Characterization of A New Staphylococcal Site-Specific Recombinase *sin* and Genetic Organization of Its Flanking Region

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A new site-specific recombinase *sin*, as a component of a putative transposon has been cloned and its base sequence has been determined. The proposed *sin* shows a high degree of homology with pI9789-*sin* and pSK1-*sin*. There is a large (16 bp) inverted repeat downstream of proposed *sin* and the postulated helix-turn-helix motif is located at the extreme C-terminus of the proposed Sin. The transposase gene (*tnpA*) and β-lactamase gene (*blaZ*) are located upstream of *sin* and arsenate reductase gene (*arsC*) and arsenic efflux pump protein gene (*arsB*) are downstream. This genetic arrangement seems to be a part of a new putative transposon because there is no known transposon with a gene arrangement of *tnpA-blaZ-sin-arsC*.

Key words: DNA invertase, *sin*, site-specific recombinase, staphylococcal transposon

Bacterial transposable elements are specific segments of DNA carrying antibiotic-resistance genes and can translocate as discrete units causing insertional polar mutations (2).

It has been analyzed that the antibiotic-resistant transposons have two basic genetic arrangements. The first one, compound transposons or class I transposons such as Tn5 and Tn10 has an antibiotic resistance determinant flanked by an IS element either as direct or as inverted repeats. In this class, IS elements take charge of the transposition of the intervening drug resistance gene(s). The second class of transposon, class II transposons or complex transposon such as Tn1 is usually flanked by 30 to 40 bp inverted repeats. Genes related to transposition and antibiotic-resistance are located between these inverted repeats (1). Another distinct class of bacterial transposable elements includes the temperate bacteriophage Mu and related phages. They are distinguished from the common transposon by the viral life style and a lack of a heritable property on the host bacterium (6). It has been demonstrated that the transposition process is independent of host *recA* function (10). Instead, it requires an element-specified function known as a transposase (8). Several other genomic rearrangements such as deletions, inversions, and excisions are also catalyzed by transposable elements in a *recA⁻* background (6).

Previously, we have cloned and analyzed a *bla*

gene containing a *Hin*dIII fragment from chromosomal DNA of ampicillin-resistant *Staphylococcus aureus*. The fact that the upstream region of *bla* structural gene is a truncated C terminus of Tn 4001 transposase (5) indicates that the *bla* structural gene was part of a transposon and is now integrated into the chromosomal DNA of *Staphylococcus aureus* as a result of transposition. The plasmid-mediated *bla* structural gene has been extensively studied in staphylococcal systems as well as in *Bacillus licheniformis* and *Escherichia faecalis* (17).

In this paper, we are reporting a new ORF with high homology to *sin*, the potential recombinases from staphylococcal plasmid pI9789 and pSK1. Both are transposon-conferring plasmids.

Materials and Methods

Strains and plasmids
Bacterial strains and plasmids used and their source are listed in Table 1.

Chemicals and enzymes
All restriction endonucleases, Klenow fragments, and T4 ligase were purchased from New England Biolabs and Promega. Fine reagents such as IPTG, X-gal, lysozyme, lysostaphin, RNase, urea, acrylamide, as well as various antibiotics such as ampicillin, chloramphenicol, and erythromycin were bought from Sigma Chemical Co. Media and com-

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Table 1. Bacterial strains, phages, and plasmids

Strains	Relevant genotypes or phenotypes	Reference or Source
Bacterial Strains		
S. aureus ATCC6538	Wild-Type	ATCC
S. aureus SBK110	Am^r, Tc^r, Km^r	Byeon et al. (1985)
E. coli HB101	F, recA13, ara14, proA2, galK2	G. Cooper
E. coli MV1190	△(lac-proAB) thi, supE44	J. A. Fuchs (Univ. of Minn.)
	△(srl-recA) 306::Tn10(Tc^r)	
	[F' traD36 proAB lac^q lacZ△M15]	
Plasmids and phages		
pBR322	Tc^r, Am^r	J. Messing
pUC119	Am^r	J. Messing
M13mp18	M13 phage	J. Messing
M13mp19	M13 phage	

Abb. : Tc, tetracycline; Am, ampicillin; Cm, chrolamphenicol; Em, erythromycin; Km, kanamycin.

ponents for culturing bacterial strains were purchased from Difco Lab.

Transformation of *Staphylococcus aureus*

For transformation of S. aureus, protoplasts were prepared as described by Gotz, et al. (7). 10 ml samples of bacterial cells grown to the stationary phase (approximately 2×10^9 colony-forming units per ml) in trypticase soy broth (Becton Dickinson Co., MD. USA) were harvested and suspended to the same volume in sucrose-maleate-MgCl$_2$-Penassay (SMMP) medium (7.5 parts of sucrose-maleate-MgCl$_2$ (SMM) buffer (1 M sucrose, 0.04 M maleate, 0.04 M MgCl$_2$, pH6.5), 2 parts of 7% Penassay broth (Difco, USA), 0.5 parts of 10% bovine serum albumin) (7). Lysostaphin and lysozyme were added to 20 µg/ml and 2 mg/ml, in final concentrations, respectively, and the cell suspensions were incubated at 37°C with gentle shaking. The absorbancy at 540 nm decreased with incubation time. Incubation was carried out until the absorbancy became constant, which for S. aureus usually occurred within 3hrs.

Cloning and identification of *bla* expression in *S. aureus*

Transformants were screened with a polyvinyl alcohol (PVA)-iodine β-lactamase plate assay (19). In the assay, PVA (0.75%; Sigma Chemical Co.) was incorporated into the agar. Colonies were placed with a toothpick onto a PVA plate and a corresponding PVA-cephalosporin C (2 µg/ml for induction of β-lactamase production) plate, and the plates were incubated overnight at 37°C. Both plates were flooded with a KI-I$_2$ solution so that a blue I$_2$-PVA complex was formed. After draining the plate, a 1% solution of penicillin was added. The penicilloic acid produced by the action of the β-lactamase on penicillin reacted with the iodine, resulting in a clearing around the β-lactamase-producing colonies. The phenotype (constitutive or inducible) of the colony could be determined by comparing the size of the clearing on the plate containing cephalosporin C with that of the clearing on the plate without cephalosporin C.

DNA sequencing and homology analysis

The nucleotide sequence of the putative transposon from *Staphylococcus aureus* SBK110 chromosomal DNA was determined by the Sanger dideoxynucleotide chain termination method (16) using a chemiluminescent Uniplex DNA Sequencing Kit (Millipore Co.).

Sequence data for homology analysis were obtained from GenBank, National Center for Biotechnology Information (NCBI), NIH, USA by 'retrieve' program and were analyzed by DNASIS program (Hitachi Co.) or 'BLASTn' of NCBI on Internet.

Results and Discussion

Cloning of *blaZ* flanking region in *S. aureus*

The 3' downstream region of *blaZ* was cloned and sequenced. Homology search of the sequence of this region identified an ORF that is a member of the *sin*, a sequence specific recombinase gene. Since the bioassay of Sin has not been established yet, the *blaZ-sin*-linked fragment was the object for the subcloning of sin gene. Presence of the *bla* gene was confirmed by measuring the β-lactamase activity of transformant. Staphylococcal or streptococcal genes could be easily cloned and maintained in *Bacillus subtilis* because all these bacteria are gram positive bacteria and because cloning vectors and methods of transformation in *Bacillus* system are well established. However, it was found that *Bacillus* is not suitable as a cloning host of *bla* gene be-
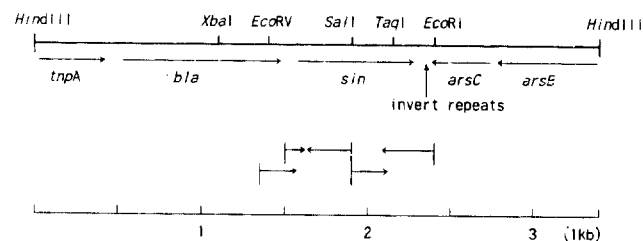
Fig. 1. Sequencing strategy of *sin* and genetic organization of the putative transposon.

Fig. 2. The nucleotide sequence of the 1.2kb *Eco*RV-*Eco*RI fragment containing *sin* gene from 3.4 kb *Hind*III fragment and deduced amino acid sequence of Sin. Two regions of inverted repeats downstream of *sin* are indicated by arrows below the nucleotide sequence.

cause it showed very delicate responses to ampicillin concentration. So we adopted a *Staphylococcus* system for *bla* gene cloning.

Base sequence determination and homology analysis of *sin*

The nucleotide sequence of the *Hind*III (3.4 kb) fragment containing the *sin* gene was determined by the dideoxynucleotide chain termination method (Fig. 2). The sequencing strategy and restriction map of the *Hind*III (3.4 kb) fragment are shown (Fig. 1). The region immediately downstream of the *bla* gene is a proposed sin showing a high degree of homology to pI9789-*sin* and pSK1-*sin*. It encodes a member of a closely related 'superfamily' of site-specific recombinases that includes the DNA invertases Hin (*Salmonella typhimurium*), Gin (phage Mu), Cin (phages P1 and P7), and Pin (*Escherichia coli*) and the resolvases of Tn3-class transposons (13). Resolvases and DNA invertases share considerable amino acid sequence homology (Fig. 3A). Analysis of resolvase and DNA invertases has shown that a hinge region that contains a conserved glycine connects two major structural domains: an N-terminal catalytic and dimerization domain and a C-terminal DNA-binding domain (13). The postulated helix-turn-helix motif is at the extreme C-terminus of the proposed Sin (Fig. 2). There is a large (16 bp) inverted repeat downstream of the proposed *sin* (Fig. 2).

Genetic organization of *blaZ-sin* conferring transposon

We have published in a previous paper that there is a truncated 3'-terminus of Tn4001-transposase (*tnpA*) upstream of *blaZ* gene on 3.4 kb *Hind*III fragment (5). Further analysis of the genetic organization of this fragment reveals that this sequence seems to be part of a putative transposon. The *Hind*III fragments were originally cloned from chromosomal DNA of *S. aureus* and it is believed that the transposase gene and β-lactamase gene found on the *Hind*III fragment are not native genes of chromosomes but of a transposon. However, there
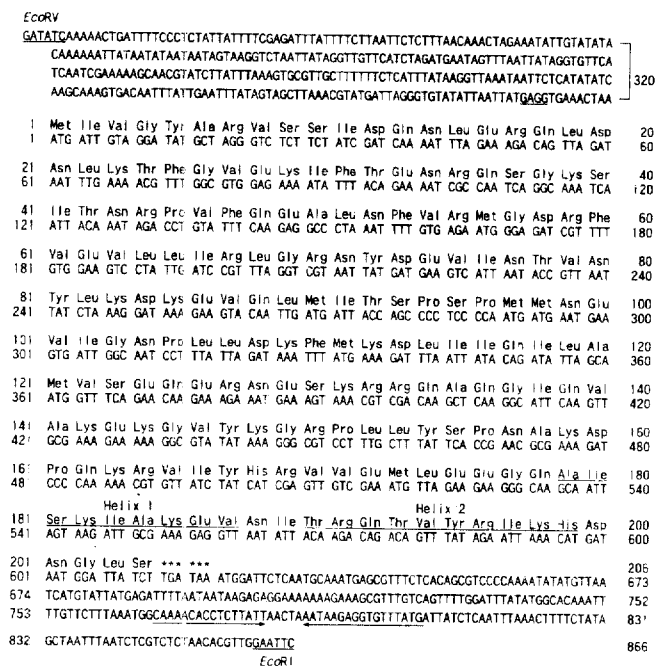
is no known transposon with a gene arrangement like ours, namely, in *tnpA-blaZ-sin* configuration. Sequence homology searches of genes on *Hind*III fragment with GenBank data enabled us to find several genes from different sources.

As mentioned earlier, the amino acid sequence of our truncated 3' region of *tnpA* was exactly same as that of Tn4001 (3). But Tn4001 has two regions that are homologous with our truncated-*tnpA* sequence: one is in the full *tnpA* sequence and the other is a truncated-*tnpA* that is same size as our sequence. It suggests that the truncated *tnpA* of our *Hind*III fragment might not be part of a functional *tnpA*, but only a drifting sequence produced by transpositional intragenic intervening or translocation. No sequence of Tn4001 other than *tnpA* has any homology with the sequence of *Hind*III fragment.

The *blaZ* homologous sequence is found on the right end of Tn552, actually in Tn552-integrated pS1 (12, 15). Immediately outside of Tn552-*blaZ* is *sin* gene with a sequence 100% identical to that of pI9789 (18). We do not have enough information whether the *blaZ-sin* link as in pS1 is a more advanced arrangement than a separate existence as in pI9789. The proposed *sin* shows significant homology with known potential recombinase genes, *sin* of pI9789 (185/204, 90.7%), and *sin* of pSK1 (186/204, 91.1%) (Fig. 3B). The *sin* gene is followed
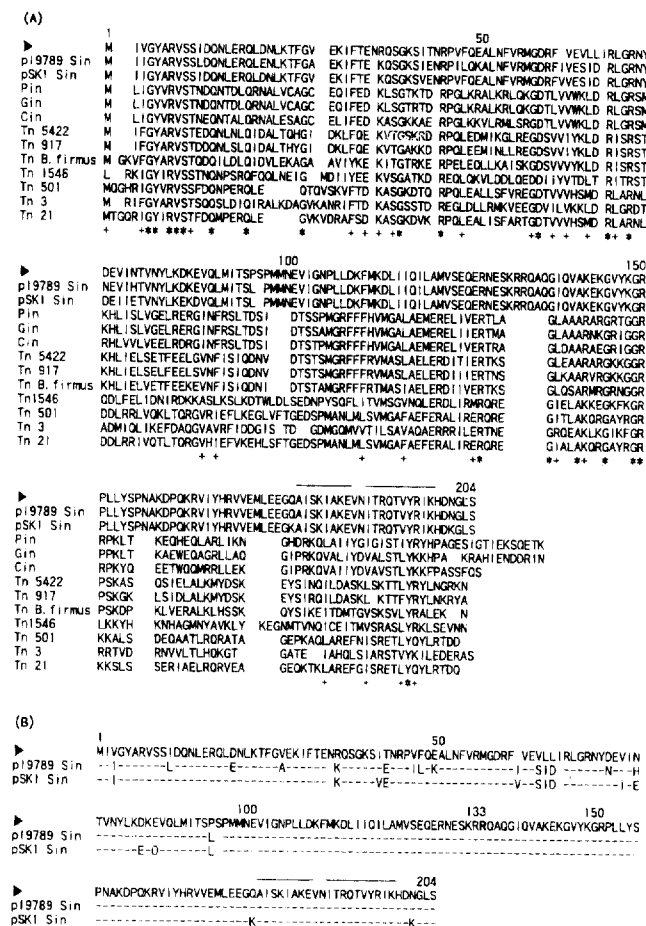
(A)

(B)

Fig. 3. (A) Amino acid sequence alignment of 13 site-specific DNA recombinases having homology with Sin. Numbers on each line refer to the positions of the amino acid. Asterisks (*) indicate position where amino acids are identical in all position, and pluses (+) indicate those where amino acids are similar. The position of the putative DNA-binding helix-turn-helix motif is indicated by a line above the sequence. The predicted amino acid sequences compared include the invertases of the putative transposon (this study, ▶), pI9789 and pSK1 from *S. aureus* (11, 17), Pin from *Shigella boydii*, Gin from phage Mu, and Cin from phage P1. The recombinases are Tn5422 from *L. monocytogenes*, Tn917 from *E. faecalis*, the putative transposon from *B. firmus*, and Tn 1546 from *E. faecium*, Tn 501 from *P. aeruginosa*, Tn3 from *E. coli*, Tn21 from *S. flexneri* (11). (B) Only three invertases from *S. aureus* are compared. "-" designates identical amino acid as Sin of the putative transposon (11, 17).

by arsenate reductase gene (*arsC*) and arsenic efflux pump protein gene (*arsB*) in a reverse direction as a *sin* (→), *arsC* (←), *arsB* (←) arrangement. The same arrangement is found on pI258, in which only the truncated, 3′ region of sin (from 133rd amino acid to the end, 204th amino acid) is linked to *arsC* gene (9) (Fig. 4). The amino acid sequences of the two overlapped regions of *sin* are identical as seen in Fig. 3, but base sequence of the two genes are not
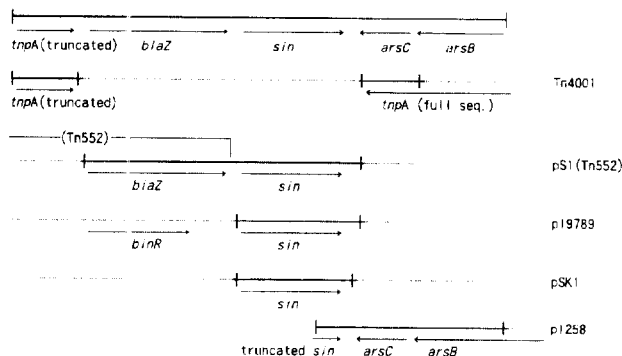

Fig. 4. Physical maps of transposons or plasmids containing genes with homology to those of the putative transposon. The regions showing homology with the putative transposon are indicated as a thick line. The *blaZ* is a component of Tn 552 and in plasmid pS1-*sin* is located next to *blaZ* (11). The sin of pS1 is exactly same as that of pI9789. pSK1-*sin* is another staphylococcal sin that shows higher homology to that of our transposon. The *sin-arsC-arsB* arrangement is recognized in pI258 (9). Truncated *sin* is part of pI9789-*sin*.

same. Two bases are different in the overlapping region (data are not shown).

There is a large (16 bp) inverted repeat downstream of the proposed *sin* which might participate in transcription termination although it is somewhat distant from the end of the gene (13). On the other hand, it may constitute part of the adjacent asa-asi-ant operon (encodes resistance to arsenate, arsenite and antimony), which is flanked by ~200bp inverted repeats and may once have been transposable (15). The helix-turn-helix structure which is located at C-terminus of Sin is believed to be a specific binding domain to any *res* site and shows a high degree of homology to other *res* sites (4). The integrity of the gene suggests that it has a function that perhaps involves the potential N-terminal strand exchange activity. The proposed *sin* is a representative of the branch of resolvases from which the DNA invertases appear to have evolved (13, 14); this might be relevant to the question of topological specificity. It is logical to suggest that our transposon seem to replicate via a co-integrate intermediate (generated by a transposase) that is resolved by the proposed *sin*.

Acknowledgement

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