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Complete Nucleotide Sequence of *Bacillus subtilis* (*natto*) Plasmid Responsible for γ -Polyglutamate Synthesis

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The complete 5,812 base pairs nucleotide sequence has been determined for *Bacillus subtilis* (*natto*) plasmid pUH1, which encodes a γ -glutamyltranspeptidase (EC 2.3.2.2) for synthesis of γ -polyglutamate. Nucleotide sequence analysis reveals two long open reading frames coding for the gene for replication (*rep*) protein and γ -glutamyltranspeptidase gene. Several putative regulatory sequences conserved were found upstream from these frames including the Shine-Dalgarno sequence, Pribnow box and the "-35 region" at preferred distances for efficient transcription. The amino acid sequences predicted from the *rep* and γ -glutamyltranspeptidase coding frames showed a significant homology, in each small segment, with those of E229 coded from the plasmid pC194 and of C403 from the plasmid pE194 of *Staphylococcus aureus*, respectively. However, no convincing homology of the pUH1 predicted γ -glutamyltranspeptidase protein could be found with *E. coli* and mammalian γ -glutamyltranspeptidases.

INTRODUCTION

"Natto" is one of the traditional non-salty fermented foods which is often served at breakfast in Japan. It is manufactured by growing *Bacillus subtilis* (*natto*) on steamed soybeans. It is an adhesive, and consists of polysaccharide (levan-form fructan) and γ -polyglutamate. The adhesive materials are mainly composed of γ -polyglutamate containing D- and L-glutamate in various proportions (Fujii, 1963). A plausible mechanism of the biosynthetic pathway of γ -polyglutamate has been proposed by Thorne *et al.* (1955) for one of the strains producing γ -polyglutamate (*B. licheniformis* ATCC 9945A). However, since it is known that various strains differ a great deal in the basic requirements for synthesis of the capsule, it is to be assumed that there is more than one biosynthetic pathway.

We showed that a 5.7-kb plasmid designated pUH1, which encodes γ -glutamyltranspeptidase (EC 2.3.2.2) gene responsible for γ -polyglutamate synthesis, is distributed widely in *Bacillus subtilis* (*natto*) isolated from a commercial product, natto (Hara *et al.*, 1983). In a previous paper (Hara *et al.*, 1991), we reported that the 2.0-kb

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Bst EII DNA fragment of pUH1 contains a gene for replication protein (*rep*) of 999-bp in size. To investigate the participation of a plasmid in γ -polyglutamate synthesis, we performed DNA sequencing of natto plasmid pUH1. In the present work, we report the complete nucleotide sequence of the plasmid pUH1, including data pertinent to the mapping of biological functions and determinants of their expression and regulation.

MATERIALS AND METHODS

Bacterial strain and plasmids

Escherichia coli JM 101 was used for a cloning host. Plasmids pUH1 (Hara *et al.*, 1982) and pATE1 (Hara *et al.*, 1991) have been previously described, respectively.

Enzyme assay

Gamma-glutamyltranspeptidase activity was assayed as previously described (Aumayr *et al.*, 1981).

DNA manipulation

The plasmid pUH1 from *Bacillus subtilis* (*natto*) was prepared and purified as described previously (Hara *et al.*, 1983). Plasmid pUH1 was sonicated to generate suitable sizes, processed with nuclease P1 and T4 DNA polymerase, and separated by electrophoresis on agarose gels. After elution from gels, the fragments were inserted at *Sma*I cloning site of M13 mp8 DNA. After transformation into *E. coli* JM101, a shotgun library was constructed by the modified method (Yasuda *et al.*, 1984) of Deininger (Deininger, 1983). The recombinant phages selected from library were sequenced.

DNA sequencing

DNA sequencing was carried out by means of the dideoxy chain termination method (Sanger *et al.*, 1977) with M13 sequencing kit. Nucleotide and deduced amino acid sequences were analyzed by the Hitachi DNASIS system.

Chemicals

Restriction endonucleases, T4 DNA polymerase, and M13 cloning and sequencing kits were obtained from Takara Shuzo Co., Ltd., and used according to the suppliers specifications. Nuclease P1 was from Yamasa Shoyu Co., Ltd. (α -³²P)dCTP was purchased from RCC Amersham.

RESULTS AND DISCUSSION

Location of biological functions on pUH1

As reported previously (Hara *et al.*, 1982), γ -glutamyltranspeptidase gene, which is responsible for polyglutamate synthesis, might be encoded on an endogeneous plasmid, pUH1. To define the bounds of a biological functional unit of pUH1, we constructed a set of plasmid derivatives using pUB110 and pATE1 (Hara *et al.*, 1991). Recombinant plasmids were selected in *B. subtilis* host by using resistance to kanamycin (Km) for pUB110 and trimethoprim (Tmp) for pATE1. The generated *Bst*EII and *Hind*III fragments of pUH1 were filled in with Klenow fragment, and cloned into *Pvu*II site of pUB110 and *Aat*I site of pATE1, respectively. The results are summarized in Fig. 1. The composite plasmid pBB2 contains a 2.0-kb *Bst*EII fragment (column BsS in Fig. 1) of pUH1 which confers autonomous replication on the plasmid

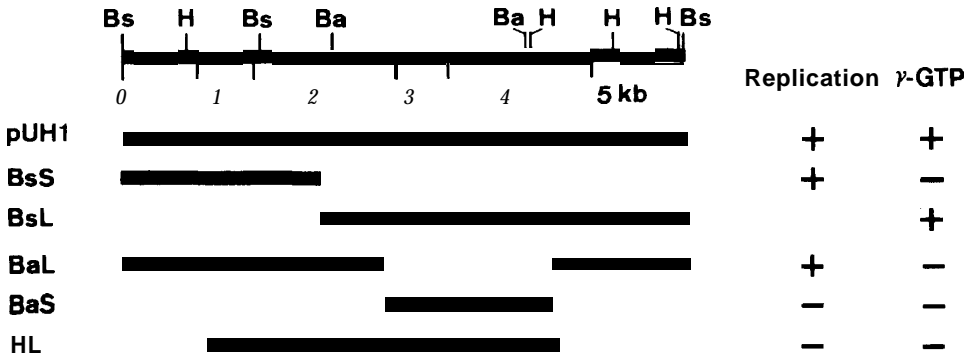


Fig. 1. Structure and location of biological functions of 5.7-kb plasmid pUH1. + and - indicate, respectively, ability and inability to replicate and to synthesis γ -glutamyltranspeptidase in the *B. subtilis* host. Modifications were made by deletion with restriction enzymes.

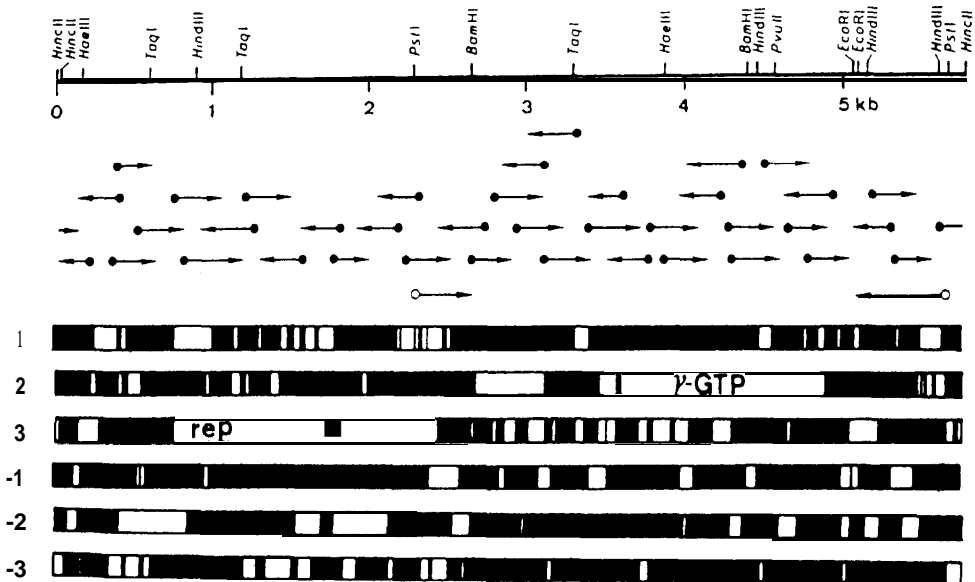


Fig. 2. Strategy for sequencing the plasmid pUH1, and the coding frames. (Upper) The physical map of pUH1 and strategy for nucleotide sequence. The 5' end of *HincII* site of pUH1 is taken as the start point for numbering the nucleotide sequence. The extent of sequencing is specified by arrows. The open and closed circles represent the 5' termini of the cloned fragments generated by restriction enzyme digestion and sonication, respectively. (Lower) The coding frame of the presented sequences: □, open coding frame; ■, noncoding region.

in the *B. subtilis* host. The 2.0-kb *Bst*EII fragment is necessary and sufficient for the plasmid replication, whose molecular structure has been previously characterized (Hara et al., 1991). The Km^r transformants harboring plasmid pPB1, which contain a 3.7-kb *Bst*EII fragment (column BsL in Fig. 1) of pUH1, can produce γ -glutamyltranspeptidase. The enzyme activity of Km^r transformants harboring pUU1, which contain the 3.6kb *Hind*III fragment (column HL in Fig. 1), could not be detected in *B. subtilis* host.

DNA sequence analysis of pUH1

The strategy for sequencing is outlined in Fig. 2. Restriction sites determined preliminarily by enzyme digestions were confirmed by sequence analysis. The complete nucleotide sequence of pUH1 DNA comprises 5,812 bp (Fig. 3). The DNA sequence contains two open translation reading frames, whose directions are the same. Of the two, the longer open reading frame which corresponds to γ -glutamyltranspeptidase, starting with ATG at nucleotide 3,650 and terminating at the stop codon at nucleotide 4,911, is capable of coding for a polypeptide of 420 amino acid residues with a *Mr* of 49,356 daltons. Upstream from the γ -glutamyltranspeptidase, putative regulatory sequences can be identified, including a Shine-Dalgarno sequence (AACGAG), which is complementary to the 3' end of 16S rRNA (3'-OH-UCUUUCCUC-CAGUAG-5') of *B. subtilis* (McLaughlin et al., 1981), at nucleotides 3,616 to 3,621, a σ^{43} RNA polymerase recognition site (TTCAAA) at nucleotides 3,555 to 3,560, and a Pribnow box (TATTAT) at nucleotides 3,578 to 3,682. The distance (17 bp) between the recognition site and a Pribnow box is shorter than the preferred one in *B. subtilis* (Moran et al., 1982). It is well known that γ -polyglutamate productivity of *B. subtilis* (*natto*) is decreased during stock on a nutrient agar slant over a long period. The instability of γ -polyglutamate synthesis might be due to inefficient regulation on the transcriptional level of γ -glutamyltranspeptidase.

The shorter frame designated rep, starting with ATG at nucleotide 816 and terminating at the stop codon at nucleotide 1,815, is capable of coding for a polypeptide of 333 amino acid residues with a *Mr* of 39,074 daltons. The nucleotide sequence upstream from the rep gene is characterized by the presence of typical regulatory signals. They include a Shine-Dalgarno sequence (AAGGAG) at nucleotides 783 to 788 for translation initiation, the sequence (TTGACA) at nucleotides 709 to 714 for a σ^{43} RNA polymerase recognition site and a Pribnow box (TATTAT) at nucleotides 733 to 738, at preferred distances (17-18 bp) from each other. To discover the sequences essential for replication, a homology search was performed to determine whether there are sequences within this region conserved among other *Bacillus* spp. and *S. aureus* plasmids known to replicate in *B. subtilis*. Results showed that a 26-bp sequence (TTTCTTATCTTGATACTATATAGAAA) at nucleotides 626 to 651 was conserved in pUH1, pBAA1 (Devine et al., 1989), pFTB14 (Murai et al., 1987), pUB110 (Muller et al., 1986), and pC194 (Dagert et al., 1984; Horinouchi and Weisblum, 1982b). Especially, the consensus sequence (CTTGATA) is found in the hairpin region of ϕ X174 shown by Shlomai and Kornberg (1980) to have origin activity. However, no significant homology was found with pE194 (Horinouchi and Weisblum, 1982a; Villafane et al., 1987), and pT181 (Khan et al., 1982).

Amino acid sequence comparison

The amino acid sequence of the predicted proteins encoded on pUH1 was compar-

GTCAACGGTAACCGGACCGTAGGGAGGATTAAGGAAGTTGACTCGCTCAGCGCCACCCGAACCCCTTCAGCACTCAAACAACCCGTTTG
 50 90
 TTTGACGCCAACCGGGCAGGGAGCCCCCGAAGAAGCGGGGGTGGGGGATTGAATGCTGGCATCCAACGGCCGCTCGTTGGTGGGTTT
 100 150
 GGGCAAAGCCAAAGAACTGTTGCAAGGCTCGTTGAGAAATAAAGAAATGCTTTTCAGGATGCTTAGAATCGTTTCTGAGAGCTTCAAATAAAA
 200 250
 AAGATGACCTTTTATAGGGGGAAGCTCTTAAAATTGAATGTAGGGGCATTTAAACACGTTTAAAAATAAAAAAGCAGACTCTTTAGAGT
 300 350
 CCGCCTTGTATTATTTAAACCCAGTGTCCATTTTCGGCTGTTGGAAATCTTTTGGATGCCGAACCATCCATTTTCTTTTGTTCATGA
 400 450
 AAAAAGTCTTTTGGATGCTTAAAAAGGCTTTTTCGTATAAAAAAGCCGATTTTTGAAAAAAAATCTCCCTGCGGGGAAGAATGGTT
 500
 TTGATCTTTGGTTTTAGGTTTAAAAAACCGGGCTGTTTTCAGCCGGCTTTTTCGATTTTGGCGGAGCCGAAATCGGGTCTTTTCT
 550 600
 TATCTTGATACTATATAGAAACATCTCAAGGCCAAAAATAGCCCCCATCCCTTATTTGTCAAGGGTTTGAACGGCTTTTGAAGTGTAGA
 650 700 (-35)
 AACTCCTCCGGCTATTATTAAGTGCCCAATAAATAAGAAATGCTAGATTACTAGCTCAGAAAGAGTTTTTTTGTTCATGTATTATC
 (-10) 750 (SD) 800
 TGAATAATGATTATATCATCCTTGAGGACAAGCCCAACAGGTAAAAAGCGGGATTGGAAGGGGAAAAAGACGGCAATCTTATGGC
 850 900
 Met Ile Ile Ser Ser Leu Arg Thr Arg Pro Gln Gln Val Lys Ser Gly Ile Gly Arg Gly Lys Arg Asp Gly Arg Ile Leu Trp L
 TGAGCACTATGAAGCTTTACAGAGTAAACTGGTATACCTTACTATGGCAAAAAGCTGAGAAATTTGTCAGTTGTGCGGAATGTCTTTC
 950
 eu Ser Thr Met Lys Leu Tyr Arg Val Lys Leu Val Tyr Leu Thr Met Ala Lys Lys Leu Arg Asn Cys Ala Val Val Arg Asn Val Phe A
 1000 1050
 GTTTAAACGAGACCCGGAGACGCAAAATTAAGTTGATCAAGCTCAGTTTTGTAAGTGAGTTATGCCGATGTGTGCGTGGCGTAGGT
 1100 1150
 rg Leu Asn Glu Thr Arg Arg Lys Leu Lys Leu Tyr Gln Ala Gln Phe Cys Lys Val Arg Leu Cys Pro Met Cys Ala Trp Arg Arg S
 CTTTAAAAATGCTTATCATATAAATAAATCGTTGAGGAAGCGAATCGGCAGTACGGTTGTTGGATGGATTTTCTCACACTGACGGTTC
 1200 1250
 er Leu Lys Ile Ala Tyr His Asn Lys Leu Ile Val Glu Glu Ala Asn Arg Gln Tyr Gly Cys Gly Trp Ile Phe Leu Thr Leu Thr Val A
 GGAATGTCGAGGGTGACGGATTAACCCATGATTGCTGCATGATGAAAGGATGGAACCGCCTTTCCGGATATAAACAGGTTAAGGTAG
 1300 1350
 rg Asn Val Glu Gly Asp Gly Leu Lys Pro Met Ile Ala Asp Met Met Lys Gly Trp Asn Arg Leu Phe Gly Tyr Arg Val Lys Val A
 CGACTTTAGTTATTTTACAGACTTTAGAGATTACCAAAAATCAGGAAGAATACATATCATCCGCATTTTCATGTGTTGTTGCCGTGTA
 1400 1450
 la Thr Leu Gly Tyr Phe Arg Ala Leu Thr Lys Asn His Glu Glu Asp Thr Tyr His Pro His Phe His Val Leu Leu Pro Val L
 AGAAAAGCTATTTTACTACATTAACATTAAAGCAGTCTGAGTGGACGAGCTTATGGAAAAGGGCGATGAAACTGGACTACACCGCGATTG
 1500 1550
 ys Lys Ser Tyr Phe Thr His Asn Tyr Ile Lys Gln Ser Glu Trp Thr Ser Leu Trp Lys Arg Ala Met Lys Leu Asp Tyr Thr Pro Ile V
 TTGATATCCGAAGAGTCAAGGGAAGAGCTAAAATTGATGCCGAACAGATTGAGAGCGATGTGCGGGAAGCCATGATGGAGCAAAAAGCTG
 1600 1650
 al Asp Ile Arg Arg Val Lys Gly Arg Ala Lys Ile Asp Ala Glu Gln Ile Glu Ser Asp Val Arg Glu Ala Met Met Glu Gln Lys Ala V
 TTCCTGAAATCTCTAAATATCCGGTTAAAGATACGGATGTTGTGCGCGCAATAAGGTGACAGAAGCAATCTGAACACCGTGTTTTATT
 1700 1750
 eu Asp Asp Ala Leu Ser Arg Arg Arg Leu Ile Gly Tyr Gly Ile Leu Lys Glu Ile His Lys Glu Leu Asn Leu Gly Asp Ala Glu A
 ACGCGATCTCGTCAAGATTGAGGAAGAAGATGACGAGGTGGGGAACGAAGCATTGAAAGTTATGGCTTACTGGCATCCAGGCATTAAAA
 1800
 sp Gly Asp Leu Val Lys Ile Glu Glu Glu Asp Asp Glu Val Ala Asn Glu Ala Phe Glu Val Met Ala Tyr Trp His Pro Gly Ile Lys A
 ATTACATAATCAGATAAAAAGCAGCGCTGTTCTCGCTTTTAACTACTCTAAATAGTCAAAATCAAGAGTTAATTTTAGATGTAATTTGTA
 1850
 sn Tyr Ile Ile Arg *** → ←

Fig. 3. Complete nucleotide sequence of pUH1.

Nucleotides are numbered from the 5' end of *HincII* site of the DNA strand of the pUH1 DNA with the same polarity as mRNA. Amino acid sequences are also shown on the coding frames. Regulatory signal sequences are underlined. Putative termination signals are indicated by arrows.

1900 1950
 GAATTAGAGTGGCTGACCAGTATTGAAACTCTTGGCTACTTTCTTAACCTTATATATAAACTATGTATATATGTGTTGTTTTTCTA
 2000 2050
 TTATTTTGATATTATTACAAGTATTGAAATTTTCTAGGAGGAAAAGTTTTTATGGTTACCACGATTGGTAAAAAGTAAGATGTGGGTAG
 2100 2150
 GTATTATTGTTGTAATTATCTTTTATTGGTATCTTTTTCGCCTGCTGTAAGGCTGATACTAAGGATAAAATATTATCTACAACCTTCTA
 2200 2250
 CCCAATCTTCAACAAAAGTTATCGTAAAGCTAATACTAGTGGGTCTATGTGAAAGTACTTAAAGCTGGACGTTCTCGTGATGTTGCTA
 2300
 TTTTCAGTTTTTGCTGATGCGAACAAAGGAAAAGGAAAGCCACATTGGGTAATGTTTCTGGTAGCGATGGCGCTACTCTGGGAAAATACG
 2350 2400
 TGACTGCAGGGCATAACATATCATCTTACAAACTATGCTGTAGAACGTTATGGAAAAGTGTTCCTATACAATTAATTTGTTTCTAATGGTT
 2450 2500
 CTGGCAAAAAGTTGAATTTTATTGGAGTCCCGATTGTAGATAGTCCAAATAGCAGATGATGAAAAAGCAGGATTAATCTCTTTTTTAT
 2550 2600
 TTTTGTTTTGGTAAAATGTGATAAGCGGGTTTTGAAATATAGAGGAGGAAATTTCTTTTGACAAAAGCAGAAGTTTTCAAAAAGAAAA
 2650
 GCTCTATTTAGAGTGGGCAAGGCTATTGTGATAGCTGTATATACTTCTCTTCTTATTCGGATTTTTGTTTGGCCATATGTGGTAGA
 2150
 AGGGAAGTCTATGGATCCCACTTTGGTTGATTCTGAAAGATTATTTGTAATAAGACTGTGAAGTATACAGGTAATTTTAAACGAGGGGA
 2800 2850
 TATAATAATTTTAAACGSAAGGAAAAAGCACACATTATGTGAAGCGATTAATTGGTTTACCTGGAGACACTGTAGAAATGAAGATGA
 2900 2950
 CCACCTTTTTTAAATGGAATGAAGTTAAGGAACCAATCTTTCTTATAATAAGAAAATGCTAAGAAAGTGGGTATAAAOCTTACAGG
 3000 3050
 AGATTTTGACCAATTAAGTTCAAAAGATAAAATATTTGTTATGGGCGATAACCGACAAGAAATCAATGGATAGTCGTAATGGGCTTGG
 3100 3150
 ACTCTTTACTAAAGATGATATTACAGGAAACCGAAGATTCTGATTTTTTCCATTTAGTAATATGCGAAAAGCTAAATAATTTTTAGACCG
 3200
 AAACAGGCTATAAGGCTCTGTTTTTTCATTTTGTATGAACATTTAGCACACAGATCAAAGTTTTCATAGTTTGAATGCTTTGATAGCAGC
 3250 3300
 AAAGGTATTTCTGATTTTCTGCGATCTCTCATCGGCGGAAAAGTCGGGTCGGCGGACAGCCGACAAGTGGCACGAACCTTTCGATGCGA
 3350 3400
 CAGCGAGAATGAGAGCGCACCGACCCGAGTCCGACGTCCAAATTTGCCATGGCATAAATTTGGTGTAGTCGTTACACCAAAGATAA
 3450 3500
 ACTTTGTGTTACCATAACCCCTATACAGTGGTGAATCGGGGTTTTTCTCATGGCAAATTTGCACTCAGGATGAAAAATACAA
 3550 (-35) (-10) 3600
 AAAAGATAGATTGAATGGAACGCAAAAACACAATCAGCGGAGTTTCAAAAAGCAAAAATGAAAAGTATTATCGGGGAGCGGACGACTTA
 (SD) 3650
 AATTATGATCTAGT GAACGAGAAACCGATTAGCTATTCAAAGCGGATTCATGAAAAAATTGAGGGGCGAGTCAAACCGAAGGTCCGAGCG
 MetLysLysLeuArgGlyGluSerAsnGlyArgSerGluAr
 3700 3750
 GATGCATGTTTTGGTCAGCGAATTTTTGATCAGCGCAAGTCTGACTATATGAATGGGTGAGCGATGAGGAGCAGCGGCGCTATTTTGA
 gMetHisValLeuValSerGluPheLeuIleThrAlaSerProAspTyrMetAsnGlyLeuSerAspGluGluGlnArgArgTyrPheGl

3800 3850
 AACAGCGGTTGATCATTGAAAGAGAAATACAGCGCTGAAAACATGCTTTATGCTACAGTCCATATGGATGAAGCGACTCCTCATATGCA
 uThrAlaValAspHisLeuLysGluLysTyrSerAlaGluAsnMetLeuTyrAlaThrValHisMetAspGluAlaThrProHisMetHi
 3900 3950
 TGTTGGTATTGTACCGATCAGAGGACGGCCGACTCTCTCGGAAAGATTTTTTAAATGGCAAATGAAGATGAAAGCCATTCAAGATGA
 sValGlyIleValProIleThrGluAspGlyArgLeuSerAlaLysAspPhePheAsnGlyLysLeuLysMetLysAlaIleGlnAspAs
 4000 4050
 TTTTCATCGGCACATGGTTGAAAACGGTTTTGACCTGGTGGCGGGCAACCAAGCGAAAAGAAGCATGAGAATGTTCCACCGATATAAAAT
 pPheHisArgHisMetValGluAsnGlyPheAspLeuValArgGlyGluProSerGluLysLysHisGluAsnValHisGlnTyrLysI
 4100
 AAATCAGCGGAAACCGGAGCTTGAAGCGCTTAATGCTGAAATGCTTTAAAGGAAAAGCAGAGAGGAACTGGAAAAGCAAAAACAAGC
 eAsnGlnArgGluProGluLeuGluArgLeuAsnAlaGluIleAlaLeuLysGluLysGlnArgGluGluLeuGluLysGlnAsnLysAl
 4150 4200
 TGTTCAAGCAGTTATAGAAGTGA AAAAAGAATCGCTGACAGCTAAGGCTGAAGAGTTGAAAATGCGCACTATTGAACATGAAAAGCGTG
 aValGlnAlaValIleGluValLysLysGluSerLeuThrAlaLysAlaGluGluLeuLysMetProThrIleGluHisGluLysAlaTr
 4250 4300
 GCTCAAAAAGGATAAAGTCAITGTGOCAGCGGGAACTCCATGCTTTGTATGCTATGCGGAGCAGAAAATAAAAAGCGGAGCGGAGTT
 pLeuLysLysAspLysValIleValProGluArgGluLeuHisAlaLeuTyrAlaTyrAlaGluGlnLysThrLysThrAlaAlaGluLe
 4350 4400
 GCGCGGGCAATTGAAGTCGAAACCGCAGGAAAAGGAGCGCTGCGCAGTCTATCGCCCGCAGAAAGCAGATCGGGCGGATGAAAAGACCAA
 uAlaGlyGlnLeuLysSerGluThrGlnGluLysGluArgTrpGlnSerIleAlaArgGlnLysGlnIleGlyArgMetLysLysThrAs
 4450 4500
 CGGCTTCAGGAACTGCAGATAGGATCCATTGAGAAGTTGAAGCGTCCAAAAGGAAATCCGCGCAAGCTTCAAAAAGGAAATTTACGGAA
 nGlyPheArgAsnCysArgValGlySerIleGlnLysLeuLysArgProLysArgLysCysGlyAlaSerLeuGlnArgAsnLeuArgLy
 4550
 GAACAAGCGTCAGGATCTTCGGCAGGAAGTGAAGAGGAACTGACGACTTTACGAAACGGAAAACGAGGAACTGTCAGCTGAAAATAAAGT
 sAsnLysArgGlnAspLeuArgGlnGluValLysGluGluLeuThrThrLeuArgThrGluAsnGlyLysArgAsnGlyGlnTyrAlaGluValLe
 4600 4650
 TTTGATCATTCAAAGAAATAGCGAAGCTGCGGAGAGCCTAAAACCTAAAACAGGAACTTGATAAGAGAAAACGGGAGTATGCTGAGGTTTT
 lLeuIleIleGlnArgAsnSerGluAlaAlaGluSerLeuLysLeuLysGlnGluLeuAspLysArgAsnGlyGlnTyrAlaGluValLe
 4700 4750
 GAGTTTCGCCAAGAAGCAGAACTCAAACGCTGAAAAGTGGCTGGAGAAAACAAGCGCTTAAAAAAGAAAATAAGCACTAAAAGAGAG
 uSerPheAlaLysLysAsnGlnThrLeuGluLysValAlaGlyGluAsnLysAlaLeuLysLysGluAsnLysThrLeuLysGluAr
 4800 4850
 AGTTGCGCTACTGGAACAATGGAAGACAAAATGGTTCAAGTGGCTTAAAGAAAATACCAAAGATGCGGAAATAGCGGCATCGTTTTT
 gValAlaValLeuGluGlnTrpLysAspLysMetValGlnTrpAlaLysGluLysLeuProLysMetArgLysLeuAlaAlaSerPhePh
 4900 4950
 CGTACGGCTGGAATGCCTAGAGAAGCCAATAAATACAAGGACAATGAATTAGAGCGGTGAAAACGGGCAATCAATTTGCCCTTCCAAAATT
 eValArgLeuGluCysLeuGluLysProIleAsnThrArgThrMetAsn***
 5000
 TCCACCACITTTTTTGGTGGTGGCATCTTTTTTGTGTCTCAAGGGACTCAAGTAAATCGACGGGTGAGTGTCTCGTGCCTTTCTT
 5050 5100
 GCTGTCTTTTTTCCGAATCGTCCATCCGTTCTGCCATGTGGCGTTGAATCTTCTCTGTCTTCATGAATTCGACTAAAGATTGTCTCT
 5150 5200
 GTAGCGATGTAGCGGTATCCGATATGGTCAGTTTAGAACGATATAAGCTTGTCTATGTGCTTTACGGTTTCGTGAGTGAGTGCATTGA
 5250 5300
 TCTTAGTCATTGTACATAGATACTCTAAAGTCTTTACGTCATCCTCGGTATAGAGTCGCCATCCTTTCGAATCTTTATTGAACGAGTAGC
 5350 5400
 CTTGTTCTCAAGCATACTGACATACTGCGGACAGTTACTGGCTCTATACCGAGGTGTTTTGCGACGTCTTTGATGATAAATTTGATTC
 5450
 CCATATCCATAAGCTATCACCTCGTAAAAAGGGTTCGCTATAGTGGGGCAAAACCCGTTTATTTAGAGCCATTTAAAAAGAAAGCTGTT
 5500 5550
 CGTCTCTTTTATCAGGTTGGGACTGTATTTAGTTTTATGGGGAGTCCCAATGAGTGATTTTGAACAAGGAATGAGATATGTAGACCAACC
 5600 5650
 CITGGCTTTGAGGTTTAGTGCGTACGGAAGAAGAGAAAAGCTTTTAGAAAAGACGGTTTCATGGAGAAATCACAGAGGAAGAATACATA

ved in a segment of 129 residues of E229 with pUH1 rep, and 51.2% in 86 residues of C403 with γ -glutamyltranspeptidase, but homology scores, in its entirety, were quite low value such as 11.9% with pUH1 rep and 37.4% with γ -glutamyltranspeptidase, respectively. The cDNAs of rat renal (Laperche et al., 1986) and human hepatic (Sakamuro et al., 1988), hepatoma (Goodspeed et al., 1989), and placental (Meytset al., 1988) γ -glutamyltranspeptidase were cloned, and their nucleotide sequences were determined. The mammalian γ -glutamyltranspeptidase, whose amino acid sequences were essentially the same, do not show any similarity at all with pUH1 γ -glutamyltranspeptidase (data not shown). More recently, Suzuki et al. (1989) performed DNA sequencing of *E. coli* γ -glutamyltranspeptidase, but no convincing homology could be found between pUH1 and *E. coli* γ -glutamyltranspeptidases, for which complete sequence data is available.

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