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Sakemi, Hirohito
Laboratory of Microbial Genetics Technology, Department of Genetics Resources Technology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Nishitake, Sayuri
Laboratory of Microbial Genetics Technology, Department of Genetics Resources Technology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Rodprapakorn, Mangkorn
Laboratory of Microbial Genetics Technology, Department of Genetics Resources Technology, Division of Agriculture, Graduate School , Kyushu University

Shirakami, Tomoyuki
Laboratory of Microbial Genetics Technology, Department of Genetics Resources Technology, Division of Agriculture, Graduate School , Kyushu University

他

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Nucleotide Sequence of Conjugative and Integrating Plasmid pSLS from *Streptomyces laurentii* ATCC31255

Hirohito SAKEMI*, Sayuri NISHITAKE*, Mangkorn RODPRAPAKORN, Tomoyuki SHIRAKAMI**, Shinpei TAKECHI*, Katsumi DOI and Seiya OGATA†**

Laboratory of Microbial Genetics, Division of Applied Genetic Resources, Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan

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The complete nucleotide sequence of a conjugative and integrative plasmid, pSLS, in the thiostrepton-producing *Streptomyces laurentii* ATCC31255 was determined. The circular DNA molecule was 15,398 bp in length and contained 70.8% G+C content. Computer-assisted analyses indicated that pSLS contained 10 open reading frames (ORFs), *orf1* to *orf10*, located on both strands of pSLS.

The *orf1* encoded for a predicted protein (200 aa) showed high similarity to TraA protein of non-integrative plasmid pJV1 in *Streptomyces phaeochromogenes* involved in plasmid transfer and pock-formation. The *orf2* coded for a protein of 660 aa that shared homology with TraB proteins encoded by other *Streptomyces* plasmids. Proteins encoded by *orf3* (170 aa), *orf4* (408aa) and *orf5* (148aa) shared homology with *Streptomyces* proteins, SpdB1, SpdB2 and SpdB3, respectively, which were also involved in plasmid spreading. The *orf8* encoded a polypeptide of 458 aa that shared homology with Int protein of integrative plasmid pSAM2 in *Streptomyces ambofacience*.

This report manifests that pSLS is unique chimeric episomal element that contains gene cluster from non-integrative plasmid and genes for site-specific integration from integrative plasmid.

INTRODUCTION

Streptomyces spp. are Gram-positive soil bacteria that undergo a complex cycle of morphological differentiation and synthesize multiple medically and industrially useful secondary metabolites (Chater, 1989; 1993). Conjugative plasmids isolated from *Streptomyces* species include autonomous circular plasmids (e.g., pIJ101 [Kieser *et al.*, 1982], pJV1 [Servín-Gonzáles, 1993] and pSN22 [Kataoka *et al.*, 1994]) and linear replicons (e.g., pBL1 [Zotchev *et al.*, 1994], pSLA2 [Qin and Cohen, 1998] and SCP1 [Yamasaki *et al.*, 2001]). In the circular plasmids, some plasmids are generated by site-specific excision of chromosomal DNA segments and capable of reintegrating site specifically into *Streptomyces* chromosomes (e.g., SLP1 [Omer *et al.*, 1988], pSAM2

* Laboratory of Microbial Genetics Technology, Division of Applied Genetic Resources, Department of Genetic Resources Technology, Graduate School of Division of Bioresource and Bioenvironmental Sciences, Kyushu University

** Laboratory of Microbial Genetics Technology, Department of Genetic Resources Technology, Division of Agriculture, Graduate School, Kyushu University

† Corresponding author (E-mail: ogata@agr.kyushu-u.ac.jp)

[Hagége *et al.*, 1993] and pSA1.1 [Doi *et al.*, 1995]). They are called integrative plasmids. During their existence as extrachromosomal replicons, the integrative plasmids, just like the non–integrative plasmids, exhibit the ability to undergo conjugal transfer and inhibit transiently the growth of plasmid recipients, yielding zones of retarded growth called "pocks" (Bibb *et al.*, 1977). Despite the functional similarities in pock formation, there were no structural similarities in the genes involved in pock formation between the integrative plasmids and non–integrative plasmids.

Thiostrepton producing *Streptomyces laurentii* ATCC 31255, wild–type strain P0, forms spontaneously developing pocks, as do *Streptomyces azureus* ATCC14921 and some other strains (Ogata *et al.*, 1992). Two plasmids, pSLS and pSLL, were related to the pock formation in strain P0 (Kinoshita–Iramina *et al.*, 1995). The integrating plasmid pSLS existed normally as a covalently closed circular plasmid in the host cytoplasm or an integrated plasmidogenic sequence ($pSLS^{int}$) in the chromosome. Another plasmid pSLL (93–kb) was also isolated from strain P0, and had a linear DNA structure carrying a protein bound to each 5' terminal of the DNA (Kinoshita–Iramina *et al.*, 1997). It was self–transmitted to the pSLL–cured strain by conjugation in solid culture. The pSLL–cured strain derived from strain P0 carried plasmid pSLS, and showed a marked decrease in spore formation and thiostrepton productivity, owing to the pSLS. However, by retransmission of pSLL, these phenomena reverted to levels in strain P0. Thus, plasmid pSLL suppressed the injurious effects of pSLS on the host mycelia. The transfer genes of *Streptomyces* conjugative plasmids such as *tra* of pIJ101 (Kendall and Cohen, 1987), *traB* of pSN22 (Kataoka *et al.*, 1991b), traSA of pSAM2 (Hagége *et al.*, 1993) and spi of pSA1.1 (Doi, *et al.*, 1995) functioned as *kill* genes (*kil*). Another gene, *kor* (*kil*–override), regulates transcription of *kil* genes (Kendall and Cohen, 1988). The gene products of *korA*, *traR*, *korSA* and *ImpSA* control the expression of *tra*, *traB*, *traSA* and *spi*, respectively. Linear plasmid pSLL would have *kor*–like gene for the repression of *kil* gene in pSLS. Attention is focused on *kil–kor* gene system in pSLS.

We report here the nucleotide sequence of plasmid pSLS, and predict the functions of genes on pSLS.

MATERIALS AND METHODS

Bacterial strains and plasmids

Streptomyces laurentii ATCC 31255 (wild–type strain P0) and its derivative strain, P1a, were used throughout this work. Strain P1a carried approximately 60 copies of circular plasmid pSLS and no linear plasmid pSLL (Kinoshita–Iramina *et al.*, 1997). *Escherichia coli* JM109 and plasmid pUC19 were used in the gene cloning.

Culture condition

Strains P0 and P1a were cultured in Bennett broth and on Rye flakes agar plate (Ogata *et al.*, 1985) at 28 °C. These strains were also grown in MG–1 broth (Ogata *et al.*, 1985) for the extraction of plasmid. The liquid cultures were incubated on a rotary shaker (250 rpm) for 48 hr and the solid cultures were incubated for 5 days. *E. coli* cells were grown at 37 °C in either LB medium or LB with 50 μg ml^{-1} ampicillin for the isolation of plasmid DNA.

DNA isolation and manipulation

Bacterial DNA was isolated and manipulated by standard procedures (Hopwood *et al.*, 1985a; Sambrook *et al.*, 1989). Restriction map of pSLS was determined by *Aat* I, *Bam*H I, *Hin*d III, *Pst* I and *Sac* I digestions. Furthermore, the fragments of pSLS were digested with convenient restriction enzymes and subcloned into pUC19 for sequencing.

Sequence analysis

DNA sequencing was performed using an automatic ALF express DNA–sequencer and Thermo Sequenase fluorescent labeled primer cycle sequencing kits with 7–deaza–dGTP (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions. Both strands of pSLS were sequenced and analyzed with the Auto assembler program of GENETYX software (Software Development Co. Ltd., Japan). Homology searches were conducted with database from the National Center for Biotechnology Information (NCBI) by use of the BLASTX algorithm.

RESULTS AND DISCUSSION

General characteristics of pSLS

Plasmid pSLS of *S. laurentii* ATCC31255 was physically and genetically mapped, and subjected to combining restriction enzyme analysis and nucleotide sequence (Fig. 1). The nucleotide sequence of pSLS revealed a total of 15,397 nucleotides. It is deposited in GenBank under an accession No. AB093554. The sequence contained 70.8% (10,901 bp) G+C contents, which was in accord with those of other *Streptomyces* plasmids. Computer analysis with the GENETYX program revealed the presence of 10 putative

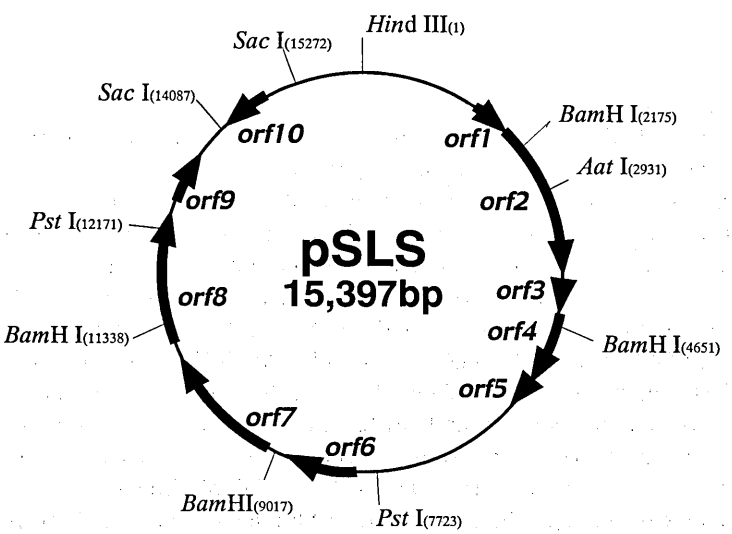

Fig. 1. Restriction map of and genetic organization of plasmid pSLS. Putative open reading frames (ORFs) are shown by arrows. The number in parentheses refers to the nucleotide position of the restriction sites.

regions encoding open reading frames (ORF 1–10) (Fig. 1). Sequences similar to the Shine–Dalgarno sequence (ribosome binding sequence) were detected closely upstream of the potential translation start sites of some ORFs. It was noticed that nine ORFs were located on the same strand except for ORF10. The characteristics of the open reading frames (ORFs) and its predicted products of pSLS are summarized in Table.

No homology between circular plasmid pSLS and linear plasmid pSLL was shown by Southern hybridization (Data not shown).

Table Deduced functions and characteristics of gene products in plasmid pSLS

	Gene	Amino acid residues	Putative function and character	Protein with the highest sequence similarity and its origin	Identity/ similarity (%)	Accession no.
ORF1	*traASL*	200	Intermycelial transfer	TraA, pJV1	69/93	PRF: 220434H
ORF2	*traBSL*	660	Intermycelial transfer	TraB, pJV1	74/84	PRF: 2204341J
ORF3	*spdB1SL*	170	Spread	SpdB1, pJV1	60/75	PRF: 2204341A
ORF4	*spdB2SL*	408	Spread	SpdB2, pJV1	59/87	PRF: 2204341B
ORF5	*spdB3SL*	148	Spread	SpdB3, pJV1	60/90	PRF: 2204341C
ORF6	*orf753*	250	Hypothetical protein			
ORF7	*orf1542*	513	Hypothetical protein			
ORF8	*int*	458	Site-specific integration	Integrase, actinophage VWB	54/67	PRF: 257416A
ORF9	*orf462*	153	Hypothetical transcription regulator			
ORF10	*mutTSL*	157	Mutator	Putative MutT-like protein, *S. coelicolor* A3(2)	53/66	GenBank: AL589707

ORF description

Seven ORFs (ORF 1, 2, 3, 4, 5, 8 and 10) showed significant similarities with genes involved in plasmid transfer, spread and integration.

The predicted protein encoded by ORF1 (200 aa) shared 69% identity (93% similarity) with the TraA protein encoded by the *traA* gene (PRF: 2204341H) of pJV1 of *Stereptomyces phaeochromogenes*, a member of the non–integrative plasmid family. ORF1 was therefore designated *traASL*, and its gene product was designated TraASL. The deduced amino acids sequence of ORF 2 (660 aa) showed similarity to TraB (PRF: 2204341J) of pJV1 with 84% similarity (74% identity). The predicted gene product of ORF2, TraBSL, showed significant similarities with *tra* gene involved in a *kil–kor* system of pJV1 of *S. phaeochromogenes* and pSN22 of *Streptomyces nigrifaciens* (Fig. 2). No significant similarities with *tra* genes of other conjugative plasmids such as pIJ101, pSAM2 and pSA1.1 were detected except ATP/GTP–binding sites (P–loop). These specific homologies among pSLS, pJV1 and pSN22 suggested that these plasmids were derived from a common ancestor.

In *kil–kor* systems, the *kil* gene (kill phenotype) specifies functions lethal to either the host or the plasmid, when unregulated by the *kor* (for *kil*– override) gene. In

```
TraBSL (pSLS)   MVKQQNIQQNLPAVSSGAGTVKSWLWKQTQPFVPPWIVTGLVGVAGGGANFAWDGSPWAG 60
TraB   (pJV1)   MVRNTQQQDGLPAVTSYGSTFKAWAWHVTKPFVPPWAVAGVTGLAGGGANLLWQGSPWAG 60
TraB   (pSN22)  -MGKDVQQQQEDRLNSGGTGMGAWLWHRAKPYTPPWIVTGAVGAAGAGAHELWGNSPWAG 59

TraBSL (pSLS)   IGLTLGSVALTAVTWTAGARSGRQLRLHSAITVASASAWTTAAAVSGPFSGPLGNLYLMG 120
TraB   (pJV1)   VGLTLGSVALTGATWAAGVKTGRQRRLHSAITVAAASAWTTAAAISGPLSGPLPDLYLMG 120
TraB   (pSN22)  VGLTLAGVGLTAATWWAGKSTGQQRRLHSAITVAAGATWFTASALSGPLTGPLPDLYLMG 119

TraBSL (pSLS)   GGILALSWNIRRVLVAGGLVETSGTSDKGLMEKVGLARTLLKNVKVEPNKVTAAYELPAG 180
TraB   (pJV1)   GAVLALSWNIRRVLAAGMAETSGESADKGLMEKVGLARTLLKNVKVEPNKVTASYELPAG 180
TraB   (pSN22)  GTSLALTWNIRQVMRSSTPEGAGSDSDKGLLEKVGLARTKLKDVKVEPNRVTVPYELPAG 179

TraBSL (pSLS)   ESDQRGPRQRPRDRLASALDVPTTAIRIEHDPDSARRGRIVIVPEDMLKDPTTWPGPYAP 240
TraB   (pJV1)   ELTN-DDLGKARDRTASALDVPTTAIRIQHDPDSARRGRIVIVPEDMLKTPTIWPGPYAP 239
TraB   (pSN22)  ELTN-DDINKAIPRIASALDVPTTAIRVQHDPDSARKGQFVIVPEDMLKQPTIWPGPFAP 238

TraBSL (pSLS)   GESVAEPLRIGVYDDGSDLLLPLLAAIHILVMGMTGSGKTEGALDLLAGAAEPARIVVVW 300
TraB   (pJV1)   GESVEVPLRIGVYDDGADLVLPLLAAIHVLVMGMTGSGKTEGALDLLLELFT-RRDVVVW 298
TraB   (pSN22)  GESVAVRCGS-RLRRRSDLVLPLLDAIHLLVMGMTGSGKTEGAVDLLLEILT-RNDVTVW 297

TraBSL (pSLS)   LADAAKAGQDFQPLVPALDWAALDTPSAAAMVASVQTVIPARTAWLRDHGYRAWEPAAAE 360
TraB   (pJV1)   LADAAKSGQDFQPLLPAMDWAALDTPSAAAMVAAVQAVIPARTAWLRDHGYRAWEAAAAR 358
TraB   (pSN22)  LADAAKAGQDFQPLVPALDWAALDTASAGAMVDAVQAVIPARTAWLRDHSYRAWEPAAAK 357

TraBSL (pSLS)   RQTDRKHSCRKDGKACGCEGIAYLLAWFEEAAKLLRELGDDVFTGIAQEARSAGVTLVVS 420
TraB   (pJV1)   RQTNPAHSCRKDGRACGCDGMAYLLAWFEEAAKLLRELGDDVFTGIAQEARSAGVSLVVS 418
TraB   (pSN22)  TQTNPAHSCASAG-ACGCPGMPYLLTWFEEAAKLLRELGDDVFTGIAQEARSAGVSLVVS 416

TraBSL (pSLS)   MQRASGYQLSTDTRASLPAAMCFGVKGDDASFALPEEVLDAGADPAAWGNKRKGYVYLAS 480
TraB   (pJV1)   MQRASGYQLSTDTRASLPAAMCFGVKGDDASFALPEEVLDAGADPAAWGNKRKGYVYLVS 478
TraB   (pSN22)  MQRASGYQLSTDHEGLAPGRMCFGVRGDDAGSPSPRSPDAVQPARGHSAATLLCRRVERL 476

TraBSL (pSLS)   AGVDEDLHATPVRTYWTGPSGEYERMAEYVVTQFETIRAAIDAVTADAATKFAGKFFTDR 540
TraB   (pJV1)   AEVDEDLHATPARTFWTGPSGEYERMAEYVVKQFANVRAALDAVTAGAAEKAVGEFFTRR 538
TraB   (pSN22)  THRDSDARRLHVAATSSSTRS----------------SCELDPVTGAAAEQAAGPLFTNR 520

TraBSL (pSLS)   RARAFG---------EAPAQGAGRILTAEQDVDQEHELVDAEDADVDPTAEILRPERTPL 591
TraB   (pJV1)   RERALGNQAASTSASEPTGDPLLDGLAEQESAQAVAALVDEEDQELDVITDLD-PVETDI 597
TraB   (pSN22)  RARAGAAS--------APARPVQEQMLLDDDGQEDGDLVEMEHDGIDLSADLP-PVENDA 571

TraBSL (pSLS)   SPPASKPGPEEARELLDELVRMLAGMGPGTVAVKDLGPYLEQLGRDRSWVSKEMKRLAEE 651
TraB   (pJV1)   ALPAAKPSPEEARELLEDMVAMLAGVGPGTVAVKDLGPYLEQLGRDRSWVSKQMSRMASE 657
TraB   (pSN22)  ELLFVKPSTEEARELLDEMVATLASVGPGTVAVRDLKPYLEQIGRDRSWVSREMKRMAEE 631

TraBSL (pSLS)   G------------LVPVLAGV 660
TraB   (pJV1)   GRLAPTAEQGVYRLVPVLAAA 678
TraB   (pSN22)  GRLAATGEEGVYRLIRTLAGV 651
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Fig. 2. Amino acid sequence alignment of TraBSL and homologues from TraB of pJV1 (PRF: 2204341J), TraB of pSN22 (PRF: 2114395E). Shading indicates identical amino acid residues. Conserved region of ATP/GTP-binding site motif A (P-loop, Walker *et al.*, 1982) are indicated by outline letters on a black background.

Streptomyces plasmids, such as pIJ101, pSAM2, pSN22 and pJV1, the *kil–kor* system is associated with transfer, the kill function being attributed to *tra* genes (transfer gene). KorSA of pSAM2 indirectly controls pSAM2 replication, integration and excision via the

regulation of the *pra* gene, which is different from the Kor protein of other *Streptomyces* plasmids that directly control plasmid transfer (Sezonov *et al.*, 2000). No sequence similar to *pra* or *kor* genes registered in the databases was observed in pSLS. Putative gene product of ORF9 had helix–turn–helix (HTH) motif observed in GntR family on C–terminal and showed lower similarity with KorSA of pSAM2 (PRF: 1920300G). In general, HTH motif is located on N–terminal of GntR family. Consequently, we could not make any conclusion on the function of ORF9 product. By Southern hybridization using *traR* gene of pSN22 and *imp* gene of pSA1.1 as probes, no signal was detected in pSLS DNA (Data not shown). Nevertheless, curing of pSLL led to the expression of a gene having a kill phenotype so far. This suggested that the *kor* gene encoded on pSLL would repress kill phenotype from *traBSL* of pSLS

Three ORFs, ORF3, ORF4 and ORF5, showed high similarities with spread genes, *spdB1*, *spdB2* and *spdB3*, of pJV1, respectively (Table). These proteins were thought to be concerned with pock size since mutations in these genes decreased their pock size (Kataoka *et al.*, 1991a). Therefore, ORF3, ORF4 and ORF5 seemed to encode the SpdB1SL, SpdB2SL and SpdB3SL. Spread genes were not required for transfer and pocking to occur but affected pock size and thus plasmid "spread" (Hopwood and Kieser, 1993). The function and mechanism of the Spd group are not clear until now (Maas *et al.*, 1998). These genes may mediate intramycelial spread of plasmids within recipient cells, such as movement across infrequent hyphal cross walls that separate the original point of transfer from other connected cell compartments. Alternatively, it is possible that plasmid spread functions instead of *tra* augment during the initial intermycelial transfer step.

ORF6 and ORF7 encoded putative proteins of 250 aa and 513 aa, respectively. The deduced amino acids sequences showed no similarities with any proteins registered in the database.

The deduced amino acids sequence of the ORF 8 (458 aa) showed significant similarities with Int proteins required for site–specific integration of plasmid into its host chromosome (Fig. 3). This gene product displayed the features of an integrase as concluded from its C–terminal region to recombinases of the integrase family (Argos *et al.*, 1986; Landy, 1989). Furthermore, ORF 8 product had a calculated pI value of 10.6 and molecular weight of 51307.4. In many *int* genes reported, there are excisionase (*xis*) genes adjacent to *int* genes. Until now, no ORF sharing similarity with the reported *Streptomyces* excisionase could be detected in the whole sequence of pSLS. The *int* gene of pSLS has significant homology with *int* genes of integrative actinomycete plasmids such as pSAM2 of *Streptomyces ambofaciens*, pSE211 of *Saccharopolyspora erythraea* (Brown *et al.*, 1990) and pSE101 of *Saccharopolyspora erythraea* (Brown *et al.*, 1994), while *tra* and *spd* genes of pSLS showed high similarities with those of non–integrative plasmid pJV1. No genetic similarities on *tra* genes and *spd* genes between integrative plasmids (pSAM2/SLP1 family) and non–integrative plasmid (pSN22/pJV1 family) were reported. Thus, it followed that pSLS has a unique chimeric episomal element containing genes from non–integrative plasmid and genes for site–specific integration from integrative plasmid.

The deduced amino acids sequence of ORF 10 (157 aa) showed similarity to putative *mutT*–like proteins of *S. coelicolor* A3(2) (AL589707) and ORF154 of pSAM2 (S39873).

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pSE211  1-183 LEVEDMQLVI (10) RYVIALALGTRQGESLALKWPRLNR 229-437  Brown et al., 1990
RP3     1-191 GARPEDAILL (10) HVLTTAFAGPRWGEGLGLHRDNTLL 237-447  Gabriel et al., 1995
SLP1    1-238 PDPRQALALL (15) FFGCMYYAAARPAEVIGLRLQDCDL 290-456  Brasch et al., 1993
λ       1-179 LTADEYLKIY (12) AMELAVVTGQRVGDLCEMKWSDIVD 227-356  Argos et al., 1986
pSLS    1-229 WTTRQVFGVR (9)  TVDVAGGCGLRQGEVFGLSEDELDF 274-276  this work
                 .  .            . * .. ..

                PatchII          PatchIII      Box B                Box C
                                              ↓  ↓                     ↓
VWB     1-286 PKSNAG--FRTVP (35) ELVFRGP (41) HDLRHVFATWLKDVG (5) TQTVMGHERGSKVTWLYQH 420-427
pSAM2   1-247 TKTRKSRRTLALP (27) GGWVFTQ (32) RELRHSFVSLLSDRG (5) ISRLVGHSGTAVTEEVYRK 366-388
pSE101  1-242 QRQAWQ-HGCDDP (99) HGLVFSS (29) HDARHTAATVLMLLR (5) ISRLVGHSGTAVTEEVYRK 428-448
pSE211  1-239 QRQTW-KHGCSDP (91) GEWMFTQ (29) HDARHTAATVLLVLG (5) ISRLVGHSGTAVTEEVYRK 417-437
RP3     1-285 TRGRG---RAAVP (27) GNWWRTF (31) RALRALHDTMQSEIG (5) ISRLVGHSGTAVTEEVYRK 399-447
SLP1    1-321 LKHRPRKAVRTVP (21) GRLFRTQ (35) YDLRHAAVSTWLSSG (5) VAARAGHS-VAVLFRVYAK 436-456
λ       1-233 SKTGV---KIAIP (25) ETIIAST (32) HELR-SLSARLYEKQ (5) AQHLLGHKSDTMAS-QYRD 345-356
pSLS    1-294 LPKGAKVRDVPLP (33) SLIFSGS (41) HALRHFYASVLLDAG (5) LSQYLGHADPGFTLRTYTH 428-457
                 .     .     .     . *   .                 *   .   *.
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Fig. 3. Amino acid sequence alignment of six domains of Int of pSLS with amino acids in the same domains of λ Int, the prototypical member of the integrase family, and other *Streptomyces* integrases. Boxes A, B and C correspond to the three major clusters with global similarities among the proteins of the integrase family described by Esposito and Scocca (1997). Patches I, II and III refer to the three additional patches of conserved sequence among the integrase family proteins found by Nunes-Düby *et al.* (1998). The numbers of amino acids spanning the different domains are indicated by numbers in parentheses. Bold letters indicate conserved residues among proteins analyzed; asterisks indicate perfectly conserved residues and dots indicate well-conserved residues among the aligned proteins. The arrows indicate the four active-site residues of the integrases.

This gene product had consensus region of MutT/nudix family protein (Fig. 4; Bessman *et al.*, 1996). On the basis of these similarities, pSLS ORF 10 was named *mutTSL*. Among *Streptomyces* plasmids, MutT/nudix family proteins identified are specific to pSAM2 and pSLS. MutT is a nucleoside triphosphatase with a preference for the syn form of dGTP, hydrolyzing it to dGMP and pyrophosphate. 8-oxodGTP is hydrolyzed 10 times faster than dGTP, making it a likely biological substrate for MutT (Fowler and Schaaper, 1997). The putative pSLS MutTSL contains all the residues that have been previously found to be irreplaceable in the MutT 23-aa phosphohdrolase module (Shimokawa *et al.*, 2000). MutT is assumed to hydrolyze 8-oxodGTP in the nucleotide pool before it can be misincorporated in *E. coli* genome. The role of MutT in plasmids is still unclear. Further investigation may reveal its functions in the plasmids.

Specific feature of gene organization in pSLS

Based on structural similarities at the level of gene organization, protein sequence, and nick site sequences, we could not detect *rep* gene in pSLS. It is suggest that the replicase of pSLS belongs to a new subfamily of replication enzymes. So, we will deter-

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MutT-like (S. coelicolor)  MARVDYFNDPNAPKANSLVPSVTAVARNEAGEVLLIHKTDNDLWALFGGGIDLGESAPDA 60
ORF131 (pSAM2)             ------------------------MVVREDGRLLAIRRADNGTWELFGGVLELDETPETG 36
ORF154 (pSAM2)             -MLLYMSQPQEATSPPLHSVSVAGVVVREDGRLLAIRRADNGTWELFGGVLELDETPETG 59
MutT (B. halodurans)       ------------------MQRVTNCIVVDHDQVLLLQKPRRGWWVAFGGKMEAGESILET 42
MutT (A. fulgidus)         ------------MKCITLTVDAIIPYQGKIVLIKRLNEPFKGHYALFGGIVEYGERVEDA 48
ORF10 (pSLS)               MGRIDYLHDPDAPPANSVVPSVVAFVQDGQGRVLMIQRSDNGRWALFGGGHDAGESISDT 60
                                                                 23-aa phosphohdrolase module

MutT-like (S. coelicolor)  AVRETKEETGFDVEVTGLVGIY-------TNPGHVMAYDDGEVRQQFSICYHARITGGEL 113
ORF131 (pSAM2)             VAREVWEETGIRVEVDELTGVY-------KNTTRGIVALVFRCKPSGGVERTSSESTAVS 89
ORF154 (pSAM2)             VAREVWEETGIRVEVDELTGVY-------KNTTRGIVALVFRCKPSGGVERTSSESTAVS 112
MutT (B. halodurans)       VKREYWEETGITVKNPELKGIFSMVIFDEGKIVSEWMLFTFKATEHEGEMLKQSPEGKLE 102
MutT (A. fulgidus)         VLREVEEETGLKGEIHSLVGVY-------------SDPNRDPRGHFVSVCFVVLPKGGEL 95
ORF10 (pSLS)               VVREVWEETGIDAEVIDVSGIY-------TDPGHVMAYDDGEIRQQFSICFRARPTGGEV 113

MutT-like (S. coelicolor)  RTSSESKEVAFVDPSKLDELNIHPSMRMRIEHGLT-DRAEPYIG------------ 156
ORF131 (pSAM2)             WLTPDEVSERMAEVYAIRLLDALDGAGPHVRSHDGKHLIPAG--------------- 131
ORF154 (pSAM2)             WLTPDEVSERMAEVYAIRLLDALDGAGPHVRSHDGKHLIPAG--------------- 130
MutT (B. halodurans)       WKKKDEVLELPMAAGDKWIFKHVLHSDRLLYGTFHYTPDFELLSYRLDPEPQMKKGV 159
MutT (A. fulgidus)         KAGSDAKEVGLFSLNELPKLAFDHEKMIKDAEVILRGILSEV--------------- 137
ORF10 (pSLS)               RTSSETTQVRWVAPADLVELDVHPTMRLRIEHAMDRTRTAPYIG------------ 157
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Fig. 4. Amino acid sequence alignment of the putative MutT–like protein of *S. coelicolor* A3(2) (putative; SCBAC5H2.05c), ORF131 of pSAM2 (PRF: 2415397A), ORF154 of pSAM2 (PIR: S39873), MutT protein of *Bacillus halodurans* (PIR: B84096) and MutT protein of *Archaeoglobus fulgidus* (PIR: A69404). Identical residues are shown by outline letters on a black background. 23–aa phosphohdrolase module was boxed.

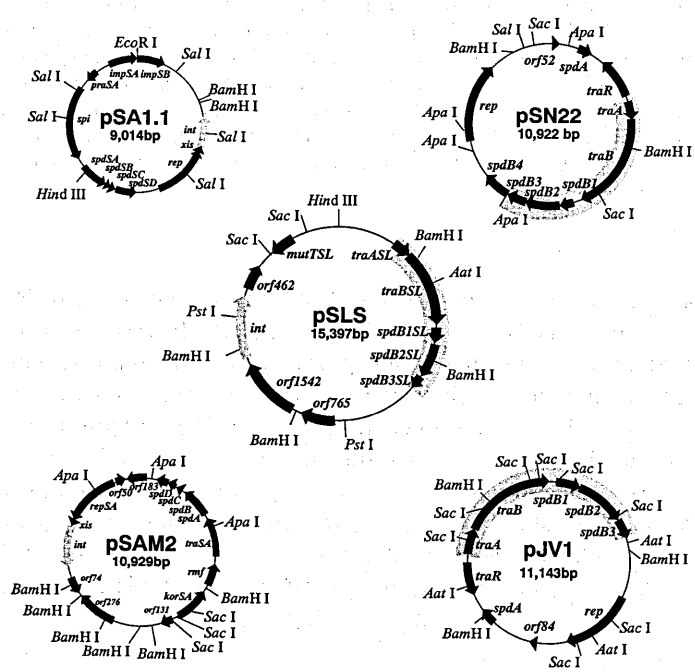

Fig. 5. Genetic relationship among *Streptomyces* conjugative plasmids. The gray shaded parts of the plasmids, pSLS of *S. laurentii*, pJV1 of *S. phaeochromogenes* and pSN22 of *S. nigrifaciens*, indicate high homologous region. The *int* genes in plasmids, pSLS, pSA1.1 of *S. azureus* and pSAM2 of *S. ambofaciens*, showed by gray arrows.

mine the minimal plasmid replicon (replication region) and single- and double-stranded origins of pSLS in the near future.

In conclusion, we report the integrative plasmid pSLS of *S. laurentii* encode *tra* and *spd* genes from non-integrative plasmid and that *tra* genes might be repressed by *trans*-acting Kor protein. In all *kil-kor* systems of *Streptomyces* plasmids, repressor protein functions by *cis*-acting like as TraR of pSN22 (Kataoka *et al.*, 1994b). It is the first report on *trans*-acting *kor* in *Streptomyces* plasmids, and this *kor* gene would locate on another linear plasmid pSLL of *S. laurentii*. Although pSLS exhibited structural specificity of non-integrative plasmid such as pJV1 and pSN22, pSLS had *int* gene for specific to integrative plasmid such as pSAM2 and pSA1.1 (Fig. 5). Based on this feature, pSLS seemed to be a novel chimeric plasmid in *Streptomyces*.

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