Transfer Function of *spi* Gene in Plasmid pSA1.1 of *Streptomyces azureus* ATCC14921

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Conjugative (sex) plasmids, such as pIJ101¹⁾,
pSA1.1²⁾, pSAM2³⁾ and pSN22⁴⁾, of the *Streptomyces*
species, are phenotypically detectable
by their ability to elicit zones of growth inhibition
or "pock" when the plasmid carrying
strains are grown in contact with plasmid-free
strains on solid media. Plasmid transfer is
almost always associated with pock formation.
Transfer regions or transfer genes are localized
on the several plasmids, but sequenced only in
the case of a few plasmids.

Plasmid pSA1.1-harboring mycelia (or
spores) of *Streptomyces azureus* ATCC 14921
elicited conjugative pocks toward the plasmid-
free mycelia at a frequency of 100%²⁾. The
pSA1.1 also inhibited the sporulation of its host
mycelia²⁾. The pock-forming function and the
sporulation-inhibitory function in the plasmid
pSA1.1 was analyzed⁵⁾. The deletion analysis of
pSA1.1 derivative plasmid indicated the sporu-
lation-inhibitory region located on the 1.5kb *Aat*
I-*Sma*I fragment of pSA1.1. The sporulation-
inhibitory gene (*spi*) was identified by the
nucleotide sequence of this fragment. The
predicted amino acid sequence of the 909bp
open reading frame was similar to that of
spoIIIE gene in *Bacillus subtilis*⁶⁾. It was char-
acteristic that there were mononucleotide bind-
ing sites in both proteins. The *spoIIIE* gene
encodes a 787-residue protein containing five to
seven putative transmembrane segments and
potential nucleotide binding sites.

Recently, it is revealed that the nucleotide

Fig. 1. Change of transfer ability of pSA1.1 by insertion and deletion. The derivatives of pSA1.1 are designated as pSAK1, pSAK52, pSAK61 and pSAK-S9. They were made by insertion of the kanamycin resistant gene and by deletion with the appropriate restriction enzymes. The interruption region of the line shows the deletion fragment of pSA1.1. The location and orientation of the *spi* gene are shown by a horizontal arrow. o, insertion site of the kanamycin resistant gene; +, transfer; -, not transfer.

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spi                                     ATGACGTGCTGCGCCGGGTG 20
                                     * * * * *
traSA GAACTGCGCTCAGGTGTGGTCGAGGTGCGGATGACCGGCTACGACGTCTCCAGCGGGTG 120

spi CCGATCCGCGGCAAGGCCCGCCGCATGGCATG-GCGTTCCGGT--CGGTGCGCGAGGAC 77
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA CAGATGCCCGCCACAGCCGAGACGCGGCCGATGCGCATCCCTGTGGCGCTGCGGGAGGAC 180

spi GG-GAGCCCTTCGAGCGGGACTA-CGCAAAGCTCCGATGGCACTCACCTGGGCGCGAAC 135
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA GGTGCGGTGCACTACCGCGACTACCGTGCCGTCCCGCACGGTCTGACCCTGGGGGCCACG 240

spi CACTCGGGCAAGTCGATGTATCAGCGCAACCTGATCAAGGGGCTGGCGCAACTGCCGGTG 195
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA GAGTCCGGGAAGTCCGTCTACCAGCGCAATCTGGTCGCGGGGCTCGCTCCGCACCATGTT 300

spi GCCCTGGTCCGTATCGACTGCAAGCGCGGTGTGGAACAGGCCGCGTTCGCGCCCCGGGTG 255
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA GCCCTGGTCCGCGATCGACTGCAAGCAAGGCGTGGAACCTGTTCCCGCTGGCGCCCGGTTG 360

spi TCGGCGCTGGTCACTCCCGATGATGCGGCCCTCGCTGCTCGGTGTGCTGGTCGCGGAG 315
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA TCCGCGCTCGCCGACAACCCCGACACCGCCCTCGATCTCCTCGAAGCGCTCGTCGGACAC 420

spi ATGGAGG-----GCGGCTTCGACCTGCTGAGC--CGTCACGGTGTC--- 354
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA ATGGAGGACGTCTACCAGCTCATCCGGGCCGAGCAGCGCATCAGCGTCGCGCTGCCGGAT 480

spi TCGG-----ACCTGTGGGAGCTGCCTGCCGAGGTGCGGCCGGTGCCGGTGGTC 402
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA GCGGAGATCGCCGCCGATATCTGGGACCTGCGCGAGGACCTGCGGCCAGTGCCGGTCTGTG 540

spi GTCCTGGTCGACGAGGTGGCGGAACTGTTCTGATCTCTCTCCAAGAAGGATGAGGAGCGC 462
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA GTCCTGGTCGACGAGGTGCGCGAACTCGCTCTCTTCGCCACCAAGGACGAAGAGAAGCGC 600

spi CGGGAACGGATCGTCACCGCGTTGATCCGACTCGCGCAGATGGCCCGGGCCATCGGTATC 522
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA CGCGACCGCATCATCTGCTTGGTCCGCTCGCCAGCTCGGCCGCGCCGCTGGCATC 660

spi CATCTGGAGATCGCGGGCAGCGCTTTGGCTCCGACCTGGGCAAGGGCGCCACCATGCTC 582
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA TACCTCGAAATCTGCGGGCAGCGCTTCGGCTCCGAACTCGGCAAGGAATCACCATGCTC 720

spi CGTGTCTAGCTACCGGCCGTGTGGTGACCGCGTCAACGACAAGCAGACCGCCGAGATG 642
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA CGTGCCAGCTACCGGCCGTACCGCCACCGCGTCAACGACGAAACGTCCGCGAACATG 780

spi GGTTTGGCGGACGTGGCCCCGACGCGGTACCGGCCGCAAGCCTGATCCCATGAACCGC 702
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA GCTTCGG-TGACGTGTACCCGATGCCGTCTGGCTGCCATCCAGATCCCCACCGACACA 839

spi CCCGGGACCGCGGTGCGCCGAGACCCCTCCGCTGGCTGGTGAAGATCCGCACCCCGGAA 762
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA CCCGGCGTCGCTGTACCGGTGACTCGACAGGCGGCTGGGCCCCGATCCGCGCCCCGCAC 899

spi ACCTACGGGACGAAGTGGTAGCCGTCTGCCGGGAGTTGCCCCACCTTATCCCGGACCTG 822
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
traSA ACCACGCTGCGCGAGGCCGTGA 922

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Fig. 2. Nucleotide sequence homology of the *spi* gene with the *traSA* gene. Identical sequences and gaps are indicated by asterisk and dashes, respectively.

constructed as shown in Fig. 1. The recombinant plasmid pSAK1 was constructed by the insertion of kanamycin resistant gene (*kmr*) from plasmid pMCP5 of *Streptomyces kanamyceticus*¹³) into the pSA1.1. The pSAK52 was constructed by the insertion of the *kmr* gene as follows. Plasmid pSA1.1 was digested with *Hin* dIII, and linearized DNA was blunted with Mung Bean nuclease and Klenow fragment. Then, the 4.0 kb *Bcl*II-digested *kmr* fragment of pMCP5 was also blunted and then ligated with the blunted pSA1.1. The pSAK61 was constructed by the insertion of the *Bcl*II-digested *kmr* gene into a unique site, *Bgl*III site, of pSA1.1. The pSAK-S9 was obtained by the deletion of the *Sma*I^a-*Sma*I^c region in pSAK1. These constructed plasmids were introduced into the plasmid-free strain PK100C by the protoplast transformation method, as described elsewhere¹⁴). The selenium resistant derivative of PK100C, strain SR58, was used as the recipient for the plasmid transfer. The plasmid trans-

To examine the transfer ability of the *spi* gene, four derivative plasmids of pSA1.1 were

Spi		MTCCAGCGSAAAPARMAWRSGRCAR	25
TraSA	MAATTDRLRHSFGVYGVTSLRELRSGVVEVRMGTGYDVLQRVQMPTAETRPMRIIPVALRED		61
Spi	TGRLRAGLRKAPMALTLGANHGGKSMYQRNLIKGLAQLPVALVGIDCKRGVEQAFAFPRL *, *, ****.,****.,***, .*****.* **..**.		85
TraSA	GAVHYRDYRAVPPEGLTLAGATESGGKVYQRNVLAVGLAPHVAIVLGIDCQGVELFLPARRF		120
Spi	SALVTTPDDAASLLGVLAEMEGRFDL-----LSREGV-SDLWELPAEVRPVFPVV ***.....*, **,....**, ..*		134
TraSA	SALADNPDTALDLLLEALVGHEMDVYLIRAEQRSISVAVPDAEIAADIWDLEDLRPPVFPVV		180
Spi	VLVDEVAELFLISSKKDEERRERIVTALIRLAQMARAIIGHLEICGRFGSDLGKGATML ***** *...*.**.****,*..* ***** *		194
TraSA	VLVDEVAELALFATKDKEEKRRDIITALVRLAQLGRAAGIYLEICGRFGSELGKGITML		240
Spi	RAQLTG RVVHRVNDKQTAE MG LADV AP DAVA AS LI PM NR PG T-- AVAAD P SG GWSKI RT ***** ,***** ,*,*, *. * * * * . * . * . * *		252
TraSA	RAQLTGRTAHRVDSETSANMASVTCHMPMSWLPSRPPTH PASLSPVTR Q AG PV SAPRT		300
Spi	PETSRDEV VAVCRE FAHL IP DL FPF LE FR PR VP AE V PA A GP S M VK PR PL TE * *		303
TraSA	PRCARP		307

Fig. 3. Amino acid sequence homology of the *spi* product with the *traSA* product. Identical residues, similar residues and gaps are indicated by asterisk, dots and dashes, respectively. The underlined residues conform to the consensus sequence for mononucleotide binding sites. The transmembrane segments are indicated by dotted underline.

formants were grown together with strain SR58 on the Rye flakes plates for 5 days at 28°C, and then double resistants were selected from the Rye flakes plates with 25 µg/ml kanamycin and 5 mM sodium selenate (Selection medium). Plasmid DNAs were extracted by using the Alkaline lysis method¹⁵⁾ from these double resistants obtained, and their features such as sizes, restriction enzyme map, and orientation of *spi* gene were reconfirmed.

The pSAK1- and pSAK52- carrying transformants were grown on the selection medium at about 1×10^5 cfu per µg of plasmid DNA, but not pSAK61- and pSAK-S9- carrying transformants. The pSAK1 and pSAK52 with the intact *spi* gene were able to transfer to the recipients, whereas the pSAK61 and pSAK-S9 with the disrupted *spi* gene could not. These results indicated that the *spi* gene (product) should play a role in plasmid transfer.

The *spi* gene and its product were also scanned for the homology with the data registered in GenBank by the GENETYX program. The *traSA* gene of conjugative plasmid pSAM2 has high homologies with *spi* gene. The value of percent identity at nucleotide sequences is 66.0% (Fig. 2). Moreover, the value of percent identity at predicted amino acid sequences are 52.3% (Fig. 3). The size of *spi* gene product and *traSA* gene product are 303 amino acids (aa) and 306 aa, and their predicted molecular weights are 32,513 and 33,441, respectively. Both of sequences have mononucleotide binding sites¹⁶⁾ and transmembrane segments¹⁷⁾ as also shown in Fig. 3. The *spi* product and TraSA also have hydrophobic stretch of amino acid residues. There are no homologies of genes and gene products among any other conjugative plasmids of streptomycetes with the *spi* gene and its product, except the mononucleotide binding sites in their products.

Some proteins such as Tra I of F element, VirB11 of Ti plasmid, and TrbB of RP4 have mononucleotide binding sites and ATPase activ-

ities¹⁸⁾. TraSA also contained an ATP-binding motif and exhibited ATPase activity as deduced from this DNA sequences. It is suggested that the DNA translocation of these elements might be mediated by ATP hydrolysis (ATP→ADP+Pi). The mechanisms of the DNA translocation seem to be similar among the bacteria. The *spi* gene product indicates a transmembrane domain as shown by the amino acid sequences. Its mononucleotide binding sites might bind ATP and utilize the free energy of ATP hydrolysis to drive plasmid transfer.

From these observations, we suppose that the *spi* gene product is incorporated in the host cell membrane and promote the plasmid transfer to the neighboring cells. Now, we are trying to analyze the function of the *spi* gene and its product in more detail.

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