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Sequence Analysis of Replication Origin of Plasmid pCTP4 in y-PGA-producing **Bacillus** Strain isolated from "Dan-douchi" in China

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The structure of a 1.6-kb SphI-HindIII DNA sequence necessary and sufficient for the replication of a 6.3-kb plasmid pCTP4 in y-PGA-producing Bacillus strain isolated from "dan-douchi" in China, which is responsible for y-polyglutamate production, has been characterized by using a trimethoprim-resistance gene derived form B. subtilis TTK24 chromosomal DNA as a selective marker. The 1.6-kb DNA sequence contains a *rep* gene encoding the protein (333 amino acids) essential for initiation of replication and a possible origin of replication. The predicted REP protein of pCTP4 has an overall homology with the REP proteins of pUH1 (73.9% identity), pLS11(87.4%), pBAA1(82.0%), and pFTB14 (76.6%) in Bacillus spp., pLP1 (46.5%) and pLAB1000 (32.4%) in Lactobacillus spp., and pUB110 (34.8%) and pC194 (21.9%) in Staphylococcus aureus, but has not any similarity with the REP protein of the staphylococcal plasmid pT181.

INTRODUCTION

"Douchi" is a native fermented product made from whole soybeans in China. There are two kinds of "douchi" as a raw fermented product : without or with salt, i. e., "dan-douchi" and "xian-douchi." "Xian-douchi" is made from steamed soybean and salt using the koji molt of *Aspergillus oryzae*, and it is found to greatly resemble "hamanatto" in Japan. "Dan-douchi," the non-salty aging product of steamed soybean by the biological action of "natto" Bacillus-like microorganisms, is an adhesive fermented soybean with a noticeable odor of ammonia, and is, then considered to be the same as Japanese "natto" (Nakao, 1972). After fermentation, the raw "dan -douchi" is sun-dried, and then the dark-colored product is mixed with red pepper and ginger and aged for a while before being eaten. However, there are some striking and possesses some acidity, whereas "natto" is predominantly sticky and contains no acid (Bo, 1984).

We have reported that a 5.8-kilobase pair (kb) plasmid designated pUH1, which encodes the y-polyglutamate-production stimulating factor (psf) gene responsible for y-polyglutamate production, is distributed widely in a number of *Bacillus subtilis* (*natto*) strains isolated from a fermented soybean food, "natto" (Hara et al., 1981 and 1983). *B. subtilis* and *B. subtilis* (*natto*) should be considered as one species, but these two bacilli are found to be classified separately on the basis of whether biotin is

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essential for growth or not (Amaha et al., 195'2; Kida *et al.*, 1963). y-Polyglutamate -producing *Bacillus* strain was isolated from "dan-douchi," which required biotin for growth and produced viscous substances, harbored a single plasmid pCTP4 (6.3 kb) (Hara et *al.*, 1993). Recently, electron microscopy showed that a heteroduplex molecule between pUH1 and pLS11 of *Bacillus subtilis* IFO 3022 contains 1.11-kb and 2.14-kb double-stranded termini (Hara et al., 1986). The 2.0-kb *Bst*EII DNA fragment of pUH1, including the 1.11-kb double-stranded region, contains a 999-bp open reading frame, a promoter for the open reading frame, and a possible replication origin upstream of the promoter (Hara et al., 1991).

Analysis of the organization of various plasmids isolated from Gram-positive bacteria, such as pT181 (Projan and Novick, 1988), pC194 (Alonso and Tailor, 1987), pE194 (Dempsey and Dubnau, 1989), pFTB14 (Murai et al., 1987), pLP1 (Perez-Martin *et al.*, 1988), pUB110 (Maciag *et al.*, 1988), pBAA1 (Devine et al., 1989), and pLAB1000 (Josson et al., 1990), has shown that all of the information necessary for replication is located on fragments of about 1.5 kb in length. These fragments harbor a *rep* gene, encoding a protein essential for the initiation of replication (REP) and its corresponding target site.

This communication reports that the 1.6-kb fragment of pCTP4 contains a 999-bp open reading frame, a promoter for the open reading frame, and a possible replication origin upstream of the promoter. Significant homology was observed between the amino acid sequence predicted from the 999-bp open reading frame and those of similar putative REP proteins encoded by the other well-known Gram-positive replicons.

MATERIALS AMD METHODS

Bacterial strains and plasmids. Escherichia coli JM101 and B. subtilis MI112 (arg -15, *leuB8*, thr-5, recE4) were used for a cloning host. The plasmids pCTP4 (Hara et al., 1993) and pATE1 (Hara et al., 1991) have been described previously, and plasmid pTL12 carrying the dihydrofolate reductase gene was kindly provided by Tanaka and Kawano (1980).

Media. LB broth and Panassay 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 those described previously (Hara *et al.*, 1991). The cells carrying trimethoprim-resistant (Tmp^r) plasmids were grown in AA medium (Tanaka and Sakaguchi, 1978) containing 1 mg/ml of Tmp.

DNA manipulations. Plasmid DNAs from *B. subtilis* were prepared and purified as described previously (Hara et al., 1991). Restriction endonucleases, 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 Ba131 (Takara Shuzo Co., Ltd.) was done by the procedure of Legerski *et al.* (1978).

Methods for transformation and assessment of ori function. E. coli was transformed by the method of Morrison (1977), and B. subtilis was transformed by using protoplasted cells (Chang and Cohen, 1979).

DNA sequencing. DNA fragments were subcloned into plasmids pUC18 and

pUC19, and DNA sequencing was done by the dideoxy chain termination method (Sanger et *al., 1977*) with Sequenase (United States Biochemical Corporation, Ohio, USA). Nucleotide and amino acid sequences were analyzed by the Hitachi DNASIS system.

RESULTS AND DISCUSSION

Delimitation of the replication origin

To facilitate the identification of the replication region of pCTP4, we used the trimethoprim-resistant (Tmp^r) dihydrofolate reductase gene of B. *subtilis* 168. A schematic presentation of the constructed plasmids is given in Fig. 1. The source of the dihydrofolate reductase-coding gene was a Tmp^r strain, TTK24, of B. *subtilis* 168 (Tanaka and Sakaguchi, 1978) and has been cloned in the pBR322 plasmid of *E. coli*. DNAs from pTL12, carrying the Tmp^r dihydrofolate reductase gene, which was constructed by Tanaka and Kawano (1980), and pBR322 were both treated with *Eco*RI and *Hind*III, mixed and ligated by T4 ligase, then pATE1 was constructed. Plasmid pCTP4 was digested with *SphI* and *Hind*III, and then the ends were filled in with the Klenow fragment to generate blunt ends. The DNA fragments were mixed and ligated to the *AatI* site of pATE1 by T4 ligase, and then added to B. *subtilis MI112* protoplasts. Several Tmp^r colonies were obtained on AA agar plates containing Tmp (1 mg/ml) and one of them was used for further study. A plasmid, pNH3, carried in such a Tmp^r colony had a molecular size of 8.2kb (Fig. 1). The physical map of pNH3 using various restriction enzymes is shown in Fig. 1.

To define the boundaries of a functional unit of pCTP4 replication, the 1.6-kb SphI -HindIII fragment of pCTP4 was digested with selected restriction endonucleases to obtain a set of overlapping DNA fragments. The digests filled in with Klenow fragment were ligated with pATE1, introduced into *E. coli* by transformation, and selected for ampicillin resistance. The plasmid DNA preparations containing each generated fragment were tested for replication in B. *subtilis*. The results are summarized in Fig. 2. The constructed plasmid with the 1.4-kb SphI-HaeIII fragment (fragment 2 in Fig. 2) could replicate in the B. *subtilis* host, but the recombinant plasmid preparations containing the small fragments (fragment 3 and 4 in Fig. 2) could not replicate.

Nucleotide sequence of the replication region

The nucleotides of the 1.6-kb SphI-HindIII fragment were sequenced by the method of Sanger (1977). Though the strategy is not shown, the nucleotides of both strands were sequenced using numerous restriction fragments to give enough overlapping regions (Fig. 3). Looking for possible open reading frames (ORFs), we found only one large frame (Fig. 2) designated rep, which consisted of 999 bp and encoded a protein molecule with 333 amino acids with a M_r of 38,582. Several conserved regulatory sequences similar to the *E. coli* and *Bacillus* consensus promoter sequences (McLaughlin *et al.*, 1981) were observed 5' upstream to the *rep* ORF.

Plasmid pNH3 was digested with BglII and then treated for 20 min with exonuclease Ba131 under the conditions in which about 50 bp per min were removed from





each end of the DNA molecule. After ligation by T4 ligase, the DNA was transformed into E. *coli* and subsequently introduced into *B. subtilis* by protoplast transformation. Tmp^r transformants, which contain 1.4-kb fragment 5 in Fig. 2, were obtained at high efficiencies with a 168-bp deletion plasmid generated with Bal31 (fragment 5 in Fig. 2), while no transformants were obtained with a similar 255-bp deletion plasmid (fragment 6 in Fig. 2). It suggests that the putative replication origin of pCTP4 is between position 168 and 255. To discover the sequences essential for replication, a homology search was done to find whether there are sequences within this region conserved in the registered Gram-positive replicons. A 34-bp sequence was conserved in four plasmids, pCTP4, pLS11, pUH1, pBAA1 and pFTB14 in *Bacillus* spp. (Fig. 4), and the 14-bp sequence within this region was conserved in all eight plasmids in Gram -positive bacteria including *Lactobacillus* spp. and Staphylococcus spp. This conserved 14-bp sequence is found in the 55-bp region of pC194 shown by Gros *et al.* (1987) to



Fig. 2. Structure and replication activity of the derived fragments of the 1.6-kb *ori* fragment.

The open arrow in the restriction map indicates an open reading frame found in the sequence data (see Fig. 3). + and – indicate, respectively, ability and inability to replicate in the *B. subtilis* host. Derivatives were made by deletion with the indicated restriction enzyme or by Ba131 digestion (B) from the *Bg1*II site of pNH3.

have origin activity. Within this 14-bp sequence, in addition, the sequence CTTGATA is the sequence at which nicking of the plus-strand occurs in the initiation of replication of the coliphage $\phi X174$ (Shlomai and Kornberg, 1980), and this conserved sequence was found in a hairpin region of $\phi X174$.

Amino acid sequence homology of *pCTP4* REP protein with different Gram-positive replication proteins

The amino acid sequence of the REP protein coding region of pCTP4 was compared with a number of amino acid sequence of proteins registered in GenBank by the homology search system GENAS (Kuhara et al., 1984). Homologies between the predicted amino acid sequence of the REP protein of pCTP4 and those of the REP protein of pUH1, pLS11, pBAA1, pFTB14, pLP1, pLAB1000, pUB110 and pC194 are illustrated in Fig. 5. The REP protein encoded on pCTP4 showed a substantial degree of homology to three REP proteins in Bacillus plasmids: 87.4% identity with REPpLS11, 82.0% with REPpBAA1, 76.6% with REPpFTB14, and 73.9% with RE-PpUH1. The REPpCTP4 is similar to REPpLP1 (46.5% identity) and REPpAB1000 (32.4%) from lactic acid bacteria but also to REPpUB110 (34.8%) and REPpC194 (21. 9%) from S. aureus. The REP protein of pFTB14 stretches for 1,017 bp, a promoter region for rep expression, and a possible replication origin for rep expression, which is upstream of the promoter. The rep product is trans-active and essential for plasmid replication (Murai et al., 1987). The tyrosine, which acts as an active site of REP in the rolling circle mechanism (Van Mansfeld et al., 1986), was conserved in all of the Gram-positive initiation proteins (Fig. 5). Khan et al. (1982) identified the start site of pT181 DNA synthesis within a 127-bp segment and showed that a 168 bp segment containing the replication start site is enough to initiate unidirectional replica-Furthermore, like REP protein of pT181 (Projan and Novick, 1988), the protein tion. of the E. coli plasmid R6K (Stalker et al., 1982; Germino and Bastia, 1982) does not

Sph I 50 5'-GCATGCTGCGGAGATAACACGGGCTGCTGCGCGCAATGATGCGGGCGCGTTGATCGTGCCGAGACTGAAAA 100 AGCCGATTTTTGAAAAAAAAATCTCCCCCCCCCGCGAAGAATGGTTTTGATCTTTGGGTTTTAGGTTTTAAAAAAAGCC 200 GGCTGTTTTCAGCCCGATTTTTCGATTTTGGCGGCGAAATCGGGTCTTTTCTTATCTTGATACTATATAGAAACAACAT 250 (-35) -10 CATTTTTCAAAAATCAGTTCAAAGTCTTGTGTGTGTCAAGGGTTAGATCGGTTTTTGACAGGTAAAAACTCCTTCTGQTATT ²350 3 5 350 (sd) 4 3 400 450 ato att ATA GCA TCC TCG ARG ATA ARA CCG CAR CAG GTA ARA AGC GGG ATT GGA AGG GAM Met Ile Ile Ala Ser Ser Lys Ile Lys Pro $\,Gln\,\,Gln\,\,Val$ Lys Ser Gly Ile Gly Arg Gly 500 AAA AGA GAC GTA CGA ACC TCA TGG CGG ACT TAC GAA GCG TTA GAG AGT AAG ATT GGG GCA Lys Arg Asp Val arg $Thr\ Ser\ Trp\ Arg\ Thr\ Tyr\ Glu\ Ala\ Leu\ Glu\ Ser\ Lys\ Ile\ Gly\ Ala$ 550 CCT TAC TAT GGC AAA AAG GCT GCC AAA CTA ATT AGT TGT GCA GAG TAT CTT TCG TTT AAG Pro Tyr Tyr Gly Lys Lys Ala Ala Lys Leu lle Ser Cys Ala Glu Tyr Leu Ser Phe Lys 600 $AGA\ GAC\ GGG$ aga cgc gac aag tta aaa ctg tat caa gcc cat ttt tgt aaa gtg agg tta arg asp arg arg Arg Gly Lys Leu Tyr Gln ala his Phe Cys Lys Val arg Leu 650 TGC CCG ATG TGT GCG TGG CGT AGG TCG TTA AAA ATT GCT TAT CAC RAT AAG TTG ATC GTA Cys Pro Met Cys Ala Trp Arg Arg Ser Leu Lys Ile Ala Tyr His Asn Lys Leu Ile Val 750 700 GAG GAA GCC AAT CCG CAG TAC GGT TCT GGA TGG ATT TTT CTC ACG CCG ATG GTT CGG AAA Glu Glu Ala Asn Pro Gin Tyr Giy Ser Giy Trpile Phe Leu Thr Pro Met Val Arg Asn 800 GTC GAG GGA GAA CGG CTG AAG CCA CAA ATT TCT GAG ATG CAT AAA GGA TTT AGG AAA CTG Val Val Glu Arg Leu Lys Pro Gln Ile Ser Glu Met His Lys Gly Phe Arg Lys Leu \$850\$ TTC CAG TAC AAA AAR GTA AAA ACT TCG GTT GCT GGA TTT TTC AGA GCT TTA GAG ATT ACC Phe Gln Tys Lys Lys Val Lys Thr Ser Val Ala Gly Phe Phe Arg Ala Leu Glu Le Thr ara AAT CAT gar gar gar aca tac CAT CCT CAT TTT CAT gag TTG ATA CCA GTA agg ara Lys AST His Glu Glu app Thr Tyr His Pro His Pho His Glu Leu lie Pro Val arg Lys 950 AAT TAT TIT GGG AAA AAC TAT ATG AAG CAG TCG GAG TCG ACG ACC CIT TGG AAA AAG TCG ASn TYr Phe Gly Lys As" tyr Ile Lys Gln Ser Glu Trp Thr Ser Leu Trp Lys Lys Ser $^{1000}_{\rm GTG$ AAA TTG GAT TAC CCT CCG ATT GTTCAT ATT CGA ACA GTG AAAGGCAAAAGCCAAC ATT ValLys Lug Asp Tyr Pro Pro Ile val Asp tle Arg Arg ValLys Gly Lys Ala Leu Ile GAC GCT GAA CTG ATT GAA AGC GAT GTG CGG GAA GCA ATG ATG GAG CAA AAA GCT GTT CTC Asp Ala Glu Leu Ile Glu Ser Asp Val Arg Glu Ala Met Met Glu Gln Lys Ala Val Leu 1150 GAA ATT TCT AAA TAT CCG GTT AAG GAT ACG GAT GTG GTG GGC GGC AAT AAG GTG ACT GAA Glu 11e Ser Lys Tyr Pro Val Lys Asp Thr Asp Val Val Arg Gly As,, Lys Val Thr Glu 1200 GAC ATT CTG AAC GCG GTG CTT TAT TTG GAT GAT GCG TTG GCA GCT CGA AGG TTA ATT GGA Asp Ile Leu Asn Ala $\forall a$ l Leu Tyr Leu Asp Asp Ala Leu Ala Ala $\forall rg$ Arg Leu Ile Gly 1250 TTC GGT GGC ATT TTG AAG GAG ATA CAT AAA AGA CTG AAT CTT GGA GAT CGG GAG GAC GGC Phe Gly Gly Ile Leu Lys Glu Ile His Lys Arg Leu Asn Leu Gly Asp Arg Glu Asp Gly 1300 1350 GAT CTG GTC AAG ATT GAG GAA GAA GAT GAC GAG ATT GCA AAC GGC GCA TTT GAG GTT ATG Asp Leu Val Lys Ile Glu Glu Glu Asp Asp Glu Ile Ala Asn Gly Ala Phe Glu Val Met 1400 HDOTT 1450 TTTTATACTCTAATAGTCAAAATGTGTTGAAAATATGTTTTGTTCGTTTTTTATCTTTGTAGCTTTTTATGATTAATTGTC 1500 1550 TATGATAACCTAGGGAATCTTTACATTGTCTGTGTTGAATAGTTACATAAACTGCTTTGCTCTTAGGATAGTTTAAATA Hindiii 1607 TACTGTTGTCATTGCTCCGAAAACAAAAGCTT-3

Fig. 3. Nucleotide sequence of the 1.6-kb SphI-HindIII DNA fragment.

Nucleotide residues are numbered in the 5'-to-3' direction, beginning with the 5'-end residue originated from the SphI site. The deduced amino acid sequence is given below the nucleotide sequence. Putative promoter elements (-35, -10 and the ribosome binding site) are underlined.



Fig. 4. (A) Homology at the origin for plus-strand synthesis among several plasmids in Gram-positive microorganisms. The nucleotides in boxes are conserved well among them. The sequence CTTGATA, which is the site at which nicking of the plus strand occurs in phage $\phi X174$, are boldface. The DNA bond between nucleotides G and A will be nicked by the REP protein and is indicated by an arrow. (B) Hairpin structure of the pC194 origin region proposed by S. D. Ehrlich and the predicted structure of origin region of plasmids pCTP4 and pUH1.

have any significant homology in its amino acid sequences with those of the REP proteins of pCTP4, pUH1, pLS11 and also pFTB14 deduced from the ORFs (data not shown). Comparison of dendrograms made on the basis of 16SrRNA hybridizations and the percentage of homology on REP protein are illustrated in Fig.6. The degree of homology of REPpCTP4 with the REP proteins of pLP1 and pLAB1000 in *Lactobacillus* spp. strains was almost the same as those with the REP proteins of pUB110 and pC194 in S. *aureus*. Taxonomic studies (based on the 16SrRNA similarities) showed that *Bacillus* spp. and S. *aureus* strains are more closely related to each other than *Staphylococcus* spp. and *Lactobacillus* spp. (Stackebrandt and Teuber, 1988). The plasmid homologies suggest an exchange of plasmid replicons by recent horizontal transfer through the different genera, including *B. subtilis (natto)*.

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REPPCTP4	10 20 30 40 50 60 MIIASSKIKPQQVKSGIGRGKRDVRTSWRTYEALESKIGAPY
REPpUH1	MIISSLRTRPOOVKSGIGRGKRDGRILWLSTMKLYRVKLVYL
REPpLS11	
REPOBAA1	
REPPFTB14	MYSSENDYRILEDKTATGKKRDWRGKKRRANLMAEHYEALEKBIGAPY
REPPLP1	MSEIPEDKTENGKVHPWRERKIENVRYARYLATLERKRAHOVR
REPpLAB1000	MSKKILKDVSRNRKERPWRERKIENTOYARYL BILNPKKANRVK
- REPpUB110	MGVSENIMCENSSIEDEKSEVIJVDKTKSGKVEDWEEKKIANVDYFEILHTLEFKKAFBUK
REPDC194	
	70 80 90 100 110 120
REPPCTP4	YGKKAAKLISCAFYLSFKROPRRGKLKLYQAHFCKVRLCPMCAWRRSLKIAYHNKLIVEE
SEPDOH1	TMAKKLRNCAVVRNVFRLNETRRRKLKLYQAQFCKVRLCPMCAWRRSLKIAYHNKLIVEE
REPPLSII	YGKKAEKLISCAEYLSFKRORRPGKLKLYQAHFCKVRLCPMCRWRRSLKIAYHNKLIVEE
REPPBAA1	YGKKAEKLISCAEYLSFKRDPETGKLKLYQAHFCKVRLCPMCAWRRSLKIAYHNKLIVEE
EPPFTB14	YGKKAERLSECAEHLSFKRDPETGRLKLYGAHFCKVRLCPMCAWRRSLKIAYHNKLIIEE
REPpLP1	GCGEVLRFRKIGEHLKLYQAHFCKVRLCPMCAWRRSLKIAYHNKLIIEE
EPpLAB1000	ECGEVLRFVADDEGRLRLYQTWFCKSRLCPLCNWRRSHGQSNQLHQVIDE
EPpUB110	DCAEILEYKONRETGERKLYRVWPCKSRLCPMCNWRRAMKHGIOSOKVVAE
REPpC194	GENOMDLVEDCNTFLSFVADKTLEKOKLYKANSCKNRFCPVCAWRKARKDALGLSLMMOY
ED-COD (130 140 150 160 170 180
CSPPCIP4	ANPOISSONI-FLIPHVRNV-VGERLEPQISEMHKGPRKL-PQYKKVKISVAGPPRALBITKN
CPPORI	ANROYGCGWI-FLTLTVRNV-EGDGLKPMIADMMKGWNRL-FGYKRVKVATLGYFRALEITKN
(SPD511	ANRQYCCGWI-FLTLTIRNV-KGERLKHQISANHEGFRKL-FQYKKVKTSVLGFFRALGIAKN
CPpBAA1	ANRQYCCCGWI-FLTLTURNU-KCERLKPQISEMMEGPRKL-POYKKVKTSVLGPPRALBITKN
EPPF IB14	ANROIGCGWI-FLILTVRNV-KGERLKPQISEMMEGFRRL-PQYKKVKTSVLGFFRALEITKN
	ANROYGKGRFLTLTVKNAHSAEELKVSLRALTXAPNKLKTRYKKVTKNLLGYIRSTEITVN
CPPLABI000	AHKQKATGKFLTLTAENA-SGENLKQEVRKMGRAISKL-FQYKKPAKNLLGYVRSTEITIN
PPACINA	VINUARIVRAUEDIDIVRAVIDEBELAKSISDRAQGERKA-RQIKKINKALVGERKATEVTIN
a.bei)4	190 200 210 220 230 240
REPPUH1	HEEDTYHPHPHELIPVRKNYFGKNYIKQSEWTSLWKKSVKLDYPPTVDIRRVKGKALIDA
REPPUH1	HEEDTYHPHFHVLLPVKKSYPTHNYIKQSEWTSLWKRAMKLDYTPIVDIRRVKGRAKIDA
REPpLS11	HEEDTYHPHFHVLIPVRKNYFGKNYIKOAEWTSLWKKAMKLDYTPIVDIRRVKGKAVLDA
REPPBAAL	HEEDTYHPHPHVLIPVRKNYFGKNYIKQAEWTSLWKKAMKLDYTPIVDIRRVKGKAKIDA
REPPFTB14	HEBDTYHPHFHVLLPVKRNYFGKNYIKQAEWTSLWKRAMKLDYTPIVDIRRVKGRVKIDA
EPpLP1	EQDGSYNQHLHVLLFVKSSYFKNSNNYLAQAEWAKLWQKALKVDYEPVVHVQAVKA-NKRKGDT
EPpLAB1000	
	KNGTYHOHNHVLLFVKPTYFKDSANYINOAEWSKLWORAMKLDYOPIVNVEAVRS-NKAKGKN
EPpUB110	-RNGTYHOHNHVLLPVKPTYFKDSAN'I NOAENSKLWORAMKLDYOP I VNVEAVRS-NKAKGKN NKDNSYNOHHHVLVCVEPTYFKNTENYVNGKON I OPMKKAMKLDYDPNDKVOM I APKNKYKSD-
REPpUB110 REPpC194	- KRGYYIQHHIVLLPV KPYYFKOSANY I NOADWSKLWORAHKLDYDP I UNVEAVRS-NAAKGKN NKDNSYNQHHIVLLVUEPTYFKNTENYVNGKON I OPWKKAMKLDYDDPNDKVQH I PRMKYKSD- KKDNSYNQHHIVLIAVNKSYFTDKRYY I SQOEMLDLNROVTC I SEI TOVOVOK I ROMNN
REPpC194 REPpC194	- KNGTVIOLINVLLPVFIFKOSANYINOSENSLUODANLLOYOPIVAVEANNES-NÄÄKSEN NKONSYNOIHIVVLOVEPYYKKOSANYINOSENSLUODANLAYOPINOKVOILASKASK KKONSYNOIHIVVLAVIKSYFTORAYYISOOFFIDIAN
REPpUB110 REPpC194 REPpCTP4 REPpUH1	- KNGTYINGINIYLLEYYFYFXGSAYINOSINGINGING LOYOPIVWEANYS - YAXKA NKDRSYNOINIYLLOYYFYFXGAYINOSING COMARAMKLOYOPIVWEANYN A SAN KKDRSYNOINIYLLAVNSYFTARAYISQCGALDLROYTCISEITOVOVGIRANNY LLESDYREANHOSKAYLCISYFYDA COMYNGINYTEDILANYU DDALAARILICC DIFROMENHOSKAYLCISYFYDRODYWEANYTEDILANYU DDALAARILICC
REPp08110 REPp0194 REPp0TP4 REPp0H1 REPp1.511	- KNGTYINDINIYLLEYKYYYKXSAYINOSINSI KUODANIKLOYOPIVWYEAVX KKONSYNOHNYULCYYEYYYKXTENYYNGKOLOFMKAAKLOYOPIVWYEAVXI REKKYK KKONSYNOHNYULAYKYYTDKAYISOOCHIDL 250 260 270 280 250 300 ELLESDYREAMEDKAYYLEISYYYKOTOYYKGHKYTEDLLAYUYLDDALAARKLIGF EDIESDYREAMEDKAYLLISYTYVKOTOYYKGHKYTEDLLAYUYLDDALAARKLIGF EDIESDYREAMEDKAYLLISYTYVKOTOYYKGHKYTEDLLAYUYLDDALAARKLIGF EDIESDYREAMEDKAYLLISYTYVKOTOYYKGHKYTEDLLAYUYLDDALAARKLIGF
REPpUBIIO REPpCI94 REPpCTP4 REPpUHI REPpLSII REPpLSII	- KNGTYINDINIVLLPVFYYFXOSAWYINOXDWS KNOQRANKLDYOP I VWVEAVNG - WAXKGKN NKONSYNOHNRVLCVEPTYFKNTENYVNGKOGIOFWKKAKKLDYOP I VWVEAVNG I RPKNYKSD- KKONSYNOHNRVL I XVNKSYFTD KRYYISOCEKILDL 1250 260 270 280 290 300 ELLESDVREAMEDCKAVLEI SYYVKOTOVVRGHKVTEDI LAAVLYLDDALAARKLIGYC EQI ENDVRAAMEDCKAVLEI SYYVKOTOVVRGHKVTEDILAAVLYLDDALAARKLIGYC EQI ENDVRAAMEDCKAVLEI SYYVKOTOVVRGHKVTEDILAYVLYLDDALAARKLIGYC
REPpUBIIO REPpCI94 REPpCTP4 REPpUHI REPpLSII REPpLSII REPpERBI4	- KNGTYINDINIYLLEYKYYYKODOVAGNYYTEDHLIYYV ILDULAARRLIGYC NONSYNOHRYULCYCETYYKNTENYNGKOGIONKKANKLOYOPINOKYOMIRPKNYKSON KKDNSYNOHRYULAYNKSYFTEKYYNGBOGGETLEL SECTOVOVOKIRON I SECTOVOVOKI ELLESDYREAMBOGAYLEISYYYKODOVAGNYYTEDHLIYYY UDDALAARRLIGYC EQIESDYREAMBOGAYLEISYYYKODOVAGNYYTEDHLIYYY UDDALAARRLIGYC ELIEDDYREAMBOGAYLEISYYYKODOVAGNYYTEDHLIYYY UDDALAARRLIGYC ELIEDDYREAMBOGAYLEISYYYKODOVAGNYYTEDHLIYYY UDDALAARRLIGYC
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12PpUB110 12PpC194 12PpUH1 12PpUH1 12PpLS11 12PpTB14 12PpLP1 12PpLAB1000 12PpUB10 12PpU44	- KNGYYNOLHWYLL Y YRYY FY FROSANY I NORIWS KLWORANK LDYOP I WWEANYS - WAKAKA NKDNS YNOLHWYL FY WRATENYWACHOL I GYMRARAK KLOYOP I WWEANYS - WAKAKA KKDNS YNOLHWYL FWYRT DRWYWACHOL I GYMRARAK KLOYOP I WWEANYS - MARKAC KKDNS YNOLHWYL FWYRT DWYNG WYT SOLANWY C SI LIWWY C DI FOUNDAL I GYM ELLESDYRRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY WYLDDAL ARRIL I GYG COL SEDWRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY WYLDDAL ARRIL I GYG ELLENDYRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY WYLDDAL ARRIL I GYG ELLENDYRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY WYLDDAL ARRIL I GYG ELLENDYRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY WYLDDAL ARRIL I GYG ELLENDYRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY YLDDAL ARRIL I GYG EDI SDWRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY YLDDAL ARRIL I GYG EDI SDWRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY YLDDAL ARRIL I GYG EDI SDWRAM HORANYL EI SY FYNN DDYWRG WYT SDLLWY YLDDAL ARRIL I GYG SL
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REPUBLIO REPCIP4 REPCIP4 REPLAI REPLAI REPLAICOO REPCIP4 REPLAICOO REPLAICOO REPLAICOO REPLIO REPLIO REPLIO REPLIO REPLIO REPLIO REPLIO REPLIN	- ANG TWO HIVLLOVEPTY FKOSANY INO KAWS KUO DANILOYOP I WWEANS - WAKKSK WKONS YWO HIVLLOVEPTY FKOSANY INO KAWSKAWC DO WAWSANS - WAKKSK KKONS YWO HIVLLOVEPTY FKOSANY I SOCKELDIA
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Fig. 5. Amino acid sequence comparison of the REPpCTP4 protein with different Gram-positive replication proteins.

The original names of these proteins have been replaced by REP, followed by a letter code indicating the plasmid from which they originate. The amino acid identities with several plasmids in Gram-positive microorganisms with REPpCTP4 are indicated (*). Amino acid numbers follow the sequence of the REP protein from the amino-terminal methionine to the carboxy terminus. Gaps have been inserted to gain maximum matching. The one-letter amino acid code has been used. The tyrosine residue (marked with arrow) probably involved in the binding of the REP protein to the DNA is indicated by an arrow.





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