

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

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

<https://doi.org/10.5109/4511>

---

出版情報：九州大学大学院農学研究院紀要. 47 (2), pp.407-417, 2003-02-01. 九州大学大学院農学研究  
院

バージョン：

権利関係：



## 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

(Received October 31, 2002 and accepted November 7, 2002)

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-González, 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<sup>int</sup>) 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 pJ101 (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 (*kill*). Another gene, *kor* (*kill*-override), regulates transcription of *kill* 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 *kill* gene in pSLS. Attention is focused on *kill*-*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<sup>-1</sup> 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, *Hind* III, *Pst* I and *Sac* I digestions. Furthermore, the fragments of pSLS were digested with convenient restriction enzymes and subcloned into pUC19 for sequencing.

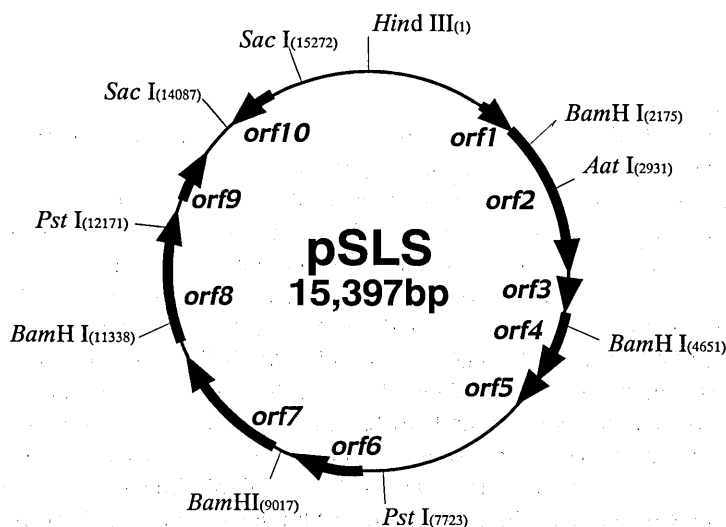
### Sequence analysis

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	<i>traASL</i>	200	Intermycelial transfer	TraA, pJV1	69/93	PRF: 220434H
ORF2	<i>traBSL</i>	660	Intermycelial transfer	TraB, pJV1	74/84	PRF: 2204341J
ORF3	<i>spdB1SL</i>	170	Spread	SpdB1, pJV1	60/75	PRF: 2204341A
ORF4	<i>spdB2SL</i>	408	Spread	SpdB2, pJV1	59/87	PRF: 2204341B
ORF5	<i>spdB3SL</i>	148	Spread	SpdB3, pJV1	60/90	PRF: 2204341C
ORF6	<i>orf753</i>	250	Hypothetical protein			
ORF7	<i>orf1542</i>	513	Hypothetical protein			
ORF8	<i>int</i>	458	Site-specific integration	Integrase, actinophage VWB	54/67	PRF: 257416A
ORF9	<i>orf462</i>	153	Hypothetical transcription regulator			
ORF10	<i>mutTSL</i>	157	Mutator	Putative MutT-like protein, <i>S. coelicolor</i> 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 *Streptomyces 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)	MYKQQNIQQNLPVSSGAGTVKSWLWKQTQPFVPPWIVTGLVGVAGGGANFAWDGSPWAG	60
TraB (pJV1)	MVRNTQQQGLPAVTSYGSTFKAWAHVTKPEVPPWAVAGVTGLAGGGANLEWQGSWAG	60
TraB (pSN22)	-MGKDVQQQEDRLNSGGTGMCAWLWHRAKPYTTPWIVTGAAGAAGAHLEWGNPSWAG	59
TraBSL (pSLS)	IGLTLGSVALTAVTWTAGARSGRQLRLHSAITVASASAWTTAAAVSGPFSGLPLGNLYLGM	120
TraB (pJV1)	VGLTLGSVALTGATWAAGVKTGRQRLHSAITVAASASAWTTAAASGPEFSGLPLDLYLGM	120
TraB (pSN22)	VGLTLAGVGLTAAATWWAGKSTGQQRRLHSAITVAAGATWFTASALSGPLTGLPLDLYLGM	119
TraBSL (pSLS)	GGI LALSNI IRRVLVAGGLVETSGTSDKGLMEKVGGLARTLLKNVKVEPNKVTAAYELPAG	180
TraB (pJV1)	GAVALASNI IRRVLAAGMAETSSESADKGLMEKVGGLARTLLKNVKVEPNKVTAAYELPAG	180
TraB (pSN22)	GTSLALTNI I RQVMRSSTPEGAGSDSDKGLLEKVGGLARTKLDKVKVEPNRVTPVELPAG	179
TraBSL (pSLS)	ESDQRGPRQRPRDLASALDVPPTTAIRI EHPDPSARRGRIVIVPEDMLKQPTIWP GPYAP	240
TraB (pJV1)	ELTN--DDL GKARDRTASALDVPPTAIRI QHPDPSARRGRIVIVPEDMLKQPTIWP GPYAP	239
TraB (pSN22)	ELTN--DDINKAI PRIASALDVPPTAIRVQHDPDSARKGQVIVPEDMLKQPTIWP GRFAP	238
TraBSL (pSLS)	GESVAEPLRIGVYDDGSDLELPELAATHI LVMGMTGSGCKTEGALDLEAGAAEPARIVVVV	300
TraB (pJV1)	GESVEVPLRIGVYDDGADLVLPLEAAIHVLMGMTGSGCKTEGALDLEELFT--RRDVVVV	298
TraB (pSN22)	GESVAVRCS--RLRRSDLVLPLEDAIHLVLMGMTGSGCKTEGAVDLELEILT--RNDVTVV	297
TraBSL (pSLS)	LADAAGAGQDFQPLVPALDWAALDTPSAAMVASVQTVI PARTAWLRDHGYRAWEPAAAE	360
TraB (pJV1)	LADAAGSGQDFQPLLPAMDWAALDTPSAAMVAAVQAVI PARTAWLRDHGYRAWEPAAAAE	358
TraB (pSN22)	LADAAGAGQDFQPLVPAEDWAALDTASAGAMVDVAVQAVI PARTAWLRDHSYRAWEPAAAK	357
TraBSL (pSLS)	RQTDRKHSCRKDGKAGCGEAIAYLLAWFEEAAKLLRELGDVFTGTAQEARSAVGLVVS	420
TraB (pJV1)	RQTNPAHSCRKDGRCAGCGMAYLLAWFEEAAKLLRELGDVFTGTAQEARSAVGLVVS	418
TraB (pSN22)	TQTNPAHSCASAG--ACGCGMPYLITWFEEAAKLLRELGDVFTGTAQEARSAVGLVVS	416
TraBSL (pSLS)	MQRASGYQLSTDTRASLPAAMCFGVKGGDASFAPEEVLADAGADPAANGNKRKGYVYLAS	480
TraB (pJV1)	MQRASGYQLSTDTRASLPAAMCFGVKGGDASFAPEEVLADAGADPAANGNKRKGYVYLAS	478
TraB (pSN22)	MQRASGYQLSTDHEGLAPGRMCFGVKGGDAGSPSPRSDAVQPARGHSAATLLCRRVERL	476
TraBSL (pSLS)	AGVDEDLHATPVRTYWTGSPSEYERMAEYVVTQFETIRAAIDAVTADAATKFAKFFETDR	540
TraB (pJV1)	AEVDEDLHATPARTFWTGPSEYERMAEYVVKQFANVRAALDAVTAGAEEKAVGEEFTRR	538
TraB (pSN22)	THRSDARRLHVAAATSSSTRS-----SCELDPVTAAGAAEQAGPLETNR	520
TraBSL (pSLS)	RARAFG-----EAPAQAGRIITAEQDVQHELVDAEDADVDPTEILRPERIPL	591
TraB (pJV1)	RERALGNQAASTSASEPTGDPLLDGLAEQESAQVAALVDEEDQELDVIITDED--PVETQI	597
TraB (pSN22)	RARAGAAS-----APARPVQEQMLLDDCGQEDGLVEMHDGIDLSADLP--PVENDA	571
TraBSL (pSLS)	SPFASKPGPEEARELDELVRMLAGMGPCTVAVKDLGPLYLEQLGRDRSWVSKEMKRLAE	651
TraB (pJV1)	ALPAAKPSPEEARELLEDNVAMLAGVGPCTVAVKDLGPLYLEQLGRDRSWVSKQMSRMASE	657
TraB (pSN22)	ELLFVKPSTEEARELDEMVALEASVGPCTVAVRDLKPYLEQLGRDRSWVSKREMKRAEE	631
TraBSL (pSLS)	G-----LVPVLAGV	660
TraB (pJV1)	GRLAPTAEQGVYRLVPLAAA	678
TraB (pSN22)	GRLAATGEEGVYRLIHTLAGV	651

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).

pSE211	1-183	LEVEDMQLVI (10)	RYVIALALGTRQGESLALKWPRLNR	229-437	Brown <i>et al.</i> , 1990
RP3	1-191	GARPEDAILL (10)	HVLTTAFAGPRWGEGLGHRDNTLL	237-447	Gabriel <i>et al.</i> , 1995
SLP1	1-238	PDRQALALL (15)	FFGCMYYAARPAEVIGLRLQDCDL	290-456	Brasch <i>et al.</i> , 1993
$\lambda$	1-179	LTADEYLKIIY (12)	AMELAVVTGQRVGDLCENKWSDIVD	227-356	Argos <i>et al.</i> , 1986
pSLS	1-229	WTRQVFGVR (9)	TVDVAGCGLRQGEVFLSEDELDF	274-276	this work
			* . . . *		
		PatchII	PatchIII	Box B	Box C
				↓ ↓	↓
VWB	1-286	PKSNAG--FRTVP (35)	ELVFRGP (41)	<b>HDLRHVFATWLK</b> DVG (5)	TQTVMGHERGSKVTWLYQH 420-427
pSAM2	1-247	TKRKSRRTLALP (27)	GGWFTQ (32)	<b>REL</b> RHSFVSLSDRG (5)	ISRLVGHSGTAVTEEVYRK 366-388
pSE101	1-242	QRQAWQ-HGCDDP (99)	HGLVFSS (29)	<b>HDARHTAATVLM</b> LLR (5)	ISRLVGHSGTAVTEEVYRK 428-448
pSE211	1-239	QRQW-KHGCSDP (91)	GEWMFTQ (29)	<b>HDARHTAATVLL</b> VLG (5)	ISRLVGHSGTAVTEEVYRK 417-437
RP3	1-285	TRGRG---RAAVP (27)	GNWRTF (31)	<b>RALRALHDTMQSEI</b> G (5)	ISRLVGHSGTAVTEEVYRK 399-447
SLP1	1-321	LKHRPRKAVRTVP (21)	GRLFRTQ (35)	<b>YDLRHA</b> AVSTWLSGG (5)	VAARAGHS-VAVLFRVYAK 436-456
$\lambda$	1-233	SKTGV---KIAIP (25)	ETIIAST (32)	<b>HEL</b> R-SLSARLYEKQ (5)	AQHLLGHKSDTMAS-QYRD 345-356
pSLS	1-294	LPKGAKVRDVLPL (33)	SLIFSGS (41)	<b>HALRH</b> FYASVLLDAG (5)	LSQYLGHADPGFTLRTYTH 428-457
				* * *	

**Fig. 3.** Amino acid sequence alignment of six domains of Int of pSLS with amino acids in the same domains of  $\lambda$  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-



MutT-like ( <i>S. coelicolor</i> )	MARVDYFNDPNAPKANSLVPSVTAVARNEAGEVLLIHKTDNDLWALF	CGGIDLCESAPDA	60	
ORF131 (pSAM2)	-----MVRVREDGRLLAIRRADNGTWELF	CGVLELDETPETG	36	
ORF154 (pSAM2)	-MLLYMSQPQEATSPPLHSVSVAGVVRVREDGRLLAIRRADNGTWELF	CGVLELDETPETG	59	
MutT ( <i>B. halodurans</i> )	-----MQRVTNCIVVDHQVLLLQKPRRGWVAF	CGCKMEAGESILET	42	
MutT ( <i>A. fulgidus</i> )	-----MKCITLTVDAIIPYQKIVLIKRLNEPFKGYALF	CGGIVEYGERVEDA	48	
ORF10 (pSLS)	MGRIDYLHDPDAPPANSVPSVAVFVQDQGRVLMIQRSNDGRWALF	CGGHDAGESISDT	60	
		23-aa phosphohydrolase module		
MutT-like ( <i>S. coelicolor</i> )	AVRETKVEETG	F DVEVTGLVGIY-----	TNPGHVMAYDDGEVRQQSICYHARITGGEL	113
ORF131 (pSAM2)	VAREVWEETG	IRVEVDELTVGY-----	KNTTRGIVALVFRCKPSGGVERTSSESTAVS	89
ORF154 (pSAM2)	VAREVWEETG	IRVEVDELTVGY-----	KNTTRGIVALVFRCKPSGGVERTSSESTAVS	112
MutT ( <i>B. halodurans</i> )	VKREVWEETG	ITVKNPELKGIFSNVIFDEGKIVSEWMLFTFKATEHEGEMLKQSPGKLE	102	
MutT ( <i>A. fulgidus</i> )	VLRVEEETG	LKGEIHSLVGVY-----	SDPNRDRPGHFVSVCFVVLKPGGEL	95
ORF10 (pSLS)	VVREVWEETG	IDAVIDVSGIY-----	TDPGHVMAYDDGEIRQQSICFRARPTGGEV	113
MutT-like ( <i>S. coelicolor</i> )	RTSSESKEVAFVDP SKLDELNIHPSMRMRIEHGLT-DRAEPIYIG-----		156	
ORF131 (pSAM2)	WLT PDEVSERMAEVYAIRLLDALDGAGPHVRSHDGKHLIPAG-----		131	
ORF154 (pSAM2)	WLT PDEVSERMAEVYAIRLLDALDGAGPHVRSHDGKHLIPAG-----		130	
MutT ( <i>B. halodurans</i> )	WKKKDEVLELPM AAGDKWIFKHVLSHSDRLLYGT FHYTPDFELLSYRLDPEPQMKGV		159	
MutT ( <i>A. fulgidus</i> )	KAGSDAKEVGLFSLNELPKLAFDHEKMIKDAEVIIRGILSEV-----		137	
ORF10 (pSLS)	RTSSETTQVRVWVAPADLVELDVHPTMRLRIEHAMDRTRTAPYIG-----		157	

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 phosphohydrolase module was boxed.

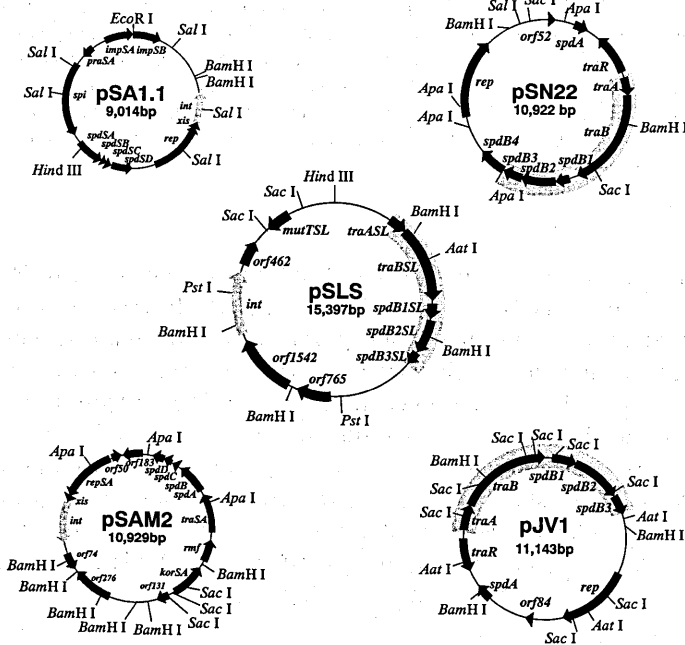


Fig. 5. Genetic relationship among *Streptomyces* conjugative plasmids. The gray shaded parts of the plasmids, pSLS of *S. laurentii*, pJV1 of *S. phaeochromogenes* and pSN22 of *S. nigrifaciens*, indicate high homologous region. The *int* genes in plasmids, pSLS, pSA1.1 of *S. azureus* and pSAM2 of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

#### ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific research (B, No. 08456055) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by the Waksman Foundation of Japan Inc. We are grateful to Dr. M. Kataoka (Shinshu University, Japan) for providing *traR* gene of pSN22. We are grateful to Dr. S. Matsuo for critical comments on the manuscript.

#### REFERENCES

- Argos, P., A. Landy, K. Abremski, J. B. Egan, E. Haggard-Lindquist, R. H. Hoess, M. L. Kahn, B. Kalionis, S. V. L. Narayana, L. S. Pierson II, N. Sternberg and J. M. Leong 1986 The integrase family of site-specific recombinase: regional similarities and global diversity. *EMBO J.*, **5**: 433-440
- Bessman, M. J., D. N. Frick and S. F. O'Handley 1996 The MutT proteins or "Nudix" hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. *J. Biol. Chem.*, **271**: 25059-25062
- Bibb, M. J., R. F. Freeman and D. A. Hopwood 1977 Physical and genetical characterization of a second sex factor, SCP2, for *Streptomyces coelicolor* A3(2). *Mol. Gen. Genet.*, **154**: 155-166
- Boccard, F., T. Smokvina, J. -L. Pernodet, A. Friedmann and M. M. Guérineau 1989 The integrated conjugative plasmid pSAM2 of *Streptomyces ambofaciens* is related to temperate bacteriophages. *EMBO J.*, **8**: 973-980
- Brasch, M. A., G. S. Pettis, S. C. Lee and S. N. Cohen 1993 Localization and nucleotide sequences of genes mediating site-specific recombination of the SLP1 element in *Streptomyces lividans*. *J. Bacteriol.* **175**: 3067-3074
- Brown, D. P., Idler, K. B. and L. Katz 1990 Characterization of the genetic elements required for site-specific integration of plasmid pSE211 in *Saccharopolyspora erythraea*. *J. Bacteriol.*, **72**: 1877-1888
- Brown, D. P., Idler, K. B., Backer, D. M., Donadio, S. and L. Katz 1994 Characterization of the genes and attachment sites for site-specific integration of plasmid pSE101 in *Saccharopolyspora erythraea* and *Streptomyces lividans*. *Mol. Gen. Genet.*, **242**: 185-193
- Chater, K. F. 1989 In I. Smith, R. A. Slepecky, and P. Setlow (ed.), Sporulation in *Streptomyces*, American Society for Microbiology, Washington, D. C, pp. 277-299
- Chater, K. F. 1993 Genetics of differentiation in *Streptomyces*. *Annu. Rev. Microbiol.* **47**: 685-713
- Doi, K., Y. Ono, E. Yokoyama, Y. Tsukagoe and S. Ogata 1998 Whole sequence of *spoIII*-like, sporulation-inhibitory, and transfer gene (*spi*) in a conjugative plasmid, pSA1.1, of *Streptomyces azureus* and detection of *spi*-like gene in the actinomycete chromosome. *Biosci. Biotechnol. Biochem.*, **62**: 1597-1600
- Eposito, D. and J. J. Scoocca 1997 The integrase family of tyrosine recombinase: evolution of a conserved active domain. *Nucleic Acids Res.*, **25**: 3605-3614

- Fowler, R. G. and R. M. Schaaper 1997 The role of the *mutT* gene of *Escherichia coli* in maintaining replication fidelity. *FEMS Microbiol. Rev.*, **21**: 43–54
- Gabriel, K. H. Schmid, U. Schmidt and H. Rausch 1995 The actinophage RP3 DNA integrates site-specifically into the putative tRNA<sub>(Arg)(AGG)</sub> gene of *Streptomyces rimosus*. *Nucleic Acids Res.*, **23**: 58–63
- Hagége, J. M., J. -L. Pernodet, G. Sezonov, C. Gerbaud, A. Friedmann and M. Guérineau 1993 Transfer Functions of the conjugative integrating element pSAM2 from *Streptomyces ambofaciens*: Characterization of a *kil-kor* system associated with transfer. *J. Bacteriol.*, **175**: 5529–5538
- Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, M. J. Ward and H. Schrempf 1985 Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Foundation, Norwich.
- Hopwood, D. A., and T. Kieser 1993 Conjugative plasmids of *Streptomyces*, In D. B. Clewell (ed.), Bacterial conjugation. Plenum Press, New York, N.Y., p. 293–311
- Kataoka, M., T. Seki and T. Yoshida 1991a Five genes involved in self-transmission of pSN22, a *Streptomyces* plasmid. *J. Bacteriol.*, **173**: 4220–4228
- Kataoka, M., T. Seki and T. Yoshida 1991b Regulation and function of the *Streptomyces* plasmid pSN22 genes involved in pock formation and inviability. *J. Bacteriol.*, **173**: 7975–7981
- Kataoka, M., Y. M. Kiyose, Y. Michisuji, T. Horiguchi, T. Seki and T. Yoshida 1994a Complete nucleotide sequence of the *Streptomyces nigrifaciens* plasmid pSN22 genetic organization and correlation with genetic properties. *Plasmid*, **32**: 55–69
- Kataoka, M., S. Kosono, T. Seki and T. Yoshida 1994b Regulation of the transfer genes of *Streptomyces* plasmid pSN22: *in vivo* and *in vitro* study of the interaction of TraR with promoter regions. *J. Bacteriol.*, **176**: 7291–7298
- Kendall, K. J. and S. N. Cohen 1987 Plasmid transfer in *Streptomyces lividans*: identification of a *kil-kor* system associated with the transfer region of pJJ101. *J. Bacteriol.*, **169**: 4177–4183
- Kendall, K. J. and S. N. Cohen 1988 Complete nucleotide sequence of the *Streptomyces lividans* plasmid pJJ101 and correlation of the sequence with genetic properties. *J. Bacteriol.*, **170**: 4634–4651
- Kieser, T., D. A. Hopwood, H. M. Wright and C. J. Thompson 1982 pJJ101, a multi-copy broad host-range *Streptomyces* plasmid: functional analysis and development of DNA cloning vectors. *Mol. Gen. Genet.*, **185**: 223–228
- Kinoshita-Iramina, C., M. Kitahara, Y. Harada, K. Doi and S. Ogata 1995 Two plasmids related to spontaneously developing pocks in *Streptomyces laurentii*. *Biosci. Biotechnol. Biochem.*, **59**: 1040–1043
- Kinoshita-Iramina, C., M. Kitahara, K. Doi and S. Ogata 1997 A conjugative linear plasmid in *Streptomyces laurentii* ATCC31255. *Biosci. Biotechnol. Biochem.*, **61**: 1469–1473
- Landy, A. 1989 Dynamic, structural, and regulatory aspects of lambda site-specific recombination. *Annu. Rev. Biochem.*, **58**: 913–949
- Maas, R. M., J. Gotz, W. Wohlleben and G. Muth 1998 The conjugative plasmid pSG5 from *Streptomyces ghanaensis* DSM 2932 differs in its transfer functions from other *Streptomyces* rolling-circle-type plasmids. *Microbiology*, **144**: 2809–2817
- Nunes-Duby, S. E., H. J. Fritsch, R. S. Tirumalai, T. Ellenberger and A. Landy 1998 Similarities and differences among 105 members of the Int family of site-specific recombinases. *Nucleic Acids Res.*, **26**: 391–406
- Ogata, S., Y. Koyama-Miyoshi and S. Hayashida 1985 Transfection and transformation systems for pock-forming and thiostrepton-producing *Streptomyces azureus*. *J. Fac. Agr., Kyushu Univ.*, **29**: 179–188
- Ogata, S., H. Matsubara, Y. Harada and A. Umeda 1992 Formation of Spontaneously developing pocks and producing of phage taillike particles in thiostrepton-producing *Streptomyces laurentii* ATCC31255. *Actinomycetol.*, **6**: 29–32
- Omer, C. A., D. Stein and S. N. Cohen 1988 Site-specific insertion of biologically functional adventitious genes into the *Streptomyces lividans* chromosome. *J. Bacteriol.*, **170**: 2174–2184
- Qin, Z. and S. N. Cohen 1998 Replication at the telomeres of the *Streptomyces* linear plasmid pSLA2. *Mol. Microbiol.*, **28**: 893–903
- Sambrook, J., E. F. Fritsch and T. Maniatis 1989 Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, NY

- Servín-González, L. 1993 Relationship between the replication functions of *Streptomyces* plasmids pJV1 and pJJ101. *Plasmid*, **30**: 131–140
- Servín-González, L., L. A. Sampieri, J. Cabello, L. Galván, V. Juárez and C. Castro 1995 Sequence and functional analysis of the *Streptomyces phaeochromogenes* plasmid pJV1 reveals a modular organization of *Streptomyces* plasmids that replicate by rolling circle. *Microbiology*, **141**: 2499–2510
- Sezonov, G., C. Possoz, A. Friedmann, J. L. Pernodet and M. M. Guérineau 2000 KorSA from the *Streptomyces* integrative element pSAM2 is a central transcriptional repressor: target genes and binding sites. *J. Bacteriol.*, **182**: 1243–1250
- Van Mellaert, L., L. Mei, E. Iammertyn, S. Schacht and J. Anné 1998 Site-specific integration of bacteriophage VWB genome into *Streptomyces venezuelae* and construction of a VWB-based integrative vector. *Microbiology*, **144**: 3351–3358
- Yamasaki, M., Redenbach, M. and H. Kinashi 2001 Integrated structures of the linear plasmid SCP1 in two bidirectional donor strains of *Streptomyces coelicolor* A3(2). *Mol. Gen. Genet.*, **264**: 634–642
- Walker, J. E., M. Saraste, M. J. Runswick and N. J. Gay 1982 Distantly related sequences in the  $\alpha$ - and  $\beta$ -subunits of ATP synthase, myosin, kinase and other ATP-requiring enzyme and a common nucleotide binding fold. *EMBO J.*, **8**: 945–951
- Zotchev, S. B., and H. Schrempf 1994 The linear *Streptomyces* plasmid pBL1: analyses of transfer functions. *Mol. Gen. Genet.*, **242**: 374–382