFLWMRAHPYDDLVVLDVTASQQLADQYUDFASH-GFHWISA	(570)
		** : *: *	
M.G. IHD	(104)	NKALMLAWORDFRRVAQOKGVIVAFEAAVAGGIPIIKAVAAGAGROPSSRVLRAI	(158)
M.G. THD	(104)	NKALIACMAMKFLPLRRKKASSSLLKLPLLVVSPLFKANREGL-AANRIENTAGI	(157)
P.A.	(107)	NKALIAVHGSRDER-QGPREGRDRAFEAAVAGGIPVIKAIREGE-SANRINNEAGI	(160)
B.S.	(104)	NKDEMAVYGSELEA-EAKENGCDIYFEASVAGGI?ILRTLEEGE-SSDRITKMAGI	(157)
C.G.	(118)	NKALVAAHSAELAD-AAEAANVDLYFEAAVAGA I IVVGPLINRSL-AGDQIQSVMGI	(181)
E.C.HD I	(578)	NEKANTSSMDYYHOLBYAAEKSRRKFLYD INVGAGLPV I ENLONLLNAGDELMKFSGI	(635)
E.C.HDII	(571)	NKLAGASDSNKYRQIHDAFEKTGRHWLYNATVGAGLPINHTVRDLIDSGDTILSISGI	(625)
		*: : * * ***	
M.G. IHD	(159)	INGTINE ILSEMREKSE AFADVLKEA-OPPL-C-EA-TRPSTSRAW-TANKLMILA-AIG	(212)
M.G. THD	(158)	INGTINFILSEMREKGLAFADVLKEA-Q-RLGYAEA-DP1FDVEG1DAAHKLMILA-AN-	(212)
P.A.	(161)	INGTONE ILSEMREKORTEPDVLAEA-O-ALGYGRGRSPIEDVEGIDAATKLITILA-SN-	(216)
B.S.	(158)	VINGTITINE IL TKIHI KEKSPYEEVLIKEA-Q-DLGFAEA-DPISDVEGLDAARIMATLARL-G	(213)
C.G.	(182)	VINGTINFILDANDSTGADYADSLAEATREGYAEA-DPIADVEGHDAASKAAILA-SIA	(227)
E.C.HD I	(636)	LSESLSY IFGKLDE-EMSESEATRLA-REM-EYTEP-DERDDLSEMEVARKLLILARETE	(691)
E.C.HDII	(626)	FSGILSWLFLQF-DGSVPETELVDQAWQQGLTEP-DPRDDLSGKOVSRELVIEAREAG	(684)
	(040)		(050)
M.G. IHD	(213)	F-GIPMUEDKATVESESKEUALDERYAEELGYKUAAWHAPSAPAR	(256)
M.G. HD	(213)		(232)
r.A.	(21/)	-LINIPEQUEURATIESESKE I SABVINTAUALGTRI KHLGVAR	(250)
8.5.	(214)	FSMNVDLEDVKV-RGBSUIIDEBBSSSSKRLGYIMKLIGI	(251)
C.G.	(228)	HIRVIADUVY-GEBERSNISAAREELAAQUAGHIIKLLAIKLLAI	(205)
E.C.HU	(692)	KELELAUIE-IEPVLPAEFNAEGUVAAFMANLSQLUULFAARVAKARDEGKVLRYVGN	(/49)
E.C.HDII	(685)	YN I EPUUVRVESLVPAHCEG-GSIDHFFENGDELNEUMVORLEAAREMGLVLRYVAR	(/40)

		•	
M.G. IHD	(257)	GVEL-ARA-POPDPRETPDROCORRHNAVLVS-DAC-RSHLV-YRPDACRR	(303)
M.G. THD	(233)	TKR-TDKGVELRVHPTLIPEKRLICOCEWRNECCAGOGRCCWPT-LY-YGAGAGAE	(285)
P. A.	(257)	R-TESGEELRVHPTLIPSDRLIANVTAVMNAVMVNGDAVGST-LY-YGAGAGME	(307)
B. S.	(252)	AORDGSKIEVSYOPTEL POHHPLSAVHNEFNAVYVYGEAVGETMFYGPGAGSM	(304)
C. G.	(266)	CEKFTNKEGKSA I SARVHPTEL PVSHPLASVNKSENA I EVEAEAAGRLMEYGNGAGGA	(323)
F.C.HD L	(750)	IDEDGVCRVK LAEVDGNDPI EKVKNGENALAEVSHYYOPI PLVI RGYGAGND	(800)
F C HDIL	(741)	EDANGKARVOWFAVREDHPLRSLLPCDNVFALESRWYRDNPLVLRGPGAGRD	(792)
	(141)		(102)
		* * ** * * *	
M G IHD	(304)	H-A-SAVVADI WYSTRTAYHRRAPAVENE I SSETSSWTCREA- IGEVSSAVVI DE RAVIDE	(359)
M G THD	(286)	PTA-SAY-ARE VOCTOOD IS	(338)
P &	(308)	PTASSVV-ARI VRVVRANTSOPENRVPH AFOPIAL SOHPLE PLEACESAVVL RIOAKOH	(366)
R C	(305)	PTATEVNS-DEVAMINED CVTCNSEVC-DOVEKNAK-SPSDIVACOFEDIHVKDE	(358)
0.0.	(324)	DELANDER OF A CONTRACT AND A CONTRACT AN	(376)
5 C UDI	(901)		(820)
	(702)		(800)
E. 0. NUTT	(193)		(003)
		• • • • • •	
	(360)	BODDCLARBEAT	(417)
M.G. THO	(330)		(304)
D A 110	(267)	DIVERSITIES DO INTERNATION DE LE CONCLUE VILLE CONTRACTORIS CONTRACTOR	(422)
F.A.	(250)	VACEOVITEOLOGIAN	(422)
0.0.	(009)		(414)
6.6.	(3//)	VORTACEAGLEGEUGIGLETINGEERDUURELEVVERGALEGULGETVELLKA	(429)
		• •••	
M.G. IHD	(418)	EPAISSSYTTE TAFFE SP	(435)
M.G. THD	(395)	EPALSCRVTRI PAFELSR	(412)
PA	(423)	EFGVSCPWVRI EVFOIEN	(439)
RS	(415)	BEVVOEVKSTVEVERMOWS	(433)
0.0.	(430)		(445)
	1 100/		
0.4.			

FIG. 6. Homology between the amino acid sequence of the HDs of *M. glycogenes* ATCC 21371 and ATCC 21276 and those of other microorganisms. Shaded letters and boldface letters show amino acid residues identical to those of *M. glycogenes* ATCC 21371 and *M. glycogenes* ATCC 21276, respectively. Conserved residues in all of the amino acid sequences are noted with asterisks, and homologous residues (I-L-V-M, D-E, R-K, S-T, F-Y) are noted with colons. Abbreviations: M.G., *M. glycogenes*; P.A., *P. aeruginosa*; B.S., *B. subtilis*; C.G., *C. glutamicum*; E.C., *E. coli*.

rounded by small hydrophobic amino acid residues, which is characteristic of the NAD(P) binding site of HDs (18, 20), is found in the N-terminal region. Like other HDs, NAD, the cofactor of the enzyme, should bind this region in M. glycogenes.

Parsot and Cohen (18) showed that the amino acid sequence between positions 200 and 221 of *B. subtilis* was well conserved in *E. coli* AK I-HD I and AK II-HD II counterparts and suggested that this region could be involved in substrate binding or could be a catalytic region. The amino acid sequence motif K-X-X-I-L-A in this region is conserved in all of the sequences compared, suggesting the importance of this region in substrate binding or catalytic activity.

The regulatory domains involved in feedback inhibition by L-threonine were postulated to be present in the C-terminal regions in *B. subtilis* (18), *C. glutamicum* (2), and *M. flagellatum* (13). The C-terminal regions of the *M. glycogenes* HDs show some homology with those of other HDs. Alteration in this region of the *M. glycogenes hom* genes by site-directed mutagenesis will provide useful information with which to investigate the regulatory domains concerned with inhibition by L-threonine, L-phenylalanine, and L-methionine.

Comparison of the predicted amino acid sequences of *thrC* **genes.** The predicted amino acid sequences of the *thrC* genes of the *M. glycogenes* strains were compared with those of other microorganisms (Fig. 7). The N-terminal peptides of two *M. glycogenes* strains are quite homologous; that is, 88

of 89 amino acid residues are identical. Comparison of the sequence of ATCC 21371 with those of *P. aeruginosa*, *B. subtilis*, *C. glutamicum*, *Serratia marcescens*, *E. coli*, and *Saccharomyces cerevisiae* showed identities of 40.3% (197 of 489 amino acid residues), 39.2% (138 of 352 amino acid residues), 29.1% (140 of 481 amino acid residues), 32.4% (139 of 429 amino acid residues), 34.3% (147 of 428 amino acid residues), and 32.5% (167 of 514 amino acid residues), respectively.

The homology observed throughout the amino acid sequences suggests that the structure of the catalytic mechanism of the threonine synthase of M. glycogenes is similar to those of the threonine synthases of other microorganisms (17). Threonine synthase is a pyridoxal phosphate enzyme. Parsot (17) found significant homology between the amino acid sequences of threonine synthase from E. coli and B. subtilis and those of other pyridoxal phosphate enzymes, such as threonine dehydratase in S. cerevisiae and serine dehydratase in E. coli. He suggested that, prior to the separation of those organisms, an ancestral microorganism had a pyridoxal phosphate enzyme with a wide substrate specificity, and subsequent mutation led it to acquire its current substrate specificity. The high degree of homology between the threonine synthase of M. glycogenes and those of other microorganisms supports his idea. The motif at positions 115 to 121, P-T-X-X-F-K-D, is well conserved in all of the sequences. Pyridoxal phosphate, the coenzyme for synthase reaction, may bind the lysine residue

118 MOTOYAMA ET AL.

M.G. I (1) MKYTSTRGOSE-ALS-FEETLEGGUAPPOGLYDEGDYDGESALALSANG-MNYRELAFALS (60) M.G. II (1) MKYTSTRGOSE-ALS-FEETLEGGUAPPOGLYDEGDYDGESODELNAMRG-MNYRELAFALS (60) C.A. (20) MKYTSTRGOSE-ALS-FEETLEGGUAPPOGLYDEGDYDGESODELNAMRG-MNYRELAFALS (60) C.G. (1) MKLYMLKOHNEOY-S-EAOAIXOGUGKXOOGEFFELDLEFELTEIDHLLE-ODFVTRSSRHS (60) C.G. (1) VOYEENDAGR-TPARTEGDUELGGUAPPOGLYDEPLDATEFILE LASVNG-LYMELEAFANK (67) E.C. (8) -VKSTRSSSEKTIS-EEEAIIOGUAPPOGLYDEPLDATEFUDJOADUSKMRE-VLANE-GYAAAA (60) F.C. (8) -VKSTRSSSEKTIS-EEEAIIOGUAPPOGLYDEPLDATEFUDJOADUSKKRE-VLANE-GYAAAA (60) M.G. II (61) MEIDOIFADDUERINDECHYRADMYAYARPGOAEDIETETYKEEDDUELSUSNEETLAFKD (121) M.G. II (61) BEIDOIFADDUERINDECHYRADMYAYARPGOAEDIETETYKEEDDUK-LDFVTRSAKILS (60) F.A. (80) PFVAG-SUADAFFFRA-GYAAAPPARAFARARP- P.A. (80) PFVAG-SUADAFFKRELEEW-G-WFAHDASGAA&P-VER-R-TNG-CV-EEFHGPELAEKD (133) S.M. (61) AFE-GEVERETAKKRELEEW-G-WFAHDASGAA&P-VER-R-TNG-CV-EEFHGPELAEKD (119) S.C. (68) LYAOEEIADAGKKKELEEW-G-WFAHDASGAA&P-VER-MINDOVSCUEFHOREKAEKD (110) S.C. (61) REV-ISLFVDBIPVEDIAITAANAFFRADEVVANVESDUVCEELFHGPELAEKD (110) S.C. (61) AFE-GEVERETAKKREUEW-G-WFAHDASGAA&P-VER-MINDOVSCUEFHOREKAEKD (110) S.C. (61) AFE-GEVERETAKKREUEW-G-WFAHDASGAA&P-VER-R-TNG-CV-EEFHGPELAEKD (110) S.C. (61) AFE-GEVERETAKKREUEW-G-WFAHDASGAA&P-VER-R-TNG-CV-EEFHGPELAEKD (110) S.C. (61) AFE-GEVERETAKKREUEW-G-WFAHDASGAA&FFA 		: * : ** **: * * : : :	
M.G. I (12) MARVESTBOSE-ALS-ETERETICS/GROBOLYLPEDYRDESODELWAMRG-DNYRDEWFARLS (50) P.A. (20) MRYLSTROAR-REN-EEUVELANGL/SDGELY/DEAL RAFELE I ANVG-LPYNELEFARLS (50) P.A. (20) MRYLSTROAR-REN-EEUVELANGL/SDGELY/DEAL RAFELE I DOLLO-DOVTRSSRUES (50) C.G. (1) VURESTBOAR-TPAREDULLAGU/SDGELYDEAL REFELTE IDTLLE-OPVTRSSRUES (50) C.G. (1) VURESTBOAR-TPAREDULLAGU/SDGELYDEAL REFELTE IDTLLE-OPVTRSSRUES (50) C.G. (1) WRLTNLKONNEOV-B-EAOAVTOGUGAPDRAEDYDEAT THDU DDAQUSKMRE-VLANE-GYAAEA (50) S.C. (8) VRSTRSSSRVT 15-EEEA I IOULATDOGUF I PUT IEUVOATE/HOWSKLSFODELANIN (50) E.C. (1) WRLTNLKONNEOV-B-EAOAVTOGUGAPDRAEDYDEAT THDU DDAQUSKMRE-VLANE-GYAAEA (50) S.C. (8) VRSTRSSSRVT 15-EEEA I IOULATDOGUF I PUT IEUVOATE/HOWSKLSFODELANIN (50) F.A. (80) PFVAG-SUADADFKKILEEW-G-WFRHOASGAAP-VERR-TNG-CV-ELFHORPELAEKD (121) S.M. (61) AFE-GEVVETAUK/ROAFEFPAWRANATARAR- (89) P.A. (80) PFVAG-SUADADFKKILEEW-G-WFRHOASGAAP-VERR-TNG-CV-ELFHORPELAEKD (103) S.G. (61) BEVISLFVDD IPVEDIKAITARATTY-PKFNSDEVTPUTEUEDIN WEGHESEGETAAFKD (112) S.C. (63) LYLAGEEHPADBIEKDLIKRSYSTRSDEVTPLVONVTGD-K-E-NLHIEELFHRETWAFKD (125) S.C. (61) AFE-GDEIFOZIENERARDAFAFAAWAAWFDSDVGVEHTEGVENTEGSFKD (103) B.S. (13) -EPVTDOTPAL TEHEGNTPL IHLPKLSEQ	M.G. I (1)	MKYISTRGOSP-ALS-FSEILLGGLAPDGGLYLPEOYPOFSADALSAMRG-MNYRDLAFTILS	(60)
P.A. (20) MRXISTRODAR-REN-ECUVELANDASDOGEFVEENLENERTLEE LASWYG-LPWHELMERVAR (73) S.M. (1) MRLYNLKONNEON-B-EADAIKOGLEKOOGEFFELDLERELTEIDLECOVTRESKIS (60) S.G. (3) VOXETENDAR-TPARESEDILGGLEKOOGEFFELDLERELTEIDLAGUSKIMPACHANDAGLAKIS (60) S.C. (6) -WRSTRSSERKTIS-EEEAIIOGUATDOGUEKADAYEDADAUSKIMPA-LANE-GYAALEA (60) S.C. (6) -WRSTRSSERKTIS-EEEAIIOGUATDOGUEKADAYEDADAUSKIMPA-LANE-GYAALEA (60) S.C. (6) -WRSTRSSERKTIS-EEEAIIOGUATDOGUEKADAYAAARADE (73) E.C. (1) WEL-DDUFFADDUERIEVADAYAAARDEGOAEDUETEYKEEDDEWELSUSAGEPLAAKKUL (121) (80) M.G. II (61) BEEDDUFFADDUERIEVADAYAAARDEGOAEDUETEYKEEDDEWELSUSAGEPLAAEKKU (121) (80) S.M. (61) AFE-ODUFFADDUERIEVADAYAAARDEGOAEDUETYKEEDDEWELSUSAGEPLAAEKKU (121) (80) S.M. (61) REVISL-VDBI PVEDIAITRAAKTA-EFFA	M.G. II (1)	MKYVSTBGOSE-ALS-ETEHLIGGIGPORGLYLPEOYPOERODELNAMBG-UNVRDLAFALLS	(60)
S.M. C 13 MRLYNLKDHNEQV-8-EAQATKQGEGKOGGEFFELDLEEDHLE-OOFVTRSSRNES C 60 S.G. C 13 VURBEREDAR-TPARESDIELGGLAPDOGENERET RYTENDLDAQUSKME-VLANE-GYAALAR C 60 S.C. C 80 -VERSTENDAR-TPARESDIELGGLAPDOGENERET RYTENDLDAQUSKME-VLANE-GYAALAR C 60 S.C. C 80 -VERSTENDAR-TPARESDIELGGLAPDOGENERET RYTENDLDAQUSKME-VLANE-GOALAFORKENERET (21) C 60 M.G. I (61) BEEDOMEADDUER INDERTWRANTATAREGOAEDHTET YKEEDDUELESUNGPTEAFKD (121) M.G. I (61) BEEDOMEADDUER INDERTWRANTATARE- PA. C 80 PFVAG-SKADADFKKILEERE'-G-WFRANKATARE- PA. C 80 C 80 PFVAG-SKADADFKKILEERE'-G-WFRANKATARE- PA. C 80 C 80 <td< td=""><th>P.A. (20)</th><td>MRY ISTRODAR-ALN-EEDVILLAG ASDIGLY VRENI PRETLEF LASWVG-LPYHELLERVUR</td><td>(70)</td></td<>	P.A. (20)	MRY ISTRODAR-ALN-EEDVILLAG ASDIGLY VRENI PRETLEF LASWVG-LPYHELLERVUR	(70)
C.G. (1) VOYBSTROASR-TP ARESOTELGGLAPHOREUTLATYREDUBLIC OUT INCLUSION (CONTACT) (60) S.C. (8)	SM (1)	WE YNI KOHNFOV-R-FAOALKOZECKOOZEFEDI DI DEELT TOULLE CONTOCODER	(60)
3 C 1 20120000000000000000000000000000000000		VDVIETDDASD_TDADESDTILIOSTICADIOCONTELECTICADES DDAOLOUWDE_VI ANE_OVAARA	(00)
3 C 0.0 ************************************		- VDERMINONON TO ANE OD RECOOLS FROM BLEE A TREAS DO AVEN MISE TO ANE A TREAS TO AND A TREAS TO	
LL. LL. <td< td=""><th></th><td>TRADROODER 15 TECER USER IRREF TET 150 VUQA LETNUNSKLSFUBEREAINK</td><td>(6/)</td></td<>		TRADROODER 15 TECER USER IRREF TET 150 VUQA LETNUNSKLSFUBEREAINK	(6/)
## ## M.G. I (61) HEIDDIFADDUE I NUDETYRADIVATARENCOAEDUET YKEEDDEVELSU.SNGPTEAFKD (12) M.G. II (61) REIDDIFADDUE I NUDETYRADIVATARENCOAEDUET YKEEDDEVELSU.SNGPTEAFKD (12) P.A. (80) PFVAG-SKADADFKKILEENY-G-WFANDXATARENCOAEDUET YKEEDDEVELSU.SNGPTEAFKD (13) S.M. (61) AFE-GEEVBETAKKRYCAAFEFA	E.G. ())	MART MERCHINE UV-D-RAUAVIUGEGRAUGEFFEMULKEESELIEIDEMER-LUFVIRSAKIES	(60)
#** #** M.G. I (61) REFDDIFADDUE I EVDETYRADAYTAKAREGOAEDUTET YKEEDDEVEUSLSNGETEAFKD (121) M.G. II (61) REFDDIFADDUE AEVDETYRADAYTAKAREGOAEDUTET YKEEDDEVEUSLSNGETEAFKD (121) M.G. II (61) REFDDIFADDUE AEVDETYRADAYTAKAREG- P.A. (80) PFVAG-SKADADEKKELEEN -G-WFRIDASSAAP-VERR-TNG-CV-EUFHBETEAFKD (133) S.M. (61) AFI-GESVETAISKKENDAFEFA S.M. (61) AFI-GESVETAISKKENDAFFFA S.C. (63) REVISLFVDD I PVEDIKAI TARAYTYBKFNSEDTVEVTELEDNI NEGHESEGETAAFKD (125) S.C. (63) LTAOEETRDABEKOLI KNSYSTFRODEVTELVONTGO-K-E-HLHI EEFHBETEAFKD (125) S.C. (63) AFI-GOENOEI EEERFRAAFFAPA			
M.G. I (61) BEIDOIPADDIE I BUDETYRAMYATARE COLEDITET YKEEDDEVELSUSNEREEFAFKD (121) M.G. II (61) BEIDOIPADDIE I BUDETYRAMYATAREP M.G. II (61) BEIDOIPADDIE I BUDETONERAMYATAREP P.A. (80) PFVAG-SILADADFKKILEE BIY-G-WFRHDASGAAAP-VERR-TNG-CV-ELFHGERIEAGEKD (133) S.M. (61) AFI-GELVIETADIEKANING ANAREFAR		** ***	
M. G. II (61) BEEL-DEFENDIOR ABDDECHYARMYATARE- (83) (80) P.A. (80) PFVAG-SEADADEFKKELEEW-G-WFRHDASCAABP-VERR-TNG-CV-EEFHGPEREAEKD (113) S.M. (61) AFE-GEEVVETALKKRUCAAFEFA	M.G. (61)	RLIDDIPADDLB I IVDKTYRADYYAYARPGQDAEDITPTYKLEDDLYLLSLSNGPTLAFKD	(121)
P.A. (80) PEVAG-SUADABKKUELEEWY-G-WFAHDASGAABP-VERP-TNG-CV-EEHPBEREAEKD (133) S.M. (61) AFE-GEVINETALKKRUDAAFEFPA	M.G.II (61)	RUI DEI PADOL RAINDRTYRADWY AYARP-	(89)
S.M. C 613 AFE-GEEVEETAEKKRWOAAFEFPA	P.A. (80)	PFVAG-S#ADADFKK#LEE##-G-#F#HDASGAA#P-VERR-TNG-CV-EEFHORTEAFKD	(133)
C.G. C 613 REVISLFVDD PVED IKALTARAATTEKFNSEDTVEVTELEDN INLGHESEGENAAFKD (119) S.C. C 613 REVISLFVDD PVED IKALTARAATTAEKFNSEDTVEVTELEDN INLGHESEGENAAFKD (119) S.C. C 613 AFE-GOLERTBAABKKOLI KRSYSTFRODEVTPLVONFGO-K-E-NLHI ELETHBERIJKARKO (102) B.S. C 133 -LEVTDOTPALTEHEGNTPLIHLPKLSEQ	S.M. (61)	AFT-GEEVEETAEKKRYQAAFEFPA	(108)
S.C. C 683 LYTAQEETPDADEKOLIKRSYSTFRSDEVTPLVONVTGD-K-E-NLIHLEGET-HEPSTYAERO (125) E.C. C 610 AFT-GDELROE IBEERVRAAFAFPA	C.G. (61)	REVISLEVINIPVEDIKALTARAYTYPKENSEDIVPVTELEDNIVEGHESEGPTAJEKN	(110)
0.0.0.1 C 000 C 0000 C 000 C 0000	S.C. (68)	I VI ANEFIDDADEKNI I KOSKSTEPSDEVTDI VONVTOD.K.E.NI U LEE EUROTVAEKO	(105)
L.U. C 01) AR SOULARDC INSCRIPTION PALTERCE INSCRIPTION PARAMETERS WOLLELPHOREMARKAND (108) B.S. C 13) -EPVTIOTRAL TEHEGATPLI HIL PKLSE0	E C (61)		(120)
B.3. (13) ************************************	DC (12)		(100)
* *::** ::: * *::** ::: M.G. I (122) MARGEERAN-EERENVEAQKGETTHI-E-GATSGDTGSAAEKAARGKOGVAVENNES (172) P.A. (134) FALCHERR-ELDHYEAQKGETTHI-E-GATSGDTGSAAEKAARGKOGVAVENNES (172) P.A. (134) FALCHERR-ELDHYEAQKGERTVIMARSGDTGSAAEKAARGKOGVAVENNES (172) P.A. (134) FALCHERR-ELDHYEAQKGERTVIMARSGDTGSAAENVARGKOGVAVENNES (172) Q.G. (120) MARGEESEEFEVEERRNETINI-E-TATSGDTGSAENVARGREGIPVENUE (157) (170) S.C. (126) VALOFVAN-LEFENFEORTNANLPEGEKKOITVVGATSGDTGSAAEPNGKUVUE (157) (157) B.S. (61) RGMTV-MAVAKAKEEGNDTITASGDTGSAAAYAARANNKCIVII (105) (173) # :::::***:::::::**:: **:: **:: **:: (173) PA. (165) PENRYSEVERCONTINENSAAKGVDOCODVMANSNDHAEMANNAENAAANA-WAARANMKCIVII (105) (173) PHOOKSREDTAOMESLODONTIENAAKKONDOCOLKAAANNAKAAAA-WAARANMKCIVII (105) * :::::**** ::::**** ::::**** **::**:* **::* (247) S.M. (168) POGKISPLOE-LLEGONTHINIAIL DEVEDDCODVKAANSNDHAEMANNAKAAWASINMARI (247) (247) (247) S.G. (171) PAGRUTPECOACMEELDONTIENTAAILOBEDAALYKOAF	D.0. (13)		(60)
M.G. I I IIII IIIIIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII			·
M.G. I (122) MARGELEME LEREVIACAKGERTINI-L-GAISGDIGSAAEKAARGKOGKVARMINGS (172) P.A. (134) FALLENER-LLOHVERAKGERTINI-L-GAISGDIGSAAEKAARGKOGKVARMISS (172) S.M. (109) FGGRFMAOMEAEVAGEQPVTI-L-TAISGDIGAVAHAFKGLNWDIFILD (184) S.M. (109) FGGRFMAOMEAEVAGEQPVTI-L-TAISGDIGAVAHAFKGLNWDIFILD (184) S.C. (120) MARGELEG-LEREVERARPVTI-L-TAISGDIGAVAHAFKGLNWDIFILD (184) S.C. (120) VALGFVGH-LEREVERARPVTI-L-TAISGDIGAVAHAFKGLNWRKWILY (184) E.C. (109) FGGRFMAOMETHIAGDKPVTI-L-TAISGDIGAVAHAFKGLNWKKVILY (184) E.C. (109) FGGRFMAOMETHIAGDKPVTI-L-TAISGDIGAVAHAFKGLNWKKVILY (184) E.C. (109) FGGRFMAOMETHIAGDKNTILKASTGNT-SAMAAA-WAARANMKCIVII (105) * :: : : : : : : : : : : : : : : : : :		* *::* * : ::	
P.A. (134) FALUERER-LLDHVERAKRBERVVIMAATSGDTGSKA EAAAVADNVD [110] (184) S.M. (109) FGGFRANDALEVAGED	M.G. I (122)	MANGELIGH-EFERVEAQKGETTNI-E-GATSGDIGSAAEYAMRGKOGVKVFMES	(172)
S.M. (109) FGGRFMAQMLAEVAGEDPVTI-E-TAISBODTGAWAHAFGGLKNNFWRITUF (157) C.G. (120) MANDELBE-EFEWELBRRNEININE-GATSBODTGASAEKANFGREBIRVENET (170) S.C. (126) VALGFVAN-EFEWFEORTNANLPEGEKKOITVVBATSBODTGASAEKANFGREBIRVENET (170) S.C. (120) MANDELBE-EFEWELBRRNDTI-E-TAISBODTGASAIKORKKONSVETILY (181) E.C. (109) FGGRFMAQMETHIADV PTI-E-TAISBODTGAAVAHAFYGLPNVKVVILY (157) B.S. (61) RGIN-MAVAKAKEEGNDTIKASSEDNE-SAAAAA-XAARAANMKCIVII (105) * : : : : ***** M.G. [173) BHOKINSREDTAGNESCODDNENIAWKOVEDDCODDIVKANSNDHAEKAKNKBAAN-MKCIVII (202) P.A. (185) PENNYSEVERRONTINICONENIAWKOVEDDCODDIVKANSNDHAEKAKNKBAAN-MKCIVII (247) S.C. (185) PENNYSEVERRONTINCONENIAMKAI EGDACHVKANSNDHAEKAKNKBAANSIBINARAI (247) S.C. (165) POGKISPLOE-KLECTLGONHTENTAILGEVEDDCODVKAANSADAEEKKONRIGAVNSINNARI (247) S.C. (165) PRGRINFEODAADMELDUBLWEDDTILSVITENCODIFALIKONKAI FLOKKENNSINNARI (247) S.C. (165)	P.A. (134)	FALOLOGR-LLDHVLAKRGERVVIMGATSGDTGSAAIEAAAVADNVDIEIID	(184)
C.G. (120) MARGELSE - EFEEVFEORTNET INT-E-GATSBDTGSSAETAWRGREGIE IVENILT (170) S.C. (126) VALOF VGN-EFEEVFEORTNANLPEGEKKOLTVVBATSBDTGSSAETAWRGREGIE IVENILT (170) S.C. (126) VALOF VGN-EFEEVFEORTNANLPEGEKKOLTVVBATSBDTGSSAEPKUKKVSVEFU (184) FGGRTMACMEITH LADK	S.M. (109)	FGGRFMAQMEAEVAGEDPVT1-L-TATSGRITGAAVAHAFYGLKNVRVVILY	(157)
S.C. (126) YALGF VØN-LEFERFEORTNANLPEGEKKOLTVVGATSEDDTBGARA IYGLIRGKKOVSVEFILY (184) E.C. (109) FGGRFMAOME THI AGDKPVTÄ-E-TATSEDDTBGAV AHAFSGLYGLIRGKKOVSVEFILY (157) B.S. (61) RGMV-MAVAKAKEEGPVTÄ-E-TATSEDDTBGAV AHAFSGLNKKVVILY (157) B.S. (61) RGMV-MAVAKAKEEGPVTÄ-E-TATSEDDTBGAV AHAFSGLNKKVVILY (157) B.S. (61) RGMV-MAVAKAKEEGNTÄ-E-AKSEDDTGAVAHAFSGLNKKVVILY (150) * ::::::::::::::::::::::::::::::::::::	C.G. (120)	MANGULGE-LEEYELRRRNET INI-L-GATSGDTGSSAEYAMRGREG I RVFML T	(170)
E.C. (109) FGGRFMAOME THIAGDKPVTI-E-TATSGDTGAAVAHAFKGLPNVKVVILY (157) B.S. (61) RGMV-MAVAKAKEEGNDTIMCASTGNT-SKAAAA-KAAA-WAARANMKCIVII (105) * :: : : : : : : : : : : : : : : : : :	S.C. (126)	VALOFVEN-LITEXFLORTNANLPEGEKKOITVVEATSEDTESKAIYGLRGKKDVSVEILY	(184)
B.S. C 611 RGHV-MAVAKAKEEGNDTHKCASTGNT-SAKAAA-YAARANMKCIVII (105) * ::::::::::::::::::::::::::::::::::::	E.C. (109)	FGGRFMAOMETHIAGDKPVT1-E-TATSCOTGAAVAHAF¥GLPNVKVVILY	(157)
* ::: *: *: *: *: *: *: *: *: *: *: *: *	B.S. (61)	RGMV-MAVAKAKEEGNDTIMCAST(NI-SAAAAA-YAARANMRCIVII	(105)
* ::: : : : : : : : : : : : : : : : : :			(,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
M.G. I (173) PHOKMSREDTACMESLODDNIENIAVKOVEDDCODEVKANSNDHAEKAKNKGAAVNSINWAAV (235) P.A. (185) PENRVSEVERROMTTIHGDNIENIAVKOVEDDCODEVKANSNDHAEKAKNKGAAVNSINWAAV (235) S.M. (156) PROKISPLGE-KLECTLGONIENIAVKOVEDDCODEVKANSNDHAEKAKNKGAAVNSINWAAV (247) S.M. (156) PROKISPLGE-KLECTLGONIENTALGENEDDCODEVKANSDADGELKGTRUVAVKSINMARI (247) S.M. (156) PROKISPLGE-KLECTLGONIENTALGENEDCODVKANSDADAEKKONRIGAVNSINMARI (247) C.G. (171) PAGNIFPCOAUMEGLODONIENTALGOEDCODVKANSDADAEKKONRIGAVNSINMARI (233) S.C. (165) PTGRISPLGE-KLECTLGONIETVØIDGONVKANSDADAEKKONRIGAVNSINMARI (247) E.C. (158) PRGKISPLGE-KLECTLGONIETVØIDGONVKANSDADAEKKONRIGAVNSINMARI (247) B.S. (106) PNGKIA-FGKLAQAVMYGAEIIAIDGNFDALKIVKOLTKOSINKAIFGENKENSKINVGAVNSINMARI (247) B.S. (106) PNGKIA-FGKLAQAVMYGAEIIAIDGNFDALKIVKGIGOUVKANSDALKIVKALVSAUNSVNYRII (159) **** * **** **** M.G. I (236) MAQUVYYFKGYFAVTADNAQOVSEAVPSGNEGOUTAGHIARM-GLPIAKLVVA (288) P.A. (248) MAQUVYYFKGYFAVTADNAQOVSEAVPSGNEGOITAGYLARM-GLPIAKLVA (289) S.M. (220) LAQUCYYFEAVADLPO-EARM-QUVIS-VPSGNEGOITAGYLARM-GLPIAKLVA		* *** * *** *** *** * ***	
P.A. (185) PRINTVSEVERRONTTI INGONENTRALEMENDODELWKARSFADOGELKOTRU VARVISINMARI (247) S.M. (158) POGRI ISPLUE-KLECTL GONHENTRALEMENDODELWKARSFADOGELKOALHUNSANSINMARI (247) C.G. (171) PAGRIUTFEODACMEELDDPHIENTRALDGVEDDODUWKAVSADADEEKKONRIGAVNSINMARI (233) S.C. (185) PIGRIUSPLUE-KLECTL GONHENTRALDGVEDDODUWKAVSADAEEKKONRIGAVNSINMARI (233) S.C. (185) PIGRIUSPLÜE-KLECTL GONHENTRALDGVEDDODUWKAVSADAEEKKONRIGAVNSINMARI (247) E.C. (185) PIGRIUSPLÜE-KLECTL GONHETVÄI DÖDEDACALVKOAFDDEELKVALGUSANSINI SRL (219) B.S. (106) PNGKI A-FGKLADAVMYGAEITÄI DÖNEDDALKINVSI (VSI CCEKSPIALVNSVNPYRI (159) * :*** : :*** :*** M.G. I (236) AAQVYYFKGYEAVTADNADOVSEAVPSONFGOVCAGHIARM-GLEVSOLIVA (288) P.A. (248) MAQI VYYFKAULOLGAPHSISVAESVPTONFGOI TAGHIARM-GLEVSOLIVA (289) S.M. (220) LAQI CYYFEAVADLPO-EARN-OLVIS-VYSONFGOI LAGHIARM-GLEVSOLIVA (289) S.G. (248) MAQI VYYFKSWIRTTTS NDOKVSESVPTONFGOI LAGHIARON-GLEVSOLIVA (280) S.G. <t< th=""><th>M.G. (173)</th><th>PHORMSOFT TAME STOTEM FRI LAV KOVETUTATIVKANSNEHA FY A VALK TO A VALKATA VALKAT</th><th>(235)</th></t<>	M.G. (173)	PHORMSOFT TAME STOTEM FRI LAV KOVETUTATIVKANSNEHA FY A VALK TO A VALKATA VALKAT	(235)
X.M. C105 Entropy Characterization in the state of t	P A (185)	PHANDVSEVED PHANT I HOW HARTS / FENERAL WERE CEADOOS / VOTO VALUE HARTS /	(247)
3. m. C1307 FUGSITIOF DECTLECT LEGITIONITY IN DEDICATIONAL TENDANT DEDICTIONAL TENSINGSITI (213) 3. G. (171) PAGRITIPEO ADMIFECTEDINE TENSING DECOMPKAI FGDKEENSKHIVGAVNSINNARI (243) S. C. (185) PTGRISPIDE ELECTLEGITISTEDINGDOLIVKAI FGDKEENSKHIVGAVNSINNARI (247) E.C. (156) PRGRISPIDE ELECTLEGITISTEDINGDOLIVKAI FGDKEENSKHIVGAVNSINNARI (247) B.S. (106) PINGRIA-FGKLAQAVMYGAEIIAI DGDFDACALVKOAFDDEELEVALGLISSANSINISRL (219) B.S. (106) PINGRIA-FGKLAQAVMYGAEIIAI DGNFDALKIVKSIF. Y *** * : * * :** * : * * :*** * M.G. I (236) AAQVYYYEKGYEAVTADNAQOVFSENYESGANGAUCAGHIARMA-GLPIAKLVVA (288) P.A. (248) MAQI VYYEHAALQLGAPHRSVAFSVPTGNFGDI FAGYLARMA-GLPISOLIVA (299) S.M. (220) LAQI CYYEFAVADL-O-EARM-QLVIS-VYSSONFGDI TAGHIARM-GLPISOLIVA (286) S.C. (248) MAQI VYYYESKI RTITSNOKVSFSVPTGNFGDI TAGYFAKKAH-LIPIEKLAIA (272) C.G. (234) MAQUYYYESKI RTITSNOKVSFSVPTGNFGDI TAGYFAKKAH-LIPIEKLAIA (272) C.G. (248) LAQU CYYEFAVADLO-EKSKKVKEVYSSONFGDI TAGYFAKKAH-LIPIEKLAIA (272) C.G. (248) LAQU CYYEFAVADLO-ERNOLVS-VYSSONFGDI TAGYFAKKAH-LIPIEKLAIA (272) S.C. (248) LAQU CYYEFAVADLO-ERNOLVS-VYSSONFGDI TAG	C M (150)	BOOKICOLOGICATIOCATER REET CONTRACTOR CONTRACTOR AND A LUNCASCERIES	(24/)
C. C. (17) PROMITER CLOPHENE CLOPHENE ALL DECOMPTANTS ALL CERVINE (247) S. C. (185) Progi By DECEMITY DENVOLUS Y DENVOLUS Y DENVEQO DIVAL FOR EXEMPSION VANNSIN MARI (247) E. C. (158) PROKI SP. DE-KLECTL GONEETVAI DEDEDAGAL VKOAFDEEL KVALGLISANSINI SR. (219) B.S. (106) PROKI A-FGKLAOAVMYGAEITAIDGNEDDALKI VKOAFDEEL KVALGLISANSINI SR. (219) B.S. (106) PROKI A-FGKLAOAVMYGAEITAIDGNEDDALKI VKOAFDEEL KVALGLISANSINI SR. (219) B.S. (106) PROKI A-FGKLAOAVMYGAEITAIDGNEDDALKI VKOAFDEEL KVALGLISANSINI SR. (219) R.G. I (236) AAQVYYEKGYEAVTADNAQOVSEAVPSGINEGOLGAGHIARDM-GLEPISOLI VA (288) P.A. (248) MAQI VYYEHAALQLGAPHRSVAFSVETORISOI FAGHIARDM-GLEPISOLI VA (289) S.M. (220) LAQI CYYEFEAVADLO-EARM-QLI'S - WSGANGOLTAGHIAROM-GLEPISOLI VA (289) S.C. (248) MAQI VYYEYENGATOKOSKKVKEYVESONFGOLTAGYTARAKA-GLEPISKRI VA (266) E.C. (220) LAQI CYYEFEAVADLO-EARM-OLVIS-VYSGANFGOLTAGYTARAKA-GLEPISKRI VA (267) S.C. (224) LAQI CYYEFEAVADLO-ERKKVKEYVESONFGOLTAGYTARAKA-GLEPISKRI VA (230) E.C. (220) LAQI CYYEFEAVADLOKOSKKVKEYVESONFGOLTAGYTARAKA-GLEPISKRI VA (287) S.C. (220) LAQI CYYEFEAVADLOKOSKKVKEYVESONFGOLTAGYTARAKA-GLEPISKRI VA (267) E.C. (220) LAQI CYYEFEAVADLOSOKKVKEYVESONFGOLTAGYTARAKA-GLEPISKRI VA (267) E.C. (220) LAQI CYYEFEAVADLOCHON SKKVKEYVESONFGOLTAGYTARAKA-GLEPISKRI AA (272) B.S. (100) CYYEFAVADLOCONSTANTINGCONSTANTING CONSTANTING CON	0.8. (130)	EVORIGINEL NEEDID HEREEL DOBED AND AND AND AND AND AND AND AND AND AN	(218)
3.0. C163) # IGHT OF LUCEUM IT VEDENVOIL SVIDI FRANKUDERNA IF GUALENSA HIV VARYBAITMAT (247) E.C. (156) PRGI ISPUEL FILLET CLOBINET VIAI DOEDAQALVKOA FDDEELKVAL LOISANSINI SRL (219) B.S. (106) PNGKI IA-FGKLAOAVMYGAEITAIDGNEDDAQALVKOAFDEELKVAL LOISANSINI SRL (219) w.G. I (236) AGVYYYEKGYEAVTADNADONSEAVPSGINEGINVCAGHIARM-GLPIAKLVVA (288) P.A. (248) MAQ VYYEKGYEAVTADNADONSEAVPSGINEGINVCAGHIARM-GLPIAKLVVA (288) P.A. (248) MAQ VYYEKGYEAVTADNADONSEAVPSGINEGINVCAGHIARM-GLPIAKLVVA (288) S.M. (220) LAQI CYYEEAVAOL QO-EARN-OL VI IS-VPSGINEGIN LAGYLARIM-GLPIAKLIAA (229) S.M. (220) LAQI CYYEEAVAOL PO-EARN-OL VI IS-VPSGINEGID LAGHIAROM-GLPIAK (226) S.C. (248) MAQUYYYSSIN RTTTSNOOKVSESVPTONEGID LAGHIAROM-GLPIEKLAIA (230) S.C. (248) LAQI CYYEEAVAOL PO-EARN-OL VIS-VPSGINEGID LAGHIAROM-GLPIEKLAIA (301) S.C. (248) LAQI CYYEEAVAOL PO-ERROL VVSVPSGINEGI LAGYFAKKM-GLPIEKLAIA (301) S.C. (240) LAQI CYYEEAVAOL PO-ERROL VVSVPSGINEGI LAGYFAKKM-GLPIEKLAIA (301) S.C. (220) LAQI CYYEEAVAOL PO-ERROL VVSVPSGINEGI LAG	0.0. (1/1)	FAURINI FERUMANEGEUUF MIERIAL DOTEDIKKU YIKATSALAEFAA UMATSAATASIMMAKL ATODI AFERMITTUDAEKUOTI OVTATEDINAASIMELEOSVERIOKUMUAANAKO MARKAI	(233)
E.C. (198) PHQRIAP-LET-LECTLEGTIGENTEL'NA LOBDELADAQALVROAF DUBELE VALCLINSANSIN ISRL (219) B.S. (106) PNGRIA-FGKLAOAVMYGAEITAI DGNFDDALKI VRSIC	3.0. (105)	FIGHIOFIVE EVALUATION VULDA INTERNANT OUR CENSRIN VARYNSINNART	(24/)
B.S. (106) PNGRIA-FGKLAQAVMYGAEIIAIDGNFDDALKIVRSICEKSPIALVNSVNPYRI (159) * :***** :***** :**** :**** M.G. I (236) AAQVYYEKGYFAVTADNADONSFAVPSGNFGNVCAGHIARM-GLPIAKLVVA (288) P.A. (248) MAQ IVYYEHAALOLGAPHRSVAFSYFTOHFGO ITAGULARM-GLPIAKLVVA (288) S.M. (220) LAQ ICYYEFAVALOLCAPHRSVAFSYFTOHFGO ITAG	E.C. (158)	PHGK I SPLOE-KLECTLGGNTET VAT DEDEDACUAL VKOAF DDEELKVALGLNSANSTN I SRL	(219)
* :* ** * : * : **** M.G. I (236) AAQVYYYEKGYFAVTADNADONSFAYPSONFGNVCAGHIARMM-GLPIAKLVVA (288) P.A. (248) MAQIVYYEHAALOLGAPHRYSAFSWFTONFGDIFAGYLARMM-GLPVSQLIVA (299) S.M. (220) LAQICYYEEAVALOLQ-EARM-OLVIS-WYSONFGDIFAGHIAROM-GLPVSQLIVA (299) S.G. (24) MAQVYYVSSWIRTTTSNOOKVSFSVFTONFGDIFAGHIAROM-GLPIORLIVA (286) S.C. (248) LAQICYYFEAVADLPO-ETRNOLVVS-VPSONFGDILAGYFAKMM-GLPIEKLAIA (301) E.C. (220) LAQICYYFEAVADLPO-ETRNOLVVS-VPSONFGDITAGYFAKMM-GLPIEKLAIA (272) S.S. (248) LAQUCYYFEAVADLPO-ETRNOLVVS-VPSONFGDITAGYFAKMA-GLPIEKLAIA (272) S.S. (200) LAQICYFEAVADLPO-ETRNOLVVS-VPSONFGDITAGYFAKMA-GLPIEKLAIA (272)	B.S. (106)	PNGKIA-FGKLAQAVMYGAEIIAIDGNFDDALKIYRSICEKSPIALVNSVNPYRI	(159)
****** :***** :**** :**** ***** :**** :**** :**** ***** :**** :**** :**** ***** :**** :**** :**** ***** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :**** :**** **** :**** :***** :***** :**** :***** :****** :***** :**** :********** :************************************			
M.G. I (236) AAQVYYYEKGYEAVTADNAQOVSEAVESGNEGUYCAGHIARMA-GLEIAKLVVA (288) P.A. (248) MAQIVYYEKGYEAUQAGHRSVARSVETGIFGGIFAGHIARMA-GLEIAKLVVA (298) S.M. (220) LAQICYYEEAVAOLPO-EARN-QLVIS-VESGNEGDIFAGLLAKSL-GLEIVKEFIAA (272) C.G. (234) MAQVYYYSSWIRTITSNDOKVSESVETGARGDICAGHIARCM-GLEIORLIVA (286) S.C. (248) LAQICYYEEAVAOLPO-ETRNOLVVS-VESGNEGDILAGYFAKKA-GLEIERLAIA (301) E.C. (220) LAQICYYEEAVAOLPO-ETRNOLVVS-VESGNEGDILAGYFAKKA-GLEIERLAIA (301) S.S. (220) LAQICYYEEAVAOLPO-ETRNOLVVS-VESGNEGDILAGYFAKKA-GLEIERLAIA (272) S.S. (220) LAQICYYEEAVAOLPO-ETRNOLVVS-VESGNEGDILAGYFAKKA-GLEIERLAIA (272) S.S. (220) LAQICYYEEAVAOLPO-ETRNOLVVS-VESGNEGDILAGYFAKKA-GLEIERLAIA (272)		* :* ** * : * **	
P.A. (248) MAQ IVYYEHAALQLGAPHRSVAFS/JETEMFGDIFAGYLARIM-GLEP/SQLIVA (299) S.M. (220) LAO ICYYEEAVAQLPQ-EARN-DLYIS-VIPSGNFGDIFAGYLARIM-GLEP/SQLIVA (299) S.M. (220) LAO ICYYEEAVAQLPQ-EARN-DLYIS-VIPSGNFGDIFAGYLARIM-GLEP/SQLIVA (299) S.G. (248) MAQUYEY/SSWIRTTTSNOOKVSFS/VERMEDIFAG (201) S.C. (248) LAQUYEY/SSFRATTSKDSKKVKEV/PSGNFGDILAGHIARGM-GLEPIEKLAIA (301) E.C. (248) LAQUYEYEAVAQLPQ-ETRNOLV/SS-VPSGNFGDILAGYFAKKM-GLEPIEKLAIA (301) E.C. (220) LAO ICYYEEAVAQLPQ-ETRNOLV/SS-VPSGNFGDILAGYFAKKM-GLEPIEKLAIA (212) S. (140) CYPEAVAQLPQ-ETRNOLV/SS-VPSGNFGDILAG	M.G. (236)	AAQYYYYEKGYEAVTADNAQOYSEAYPSGNEGNVCAGHIARMM-GLPIAKLVVA	(288)
S.M. (220) LAOI CYYFEANAOL PO-EARN-QUVIS-WPSCHREDI TAGULAKSL-BUPYKRFI AA (222) C.G. (234) MAQWYYYSWIRTTTSNDOKVSESVPTONEGDI LAGHIARON-GUPIDRLIVA (286) S.C. (248) LAOU YYYY SFOATNOKOSKKVKFWYPSCHREDI LAGYFAKKM-BLPIEKLAIA (201) E.C. (220) LAOI CYYFEANAOL PO-ETRNOL WYS-WPSCHREDI LAGYFAKKM-BLPIEKLAIA (201) S.S. (120) EADI CYYFEANAOL PO-ETRNOL WYS-WPSCHREDI LAGYFAKKM-BLPIEKLAIA (220) S.S. (120) EADI CYYFEANAOL PO-ETRNOL WYS-WPSCHREDI LAGYFAKKM-BLPIEKLAIA (221)	P.A. (248)	MAGIVYYEHAALQLGAPHRSVAFSYPTGNEGDIFAGYLARNM-GLPVSQLIVA	(299)
C.G. (234) MAQVYYYYSSWIRTTTSNDXKVSESVPTONEGDICAGHIARON-GUPIDRLIVA (286) S.C. (248) LAQVYYYYSFOATNGKOSKKVKFVVPSONEGDILAGYFAKKM-BUPIEKLAIA (301) E.C. (220) LAQICYYFEAVAOLQO-ETRNOLVVSVPSONEGDILAGYILAKSL-GUPYKRF1AA (272) S. (160) ECRYTAAEFVCF01CLAPDVI-A UVVACUTAVVVCFVFVFVFVRV1AA (2712)	S.M. (220)	LAQICYYEEAVAQLPQ-EARN-QLVIS-VPSGNEGDLTAGLLAKSL-GLPVKRFIAA	(272)
S.C. (248) LAQMTYYEYSFEQATNGKDSKKVKFVYPSONFGDILAGYFAKKM-BLPIEKLAIA (301) E.C. (220) LAQICYYFEAVAOLPO-ETRNOLVYSYPSONFGDITAGLIAKSL-GLPYKRFIAA (272) S. (160) EQRITABEVEPICI-C-A-DPUI-A LIVENARDII TAVWCFFYFUEVRCIA PUID-(212)	C.G. (234)	MAQYYYYYSSWIRTTTSNDQKYSFSYPTGNFGDICAGHIAROM-GLPIDRLIVA	(286)
E.C. (220) LAQICYYFEAVAQLPQ-ETRNOLVVSVPSGNFGDLTAGLLAKSL-GLPVKRFIAA (272) B.S. (160) FGNTAAFEVEDIGFAPDVI-AIPVGAGNITAVWKGEKEVHEVAGTA	S.C. (248)	LAOMTYYFYSFFOATNGKDSKKVKFVVPSGNFGDILAGYFAKKM-GLPIEKLAIA	(301)
B S (160) EGOKTAAFEVCEOLGEAPOVL-ALPVGNAGNITAYWKGEKEYHEKNCTGLDKMP (212)	E.C. (220)	LADICYYFEAVAOL PO-ETRNOL VYSYPSGNEGDL TAGLI AKSL-GLPVKRFIAA	(272)
	B.S. (160)	EGOKTAAFEVCEOLGEAPOVL-AIPVGNAGNITAYWKGFKEYHFKNGTGIPKMR	(212)

APPL. ENVIRON. MICROBIOL.

			* * : * :	
M.G.	I	(289)	TNENDVLDEFFKTGVYRPRGSANTYHTSSPSMDISKASNEERFVFDLVGRD-AAKVRELWGKV	(350)
P.A.		(300)	RNRNDILHREM-SGNASTRHTL-I-PSVSPSMDIMVSSNEERLLFDLHGRN-GKAVAELLDAF	(358)
S.M.		(273)	TNANDTVPRELTSGOWORHATVATLSNAMDVSOPNNWPR-VEELFRRK-VWOLKEL-GHA	(329)
C.G.		(287)	THENDYLDEFFRIGDYRVRSSADTHEISSPSHDISRASHFERFIFDLLGRD-ATRINDLFGTO	(348)
S.C.		(302)	TNEND I LORELKSGLEE-R-SDKVAATLSPANDIL I SSNEERLLWYE-AREYLANGDDLKAGE	(361)
E.C.		(273)	TNVNDTVPRELHDGOWSPKATQATLGNAMDVSOPNNWPR-VEEL-FRRKIWOLKEL-GY-	(328)
B.S.		(213)	GEEAEGAAKIVRNEVIENPETIATAIBIGNP-KSWDKAVKAAE	(254)
NG		(351)	DACACEDEND_COWENEVA_BACEVERCENDENDERVATEDATED	(100)
P A	1	(350)	VIGOLDEND - GOWEAR VR - DIGE V GOOGNAMMINGWELERA I NED TORIERU I - TERU GENERA VIGOLDEND - OGWEAR VR - DIGE V GOOGNAMMINGWELERA I NED TORIERU I - TERU GENERA VIGOLDEND - OGWEAR VR - DIGE V GOOGNAMMINGWELERA I NED TORIERU I - TERU GENERA	(408)
с м		(330)		(414)
0.M.		(240)		(361)
0.6.		(348)	VICAGUESEAU-DANELKAAALTGEAGEKSIHADEVATTAUVESELDVLTDP-HTADGVHTA	(407)
5.0.		(302)	IVNNWEVELKINGKEVVBROIIEGAOK-	(389)
E.C.		(328)	-RAVD-DETTQRAVAYRA	(360)
B.S.		(255)	ESNGK IDEVIDUEILHAYQL#ARVEGWFAEPGSCASIAGVLKQ	(297)
			::* * *: *	
M.G.	L	(409)	LEHREAGTPMLVLETALPAKFEDAIVEALGHKP-ERPHSLEGLESLPORFEVMEADA	(464)
P.A.		(415)	RECORSLEVPHIVTEGTAHEVKEPEA-VEKAGIGOAP-ALPAHLADEFEREERCTVLPNEL	(472)
S.M.		(362)	RDOLQEGEFGLFLGTAHPAKFKES-VEA-ILGOEL-PLPKALALRAELPLLSHTLPASF	(417)
C.G.		(408)	ROWRD-EVNTRIIVLETALPVKFADTIVEAIGEAP-OTPERFAAIMDAPFKVSDLPNDT	(464)
E.C.		(362)	EROOLNP-GEYGEFEGTAHPAKEKES-VEA-IEGE-TLDLPKEEAERADEPLLSHNLPADE	(418)
B.S.		(298)	VKSGEIPKGSKVVAVLTGNGLKOPNTA¥D-ISEI-KRVTLRTDEDSILEYVKGAARV	(352)
		(
M.G.	I.	(405)	AVIKALINEHI	(475)
P.A.		(473)	AKVUARVSUN	(482)
S.M.		(418)	GELRKELM	(425)
C.G.		(465)	DAVKOYIVDAI-	(475)
E.C.		(419)	AALKLMMNHQ	(428)

FIG. 7. Homology between the amino acid sequence of the threonine synthase of *M. glycogenes* ATCC 21371 and those of other microorganisms. Shaded letters show amino acid residues identical to those of *M. glycogenes* ATCC 21371. Conserved residues in all of the amino acid sequences are noted with asterisks, and homologous residues (I-L-V-M, D-E, R-K, S-T, F-Y) are noted with colons. S.M., *S. marcescens*. All other abbreviations are as defined in the legend to Fig. 6.

at position 120 by analogy with other threonine synthases (17).

ACKNOWLEDGMENTS

We are grateful to K. Hasegawa for technical assistance, Y. Sato for homology search of amino acid sequences, A. Iida and Y. Yonetani for experimental procedures with Northern blot analysis, and N. Hanai, A. Furuya, and M. Oda for preparation of antiserum.

REFERENCES

- Aiba, H., S. Adhya, and B. de Crombrugghe. 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. 256:11905–11910.
- Archer, J. A. C., D. E. Solow-Cordero, and A. J. Sinskey. 1991. A C-terminal deletion in *Corynebacterium glutamicum* homoserine dehydrogenase abolishes allosteric inhibition by L-threonine. Gene 107:53-59.
- Baur, H., E. Luethi, V. Stalon, A. Mercenier, and D. Haas. 1989. Sequence analysis and expression of the arginine-deaminase and carbamate-kinase genes of *Pseudomonas aeruginosa*. Eur. J. Biochem. 179:53-60.
- Chen, E. Y., and P. H. Seeburg. 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA 4:165–170.
- Clepet, C., F. Borne, V. Krishnapillai, C. Baird, J. C. Patte, and B. Cami. 1992. Isolation, organization and expression of the *Pseudomonas aeruginosa* threonine genes. Mol. Microbiol. 6:3109–3119.
- Cohen, G. N., and I. St.-Girons. 1987. Biosynthesis of threonine, lysine, and methionine, p. 429-444. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typh*-

imurium. Cellular and molecular biology. American Society for Microbiology, Washington, D.C.

- 7. Follettie, M. T., H. K. Shin, and A. J. Sinskey. 1988. Organization and regulation of the *Corynebacterium glutamicum homthrB* and *thrC* loci. Mol. Microbiol. 2:53-62.
- Furuya, A., M. Ohtomo, T. Inada, and H. Yoshida. 1987. Generation and application of monoclonal antibodies against salmon somatotropin (salmon growth hormone) and salmon prolactin. Agric. Biol. Chem. 51:2331–2335.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
- Lidstrom, M. E., and Y. D. Tsygankov. 1991. Molecular genetics of methylotrophic bacteria, p. 273–304. *In* I. Goldberg and J. S. Rokem (ed.), Biology of methylotrophs. Butterworth-Heinemann, Stoneham, Mass.
- 11. Lipman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. Science 227:1435-1441.
- 12. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- 13. Marchenko, G., and Y. Tsygankov. 1992. Genes for threonine biosynthesis in *Methylobacillus flagellatum*, abstr. B79. *In* Proceedings of the 7th International Symposium on Microbial Growth on C_1 Compounds, 15 to 20 August 1992, Warwick, England.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208-218.
- 15. Motoyama, H., H. Anazawa, R. Katsumata, K. Araki, and S. Teshiba. 1993. Amino acid production from methanol by Methylobacillus glycogenes mutants: isolation of L-glutamic acid hyper-producing mutants from M. glycogenes strains, and derivation of L-threonine and L-lysine-producing mutants from

them. Biosci. Biotechnol. Biochem. 57:82-87.

- Motoyama, H., H. Anazawa, and S. Teshiba. 1993. Characterization of the aspartate family amino acids biosynthetic enzymes in L-threonine- and L-lysine-producing mutants of *Methylobacillus glycogenes*. Biosci. Biotechnol. Biochem. 57:461– 466.
- Parsot, C. 1986. Evolution of biosynthetic pathways: a common ancestor for threonine synthase, threonine dehydratase and D-serine dehydratase. EMBO J. 5:3013–3019.
- Parsot, C., and G. N. Cohen. 1988. Cloning and nucleotide sequence of the *Bacillus subtilis hom* gene coding for homoserine dehydrogenase: structure and evolutionary relationships with *Escherichia coli* aspartokinases-homoserine dehydrogenases I and II. J. Biol. Chem. 263:14654–14660.
- Peden, K. W. C. 1983. Revised sequence of the tetracyclineresistance gene of pBR 322. Gene 22:277-280.
- Peoples, O. P., W. Liebl, M. Bodis, P. J. Maeng, M. T. Follettie, J. A. Archer, and A. J. Sinskey. 1988. Nucleotide sequence and

fine structural analysis of the Corynebacterium glutamicum hom-thrB operon. Mol. Microbiol. 2:63-72.

- Thèze, J., D. Margarita, G. N. Cohen, F. Borne, and J. C. Patte. 1974. Mapping of the structural genes of the three aspartokinases and of the two homoserine dehydrogenases of *Escherichia coli* K-12. J. Bacteriol. 117:133–143.
- 22. Tinoco, I., Jr., P. N. Borer, B. N. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla. 1974. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. 246:40–41.
- Turano, F. J., R. L. Jordan, and M. F. Matthews. 1990. Immunological characterization of *in vitro* forms of homoserine dehydrogenase from carrot suspension cultures. Plant Physiol. 92:395-400.
- Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119.