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Cloning and Nucleotide Sequence of γ-Polyglutamate Production Stimulating Factor on *Bacillus subtilis* (*natto*) Plasmid, pUH1

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The gene coding for γ-polyglutamate production stimulating factor (*psf*) from Bacillus *subtilis* (*natto*) plasmid pUH1 was cloned and sequenced in *Bacillus subtilis* host. The activity of y-glutamyltranspeptidase (γ-GTP) in *B. subtilis* host, which was introduced the *psf* gene was relatively high level, while its activity in *E. coli* host could not be detected. The nucleotide sequence of *psf* gene was determined and an open reading frame encoding a polypeptide composed of 420 amino acid residues (Mr, 49, 356) was identified. The putative -35 and -10 sequences, TTCAAA and TATTAT, were observed as the consensus sequence for the promoter recognized by the σ^{43} RNA polymerase of *B. subtilis*, and the ribosome binding site, whose sequence was AACGAG, was complementary to the binding sequence of *B. subtilis* 16S rRNA except for one base. The amino acid sequence of *psf* with the segment of putative protein C403 of staphylococcal plasmid pE194 indicates homology, whereas that with *E. coli* and mammalian γ-GTPs does not show any similarity at all.

INTRODUCTION

"Natto" is a Japanese traditional fermented food, manufactured by growing *Bacillus subtilis* (*natto*) on steamed soybeans. It is an adhesive, and consists of polysaccharide (levan-form fructan) and y-polyglutamate (γ-PGA). The adhesive material is mainly composed of γ-PGA containing D-and L-glutamate in varying proportions (Fujii, 1963). A plausible mechanism of the biosynthetic pathway of γ-PGA has been proposed by Thorne et al. (1955) for one of the γ-PGA-producing strains (*B. licheniformis* ATCC9945A). However, since it is known that various strains differ a great deal in the basic requirements for γ-PGA synthesis as a capsule, it is to be assumed that there is more than one biosynthetic pathway. We reported that a 5.8-kb plasmid designated pUH1, which is responsible for y-PGA synthesis, is distributed widely in *B. subtilis* (*natto*) strains isolated from a non-salty fermented soybean food, natto (Hara et al., 1983 ; 1993 ; unpublished results).

y-Glutamyltranspeptidase (γ-GTP, EC2.3.2.2) catalyzes the transfer of the γ-glutamyl residue from y-glutamyl compounds, such as glutathione, to amino acids and peptides, and the hydrolysis of y-glutamyl compounds (Tate and Meister, 1981), but its physiological role still remains controversial. The cDNAs of rat renal (Laperche *et al.*, 1986) and human hepatic (Sakamuro et *al.*, 1988) γ-GTPs were cloned, and the nucleotide sequences were determined. Recently, Suzuki et *al. (1989)* reported the

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nucleotide sequence of *Escheyichia coli K-12* y-GTP. However, the homology percent of amino acid sequence between the *E. coli* and mammalian γ-GTPs was quite low.

We have succeeded in expressing γ-GTP activity in *B. subtilis* host strains, which were introduced a γ-PGA production stimulating factor (*psf*) gene. The *psf* gene might be responsible for γ-PGA synthesis in B. *subtilis* (*natto*). In the present work, we have sequenced the *psf* gene and compared with the *E. coli* and mammalian γ-GTPs.

MATERIALS AND METHODS

Bacterial strains and **plasmids**

Escheyichia coli JMlOl (*supE*44, *thi*, Δ(*lac-proAB) [F', proAB, lacIqZ*ΔM15, *traD*36], *mcrA*$^+$) and *Bacillus subtilis MI112 (ayg-15, leuB8, thy-5, recE4)* were used as hosts for cloning and nucleotide sequencing. Plasmid pUH1 has been previously described (Hara *et al.,* 1983). Plasmid pUB110 was used as a vector for *B. subtilis* host, and plasmids pATE1 (Hara *et al.,* 1991) was for *E. coli.*

Media

LB broth and Panassary broth (Difco) for both *B. subtilis* and *E. coli*, Spizizen minimal medium for *B. subtilis,* and M9 minimal medium for *E. coli* were the same as described previously (Hara *et al.,* 1991). The cells carrying Km' plasmids were grown in AA medium (Tanaka and Sakaguchi, 1978) containing kanamycin *(50* μ*g*/ml).

DNA manipulations

The plasmid pUH1 from *B. subtilis* (*natto*) strain Asahikawa and its derived plasmids from Km' transformants were prepared and purified as previously described (Hara *et al.,* 1983) was digested with *Bst*EII, and then the ends were filled in with Klenow fragment to generate blunt ends. The DNA fragments were ligated with *Bam*HI liker, inserted at the *Bam*HI site of pUB110 with T4 ligase and then added to *B. subtilis* MI112 protoplasts. Restriction enzymes, T4 DNA ligase and bacterial alkaline phosphatase were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan), and used as recommended by the manufacture. Degradation of DNA with exonuclease Ba131 (Takara Shuzo Co., Ltd.) followed by procedure of Legerski *et al.* (1978).

Transformation procedure

E. coli JM101 was transformed by the method of Morrison (1977), and *B. subtilis MI112* was transformed by using protoplasted cells (Hara *et al.,* 1991).

Assessment of y-glutamyltranspeptidase assay

After cultivation at time indicated in the medium described by Fujii (1963), cells were harvested, suspended in 0.9% NaCl, and disrupted with a sonic oscillator (Branson Sonifer 185). The sonicated cells were centrifuged and the supernatant solution was used as intracellular enzyme preparation. The extracellular enzyme activity was measured in the culture fluid removed the cells by centrifugation. γ-GTP activity was assayed as previously described (Aumayr *et al.,* 1981). One unit of γ-GTP activity is defined as the amount of enzyme which liberates 1μmol of a-naphthylamine per min at 37°C.

DNA sequencing

DNA fragments were subcloned into plasmids pUC18 and pUC19, and then, if need, the Bal31-deleted derivatives were obtained by the stepwise deletion method of McCutchan et ***al.*** (1984). DNA sequencing was carried out by means of the dideoxy chain termination method (Sanger et ***al.,*** 1977) with Sequenase Version 2.0 DNA Sequencing Kit (United States Biochemical Corporation, Cleveland, Ohio). Nucleotide and amino acid sequences were analyzed by the Hitachi DNASIS system.

Chemicals

Restriction enzymes, **T4 DNA** ligase and bacterial alkaline phosphatase were purchased from Takara Shuzo Co., Ltd., and used as recommended by the manufacture. Degradation of DNA with exonuclease Bal31 (Takara Shuzo Co., Ltd.) followed by procedure of Legerski et ***al.*** (1978).

RESULTS AND DISCUSSION

Cloning of *psf* gene in B. *subtilis*

As reported previously (Hara et ***al.,*** 1981), the *psf* gene is encoded on an endogenous plasmid, pUH1 from *B.* ***subtilis*** (*natto*). **We** constructed a set of derived plasmids using pUB110 as a vector, in which a series of pUH1 fragments were inserted. Figure 1 shows the subclones of *B.* ***subtilis*** (*natto*) plasmid pUH1 and their expression of *psf* in *B.* ***subtilis*** MI112. The *psf* activity was detected in several Km’ transformants, which carried plasmid pPB1 (column BsL in Fig. 1) with molecular size of 8.2 kb, including 3.7-kb BstEII fragment of pUH1. To define the boundaries of a functional unit of inserted DNA, a 3.7-kb BstEII fragment of pUH1 was digested with selected restriction endonucleases to obtain a set of overlapping DNA fragments. The plasmid DNA preparations containing each generated fragments were tested for the enzyme activity in *B.* ***subtilis.*** The deletion experiments revealed that a 1.7-kb

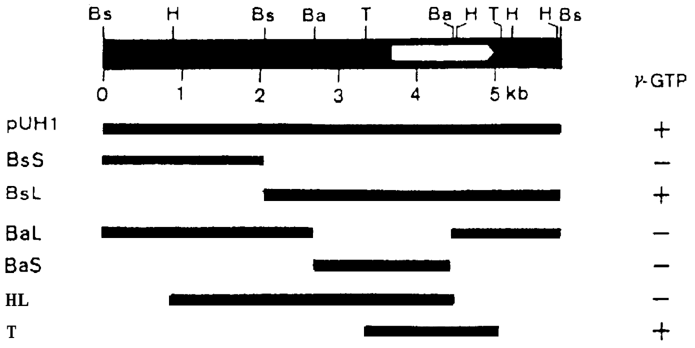

Fig. 1. Subclones of *B. subtilis* (*natto*) plasmid pUH1 and their expression of γ-GTP in *B. subtilis M1112.*

The open *arrow* in the BstEII fragment indicates an open reading frame found in the sequence data (see Fig. 3). The activity of γ-GTP expressed was assayed as previously described (Aumayr *et al.,* 1981). Abbreviations for restriction sites are : Ba, *Bam*HI ; Bs, BstEII ; **H,** Hind111 ; T, *Taq*I.

Table 1. γ-GTP activities in *B. subtilis* and *E. coli* strains carrying *psf* gene.

Strain		*psf*	γ-GTP activity”	
			Intracellular	Extracellular
B. subtilis	Asahikawa	+	91.8	423
B. subtilis	MI112		10.1	22.5
	MI112 (pBS)	+	93.5	91.5
E. coli	JMlOl	–	8.3	10.6
	JMlOl (pHN-1)	+	8.0	10.5

[a] Enzyme activities were measured under the conditions described in Materials and Methods.

*Taq*I fragment (column T in Fig. 1) is necessary for the expression of *psf* gene in *B. subtilis* MI112.

Expression of *psf* gene in B. *subtilis* and *E. coli*

The presence of *psf* gene on plasmid pUH1 was confirmed by measuring the γ-GTP activity in strains carrying the *psf*. These results are summarized in Table 1, which includes the enzyme productivity of *B. subtilis* (*natto*) strain Asahikawa. The *B. subtilis MI112* (pBS) carrying recombinant plasmid pBS expressed very high γ-GTP activity intracellularly at 93.5 mU/ml in the PY culture medium growth at 37°C for 24 hr, while the strain MI112 (pBS) accumulated extracellularly γ-GTP at level of 91.5 mU/ml for 5 days. In order to compare the productivity of γ-GTP in *E. coli* with that in *B. subtilis*, *we* subcloned the 1.7-kb *Taq*I fragment of pUH1 into pATE1 constructed from pTL12 (Tanaka and Sakaguchi, 1982) (the recombinant plasmid obtained was designated pHN1). However, *E. coli* JMlOl carrying pHN1 did not express intracellularly the γ-GTP activity in LB broth grown at 30°C for 24hr. Also, γ-GTP activity could not detect extracellularly after even 5-days cultivation.

Nucleotide sequence of pUH1 *psf* gene

Figure 2 shows the physical map and sequencing strategy of 1.7-kb *Taq*I fragment including the *psf* gene of *B. subtilis* (*natto*) plasmid pUH1. The nucleotide sequences of both strands of the 1.7-kb *Taq*I fragment were determined by the dideoxynucleotide chaintermination method (Sanger et *al.*, 1977) after successive Ba131 exonuclease deletions (Davis *et al.*, 1986). The nucleotide sequence was determined for both strands by using numerous restriction fragments to give enough overlapping regions. Figure 3 shows the nucleotide sequence of the 1.7-kb *Taq*I fragment. By examination of possible open reading frames (ORFs), we found only one large frame, which consists of 1,260 bp and encodes a protein molecule with 420 amino acids and an *M*r of 49,356. The *psf* ORF was found to be preceded by a putative σ^{43} RNA polymerase promoter of *B. subtilis*. The 5’ upstream region of *psf* gene contains a 5’-AACGAG-3’ sequence (indicated as SD in Fig. 3) complementary to the 3’ end of 16S rRNA (3’-OH-UCUUUC-CUCCAGUAG-5’) of *B. subtilis* (McLaughlin et al., 1981) at nucleotides 295 to 300 for translation initiation. There is a 5’-TATTAT-3’ sequence (-10 in Fig. 3) resembling a Pribnow box (Moran et *al., 1982)* at nucleotides 257 to 262, and at a site of 23 bp upstream of this -10 sequence, there is a 5’-TTCAAA-3’ sequence resembling the -35 sequence (Moran *et al.*, 1982) of *B. subtilis* gene. The observed distance (17 bp)

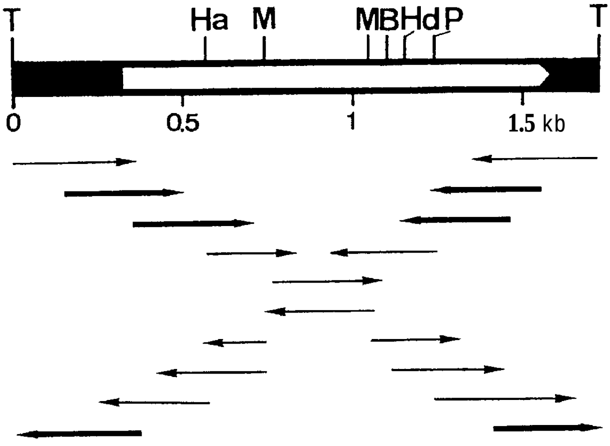

Fig. 2. Restriction map and sequencing strategy for the *psf* gene of *B. subtilis* (*natto*) plasmid pUH1.

The open arrow in the restriction map indicates an open reading frame found in the sequence data (see Fig. 3). Below the map, arrows indicate the direction and extent of sequencing either of subcloned restriction fragments (thin arrows) or of a series of deletion fragments prepared by Bal31 (thick arrows). Abbreviations for restriction sites are: B, *Bam*HI; Ha, *Hae*III; Hd, *Hin*dIII; M, *Msp*I; P, *Pvu*II; T, *Taq*I.

between the -10 and -35 sequences accords well with that observed generally in *B. subtilis* genes (17-18 bp) (Moran *et al.*, 1982).

Homology of PSF to other y-GTP genes

The amino acid sequence of the predicted protein PSF encodes on pUH1 was compared with a number of protein sequences registered in GenBank with used of the homology search system of GENAS (Kuhara et *al.*, 1984). As shown in Fig. 4, sequence homologous to the pUH1 PSF were found to in putative protein C403 (420 residues ; Horinouchi and Weisblum, 1982) with 48,300 daltons encoded in staphylococcal plasmid pE194. Approximately, 51.2% amino acid homologies with pUH1 PSF was observed in a segment of 86 residues of C403, but percent match of both proteins, in its entirety, was quite low value such as 11.9%. The cDNAs of rat renal (Laperche *et al.*, 1986) and human hepatic (Sakamuro et al. 1988), hepatoma (Goodspeed et *al.*, 1989), and placental (Meyts et al., 1988) γ-GTPs were cloned, and the nucleotide sequences were determined. The mammalian γ-GTPs, whose amino acid sequences were essentially the same, are synthesized as single polypeptides and then processed into the large and small subunits (Matsuda and Katsunuma, 1984), but did not show any similarity at all with pUH1 PSF from *B. subtilis* (*natto*). Percent match of amino acid sequence of pUH1 PSF for human hepatic y-GTP was 8.4%. Recently, Suzuki et *al.* (1989) performed DNA sequencing of *E. coli* y-GTP, and they suggested that the *E. coli* y-GTP might be also processed posttranslationally. The homology of amino acid

```
                                    TaqI       .               .                    50
                                    TCGATGCGACAGCGAGAATGAGAGACGCACCAGCACCGCAGGTCGCACGTCCAAATTT

                                   .     100             .                     .                   1
GCCATGGCATAATTTGGTGTAGTGCGTTACACCAAAGATAAACTTTGTGTTACCATAACCCCTATACAGTGGTCTGAATCGGGGGTTTTT

50                        .         .          .       200          .          .         .     (-35)
CTCATGGCAAATTATGCAGTCATCAGGATGGAAAAATACAAAAAAGATAGATTGAATGGAAC CGCAAAAACACAATCAGCGGGAGTTTCAA

 .         250       (-10)         .         .           .       (SD)          .          .
AAAAGCAAAAATGAAAAGTATTATCGGGAGCGGACGTCCTAAATTCTGATCTGGTGAACGAGGAACCGATTAGCTATTCAAAAGCGGTTC

            .          350                                 .             400
ATGAAAAAATTGAGGGTCGGGTCAAACGGAAGGTCCGAGCGGATGCGTGTTTTGGTCAGCGAATTTTTGATCACGGCAAGTCCTGACTAT
MetLysLysLeuArgValGlySerAsnGlyArgSerGluArgMetArgValLeuValSerGluPheLeuIleThrAlaSerProAspTyr
                                  450                              .                    500
ATGAAAGGGCTGAGTGATGAGGAGCAGCGGCGCTATTTTGAAACAGCGGTTGATCATTTGAAAGAGAAATACAGCGCTGAAAACATGCTT
MetLysGlyLeuSerAspGluGluGlnArgArgTyrPheGluThrAlaValAspHisLeuLysGluLysTyrSerAlaGluAsnMetLeu
                                             550                    .                         6
TATGCCACAGTCCATATGGATGAAGCGACTCCCCATATGCATGTTGGTATTGTACCGATCACGGAGGACGGCCGACTCTCTGCGAAAGAT
TyrAlaThrValHisMetAspGluAlaThrProHisMetHisValGlyIleValProIleThrGluAspGlyArgLeuSerAlaLysAsp
00                                                      650
TTTTTTAATGGCAAATTGAAGATGAAAGCCATTCAAGATGATTTTCATCGGCACATGGTTGAAAACGGTTTTGACCTGGTGCGCGGCGAA
PhePheAsnGlyLysLeuLysMetLysAlaIleGlnAspAspPheHisArgHisMetValGluAsnGlyPheAspLeuValArgGlyGlu
            700                                .                    750
CCGAGCGAAAAGAAGCATGAGAATGTTCACCAGTATAAAATCAATCAGCGGCAAGCGGAGCTTGAGCGGCTTAATGCTGAAATTGCTTTA
ProSerGluLysLysHisGluAsnValHisGlnTyrLysIleAsnGlnArgGlnAlaGluLeuGluArgLeuAsnAlaGluIleAlaLeu
                       800         .                                   850
AAGGAAAAGCAGAGAGAGGAACTGGAAAAGCAAAACAAAGCTGTTCAAGCAGTTATAGAAGTGAAAAAAGAATCACTGACAGCTAAGGCT
LysGluLysGlnArgGluGluLeuGluLysGlnAsnLysAlaValGlnAlaValIleGluValLysLysGluSerLeuThrAlaLysAla
             .         .          900          .           .                     .          950
GAAGAGTTGAAAATGCCGACTATTGAACATGAAAAAGCGTGGCTCAAAAAGGATAAAGTCATTGTGCCGGAGCGGGAACTCCATGCTTTG
GluGluLeuLysMetProThrIleGluHisGluLysAlaTrpLeuLysLysAspLysValIleValProGluArgGluLeuHisAlaLeu
                        .                   1000            .                                10
TACGCCTATGCGGAGCAGAAAACTAAAACGACAGCCGAGCTGGCGGGGCAATTGAAGTCAGAAACGCAGGAAAAGGAGCGCTGGCAGTCT
TyrAlaTyrAlaGluGlnLysThrLysThrSerAlaGluLeuAlaGlyGlnLeuLysSerGluThrGlnGluLysGluArgTrpGlnSer
50                                                     1100         .          .
ATCGCCCGGCAGAAGCAGATCGGGCAGATGAAAAAGACCAACGGCTTCAGGAACTGCAGAGTAAGATCCATTCAGAAGTTGAAGCGTCCA
IleAlaArgGlnLysGlnIleGlyGlnMetLysLysThrAsnGlyPheArgAsnCysArgValArgSerIleGlnLysLeuLysArgPro
           1150         .                    .                    1200
AAAAGGAAATGCGGCGCAAGCTTGCAAAGGAATTTACGGAAGAGCAAGCGTGAGGATCTTCGGCAGGAAGTGAAAGAGGAGCTGACGGCT
LysArgLysCysGlyAlaSerLeuGlnArgAsnLeuArgLysSerLysArgGluAspLeuArgGlnGluValLysGluGluLeuThrAla
                      1250                                         .          1300
TTACGAACGGAAAATAAGGAACTGCTAGCTGAAAATAAAGTCTTGATCATTGAGAGGAATCGTGAAGCTGAGGAAAACTTAAAACTAAAA
LeuArgThrGluAsnLysGluLeuLeuAlaGluAsnLysValLeuIleIleGluArgAsnArgGluAlaGluGluAsnLeuLysLeuLys
             .                   1350                              .          .          1400
CAGGAACTTGATAAGAGAGACGGGCAGTATGCTGAGGTTTTGAGTTTCGCCCAGAAGCAGAACCAAACACTTGAAAAAGTGGCTGGAGAA
GlnGluLeuAspLysArgAspGlyGlnTyrAlaGluValLeuSerPheAlaGlnLysGlnAsnGlnThrLeuGluLysValAlaGlyGlu
                        .                   1450            .          .                     15
AACAAGGCATTAAAAAAAGAAAATAAGACACTAAAAGAGAGAGTTGCTGTATTAGAAGAGTGGAAAGATAAAATGGTGCAGTGGGCTAAA
AsnLysAlaLeuLysLysGluAsnLysThrLeuLysGluArgValAlaValLeuGluGluTrpLysAspLysMetValGlnTrpAlaLys
00                                            .         1550
GAAAGGTTACCAAAGATGCGGAAATTAGCGGCATCGTTTTTCAATACAGCAGGTATCGTCGAGAAGCTGCTAAATATAAGGACAATGAAT
GluArgLeuProLysMetArgLysLeuAlaAlaSerPhePheAsnThrAlaGlyIleValGluLysLeuLeuAsnIleArgThrMetAsn
           1600                                                     1650
TGGAGCGATAAATGGTTTTCTTAAGCGGTGAAAACGGGCAAGTGAATTAGTCTTCCAAAATTGCCACCACTTTTTTTGTTTAGTGGCTGC
TrpSerAspLysTrpPheSer***                 ------>                <------
                      1700         .     TaqI
AATCATTTTTTGTGTCTCCAGTGATTCTCGTAAAGCTTCGA
```

Fig. 3. Nucleotide sequence and the deduced amino acid sequence of *psf* gene of *B. subtilis* (*natto*) plasmid pUH1.

The putative -35, -10 and SD sequences were indicated by underlines and the possible terminator sequences are shown by arrows. Nucleotides are numbered from the 5' end of *Taq*I site of the 1.7-kb DNA strand with the same polarity as mRNA. Amino acid sequences are shown below the coding frame.

```
                                  10        20        30        40        50
1 S. aureus pE194 C403    MSHSILRVARVKGSSNTNGIQRHNQRKNKNYNNKDINHEETYKNYDLINA
2 pUH1 PSF
3 pNKH PSF
4 E. coli K-12 γ-GTP         MIKPTFLRRVAIAALLSGSCFSAAAAPPAPPVSYGVEEDVFHPVRAKQ
5 Human hepatic γ-GTP                  VKKKLWLGLLAVVLVLVIVGLCLWLPSASKHPDNHVYTR

          60        70        80        90       100       110       120
1 QNIKYKDKIDETIDENYSGKRKIRSD-AIRHVDGLVTSDKDFFDDLSGEEIERFFKDSLEFLENEYGKEN
2             MKKLRGESNGRSERMHVLVSEFLITASPDYMNGLSDEEQRRYFETAVDHLKEKYSAEN
3             MKKLRGESNGRSERMRVLVSEFLITASPDYMKGLSDEEQRRYEETAVDHLKEYYSAEN
4 GMVASVDATATQVGVDILKEGGNAVDAAVAVGYALAVTHPQAGNLGGGGFMLI-RSKNGNTTAIDFR-EM
5 AAVAADAKQCSKIGRDALRDDDSAVDAAIAALLCVGLMNAHSMGTGGGLFLTIYNSTTRKAEVINAR-EV

         130       140       150       160       170       180       190
1 MY-LAT----------VHLDERVPHMHFGFVPLTEDGRLSAKEQLGNKKDFTQLQDRFN--EYVNEKGYE
2 ML-YAT----------VHMDEATPHMHVGIVPITEDGRLSAKDFFNGKLKMKAIQDDFH--RHMVENGFD
3 ML-YAT----------VHMDEATPHMHVGIVPITEDGRLSAKDFFNGKLKMKAIQDDFH--RHMVENGFD
4 APAKATRDMFLDDQGNPDSKKSLTSHLASGTPGTVAGFSLALDKYGTMPLNKVVQPAFKLARDGFIVNDA
5 APRLAFATMF-NS-SE-QSQKG--G-LSVAVPGEIRGYELAHQRHGRLPWARLFQPSIQLARQGFPVGKG

         200       210       220       230       240       250       260
1 LERGTSKEVT-EREHKAMDQYKKDTVFHKQELQEVKDELQKANKQLQSGIEHMRSTKPFDYENERTGLFS
2 LVRGEPSEKKHENVHQYK-INQREPELERLNAEIALKEKQREELEKQNKAVQAVIEVKKESLTAKAEELK
3 LVRGEPSEKKHENVHQYK-INQRQAELERLNAEIALKEKQREELEKQNKAVQAVIEVKKESLTAKAEELK
4 LADDLKTYGSEVLPNHENSKAIFWKEGEPLKKGDTLVQANLAKSLEMIAENGPDEFYKGTIAEQIAQEMQ
5 LAAALEN-KRTVIEQQPVLCEVFCRDRKVLREGERLTLPQLADTYETLAIEGAQAFYNGSLTAQIVKDIQ

         270       280       290       300       310       320       330
1 GREETGRKILTADEFERLQETISSAERIVDDYENIKSTDYYTENQELKKRRESLKEVVNTWKEGYHEKSK
2 MPTIEHEKAWLKKDKVIVPERELHALYAYAEQKTKTAAELAGQ-LKSETQE-KERWQSIARQEADR-ADE
3 MPTIEHEKAWLKKDKVIVPERELHALYAYAEQKTKTTAELAGQ-LKSETQE-KERWQSIARQEADR-ADE
4 KNGGLITKEDLAAYKAV-ERTPISGDYGYQVYSMPPPSSGGIHIVQILNILENFDMKKYGFGSAD--AM-
5 AAGGIVTAEDLNNYRAELIEHPLNISLGDAVLY-MPSAPLSGPVLALILNILKGYNFSRESVESPEQKGL

         340       350       360       370       380       390       400
1 EVNKLKRENDSLNEQLNVSEKFQASTVTLYRAARANFPGFEKGFNRLKEKFFNDSKFERVGQFMDVVQDN
2 KDQRLQELQSRIHSEVEASKKEMRRKLAKEFD---T---EEQRQDLRQEVKEELTTLRTENEELSAENKV
3 KDQRLQELQSKIHSEVEASKKEMRRKLAKEFD---T---EEQREDLRQEVKEELTALRYENKELLAENKV
4 -QIMAEAEKYAYADRSEYLGDPDFVKVP-W--WALTNKAYAKSIADQIDINKAKPSSEIRPGKLAPYESN
5 TYHRIVEAFRFAYAKRT-LLGDPKFVDVTEVVRNMTSEFFAAQLRAQISDDTTHPISYYKPEFYTP-DDG

         410       420       430       440       450       460       470
1 VQKVDRKREKQRTDDLEM
2 LIIQRNSEAAESLKLKQELDKRNGQYAEVLSFAKKQNQTLEKVAGENKALKKENKTLKERVAVLEQWKDK
3 LIIERNREAEENLKLKQELDKRDGQYAEVLSFAQKQNQTLEKVAGENKALKKENKTLKERVAVLEEWKDK
4 QTTHYSVVDKDGNAVAVTYTLNITFGTGIVAGESGILLNNQMDDFSAKPGVPNVYGLVGGDANAVGPNKR
5 GTAHLSVVAEDGSAVASTSTINLYFGSKVRSPVSGILFNNEMDDFSS-PSITNEFGVPPSPANFIQPGKQ

         480       490       500       510       520       530       540
2 MVQWAKFKLPKMRKLAASFFVRLECLEKPINTRTMN
3 MVQWAKEKLPKVRRLAVSFFNTAGIVEKLLNIRTMNWSDKWFS
4 PLSSMSPTIVV-KDGKTWLVTGSPGGSRIITTVLQMVVNSIDYGLNVAEATNAPRFHHQWLFDELRVEKG
5 PLSSMCPTIMVGQDGQVRMVVGAAGGTQITTATALAIIYNLWFGYDVKRAVEEPRLHNQLLFNVTTVERN

         550       560       570       580       590       600
4 FSPDTLKLLEAKGQKVALKEA-MGSTQSIMVGPDGELYGASDPRSVDDLTAGY
5 IDQAVTAALETRHHHTQIASTFIAWQAIVRTAGG-WAAASDSRKGGE-PAGY
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Fig. 4. Comparison of the amino acid sequences of the pUH1 and pNKH PSFs, the putative protein C403 of pE194 and the *E. coli* and human hepatic γ-GTPs.

The putative C403 encodes on staphylococcal plasmid pE194 is from Horinouchi and Weisblum (1982). The pNKH PSF is from Hara *et al.* (1994). The *E. coli* y-GTP is from Suzuki et al., (1989). The human hepatic y-GTP sequence is from Sakamuro *et al.*, (1988). Gaps have been inserted to gain maximum matching. The one-letter amino acid code has been used. Percent match of amino acid sequence of pUH1 PSF for C403 on plasmid pE194 was 11.9%, and for *E. coli* and human hepatic γ-GTPs were 7.8% and 8.4%, respectively.

sequences between the *E. coli* y-GTP and pUH1 PSF is relatively low, and percent match was only 7.8%. Furthermore, the percent match of amino acid sequence among mammalian and *E. coli* γ-GTPs was not so high (ca. 30%). Though mammalian γ-GTPs are connected with metabolism of glutathione-related compounds, but the organisms cannot procedure γ-PGA, like B. *subtilis* (*natto*) strains. It is, therefore, to be assumed that there are more than one enzyme catalyzes the transfer of the y-glutamyl residue from γ-glutamyl compounds, and that one of them plays a important role in y-PGA synthesis in B. *subtilis* (*natto*).

Recently, the B. *subtilis* y-GTP was cloned and sequenced by 'Katsumata et al. (1991). The sequence contains a single open reading frame encoding the signal peptide and large and small subunits, in that order, as is the case with *E. coli* y-GTP (Suzuki *et al.*, 1989), but should be concerned with y-PGA production in *B. subtilis* (*natto*).

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