Nucleotide sequence and organization of the multiresistance plasmid pSCFS1 from *Staphylococcus sciuri*

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**Objectives:** The multiresistance plasmid pSCFS1 from *Staphylococcus sciuri* was sequenced completely and analysed with regard to its gene organization and the putative role of a novel ABC transporter in antimicrobial resistance.

**Methods:** Plasmid pSCFS1 was transformed into *Staphylococcus aureus* RN4220, overlapping restriction fragments were cloned into *Escherichia coli* plasmid vectors and sequenced. For further analysis of the ABC transporter, a ~3 kb EcoRV–HpaI fragment was cloned into the staphylococcal plasmid pT181MCS and the respective *S. aureus* RN4220 transformants were subjected to MIC determination.

**Results:** A total of 14 ORFs coding for proteins of >100 amino acids were detected within the 17 108 bp sequence of pSCFS1. Five of them showed similarity to recombination/mobilization genes while another two were similar to plasmid replication genes. In addition to the previously described genes *cfr* for chloramphenicol/florfenicol resistance and *erm*(33) for inducible resistance to macrolide-lincosamide-streptogramin B resistance, a Tn554-like spectinomycin resistance gene and Tn554-related transposase genes were identified. Moreover, a novel ABC transporter was detected and shown to mediate low-level lincosamide resistance.

**Conclusion:** Plasmid pSCFS1 is composed of various parts which show similarity to sequences known to occur on plasmids or transposons of Gram-positive, but also Gram-negative bacteria. It is likely that pSCFS1 represents the result of inter-plasmid recombination events also involving the truncation of a Tn554-like transposon.

Keywords: macrolide resistance, ABC transporter, low-level clindamycin resistance, florfenicol resistance, spectinomycin resistance

**Introduction**

Members of the species *Staphylococcus sciuri* are common inhabitants of the skin of rodents, insectivores, ruminants and ungulates, and have rarely been associated with diseases in animals or humans. The finding that *S. sciuri* strains harbour *mecA*-related sequences led to the assumption that the staphylococcal gene *mecA*, responsible for methicillin resistance, may originate from *S. sciuri*.¹ Antimicrobial resistance is common among *S. sciuri* isolates and several small resistance plasmids isolated from *S. sciuri* have already been described.² Plasmid pSCFS1 is the first multiresistance plasmid detected in *S. sciuri* and has been reported to mediate resistance to chloramphenicol and its fluorinated analogue florfenicol, to macrolide, lincosamide and streptogramin B (MLS₉) antibiotics as well as to spectinomycin.³⁴ Two novel resistance genes, *cfr* for resistance to chloramphenicol/florfenicol and *erm*(33) for resistance to MLS₉ antibiotics, have already been detected on this plasmid.³⁴ Since *erm*(33) in part closely resembled *erm*(A) of transposon Tn554, and Tn554 also carried a spectinomycin resistance gene in close proximity to *erm*(A), the pSCFS1 sequence was analysed for sequences similar to that of Tn554.⁵

To gain detailed insight into the structure of plasmid pSCFS1, an approach was made to sequence this plasmid completely and to analyse the predicted reading frames for their similarities to other sequences deposited in the databases.

**Materials and methods**

The *S. sciuri* isolate harbouring plasmid pSCFS1 was obtained from the nasal swab of a calf suffering from a respiratory tract infection. Plasmid pSCFS1 was transformed into *Staphylococcus aureus*...
 Antibiotic multiresistance in S. sciuri

RN4220 and the transformants were checked for their in vitro susceptibility to antimicrobial agents.\(^3,4\) MICs were determined by either the microdilution or the macrodilution broth method according to NCCLS document M31-A2.\(^6\) Restriction mapping of plasmid pSCFS1 with various restriction endonucleases and cloning of the Eco-RV, PstI, ClaI and BclI fragments into either pH Bluescript II SK\(^+\) (Stratagene, Amsterdam, The Netherlands) or pCR Blunt II TOPO (Invitrogen, Groningen, The Netherlands) followed standard protocols. Sequencing of these overlapping fragments was achieved by primer walking on both strands starting with the M13 universal and reverse primers. For separate analysis of ORF3, apart from the other resistance genes, a 3 kb Eco-RV–HpaI fragment containing the entire ORF3 and its adjacent sequences was initially cloned into pCR Blunt II TOPO and later cloned into pT181MCS.\(^7\) The recombinant plasmids, designated pT181MCS-ABC, was transformed into S. aureus RN4220. Transformants were checked for their MICs of chloramphenicol, florfenicol, spectinomycin, erythromycin, tilmicosin, clindamycin and quinupristin/dalfopristin. The complete MICs were represented by the two previously described genes cfr (ORF4), coding for a putative oxidoreductase that mediates combined resistance to chloramphenicol and florfenicol, and the gene erm(33) (ORF7), a novel MLS\(_B\) resistance gene that represents an in vivo derived 'in-frame' recombination product of the MLS\(_B\) resistance genes erm(C) and erm(A).\(^3,5\) The third resistance gene is the gene spc (ORF8) which codes for a spectinomycin adenyltransferase identical to that of transposon Tn554.\(^5\)

Immediately downstream of spc, two more Tn554-related reading frames were found: the complete reading frame for the 125 amino acid transposase C (ORF9) and a truncated reading frame (ORF10) whose deduced C-terminal 45 amino acids were identical to the C terminus of transposase B of Tn554. The 2030 bp segment located from bases 10182 to 12211 showed 99% identity to the corresponding part of Tn554 and includes the 3' end of erm(33) and the genes spc, tnpC and ΔtnpB.

The putative products of the five ORFs 1, 2, 5, 12 and 13 showed more or less extended similarities to DNA-binding proteins which may play a role in integration, recombination or mobilization processes. The ORF1 product exhibits 44% identity and 64% similarity to an integrase protein of Enterococcus faecium (accession no. ZP_00036382). The ORF2 protein shows 29% identity and 51% similarity to a putative DNA-binding protein of Bacillus subtilis (accession no. NP_053778). The product of ORF5 shows 52% identity and 66% similarity to the N terminus of a recombinase/mobilization protein from S. aureus (accession no. AAF85649) whereas the product of ORF12 exhibits 35% identity and 56% similarity to the C terminus of the same recombinase/mobilization protein. The product of ORF13 shows 71% identity and 83% similarity to an internal 153 amino acid segment of the 361 amino acid recombinase/mobilization proteins of Lactobacillus plantarum and Lactobacillus hilgardii (accession nos. AAA25252 and AAA98162).

Two reading frames were detected whose products showed similarities to plasmid replication proteins: that of ORF11 shows 38% identity and 55% similarity to the Rep protein of plasmid pTS1 from the oral spirochaete Treponema denticola (accession no. NP_073755) while that of ORF14 exhibits 29% identity and 46% similarity to a 220 amino acid internal segment of the Rep protein of the Mannheimia varigera plasmid pMVS201 (accession no. NP_573540). Finally, the deduced amino acid sequence of ORF6, which in part overlaps ORF5 (Table 1), revealed low levels of similarity to small hypothetical proteins detected during whole-genome sequencing of S. aureus strain N315.

**Results and discussion**

**Organization of plasmid pSCFS1 and analysis of the reading frames**

Sequence analysis confirmed the size of plasmid pSCFS1 to be 17 108 bp. A total of 14 reading frames potentially coding for proteins of >100 amino acids was detected (Figure 1, Table 1). Similarity searches allowed the grouping of 13 of the 14 reading frames into any of five categories: resistance genes, transposase genes, recombinase/integrase/mobilization genes, plasmid replication genes and hypothetical genes.

![Figure 1. Circular map of plasmid pSCFS1. The reading frames are numbered with reference to Table 1; grey-shaded reading frames are involved in antimicrobial resistance.](https://academic.oup.com/jac/article-abstract/54/5/936/812075/)

The resistance genes were represented by the two previously described genes cfr (ORF4), coding for a putative oxidoreductase that mediates combined resistance to chloramphenicol and florfenicol, and the gene erm(33) (ORF7), a novel MLS\(_B\) resistance gene that represents an in vivo derived 'in-frame' recombination product of the MLS\(_B\) resistance genes erm(C) and erm(A).\(^3,5\) The third resistance gene is the gene spc (ORF8) which codes for a spectinomycin adenyltransferase identical to that of transposon Tn554.\(^5\)

**Analysis of the ABC transporter and its role in antibiotic resistance**

All ORFs described previously either played a defined role in resistance to chloramphenicol/florfenicol, MLS\(_B\) antibiotics or spectinomycin, or were supposed to have other functions based on the results of the structural comparisons. The only remaining reading frame, ORF3, was considered a candidate for a putative role in antimicrobial resistance. ORF3 codes for an ABC transporter of 492 amino acids. The Walker A motif (GRRGFRKRT) was detected at positions 38–45 and the Walker B motif (FLLLID) at positions 137–142 in the deduced amino acid sequence. Both motifs characterize the ATP-hydrolysing domains and are supposed to constitute a nucleotide-binding
S. aureus RN4220 and the S. aureus pT181MCS-ABC did not differ from the original S. aureus produced into cfr. Not included any of the aforementioned three resistance genes, compounds. To determine whether the substrate spectrum of E. faecalis resistance of observed. Lsa was shown to be responsible for the intrinsic identity and 90% similarity to an ABC transporter with unknown function found during whole-genome sequencing of Bacillus anthracis strain Ames (accession no. NP_844899). Moreover, a lesser degree of 41% identity and 64% similarity to the ABC transporter from plasmid pSCFS1 comprised of the ABC transporter Lsa from Bacillus subtilis. Based on the similarities in structure and substrate spectrum, the novel ABC transporter confers low-level clindamycin resistance. An increase in the MIC of clindamycin. Since A and B compounds of the streptogramins were not commercially available separately, we were only able to test the combination quinupristin/dalfopristin. The MIC of 0.5 mg/L for the transformant was only slightly higher compared with the other strains for which the MICs were 0.25 mg/L. From these data we conclude that the ABC transporter confers low-level clindamycin resistance. Based on the similarities in structure and substrate spectrum, the novel ABC transporter gene received the designation lsa(B) from the Nomenclature Center for MLS Resistance Genes (http://faculty.washington.edu/marilynr/).

In summary, plasmid pSCFS1 is the first plasmid from S. sciuri for which the complete sequence has been determined and deposited in the databases (http://www.ncbi.nlm.nih.gov/genomes/static/eub_p.html). The few resistance plasmids so far detected in S. sciuri differed from those of other staphylococci in size and structure. The recent finding that plasmid pSCFS1 from bovine S. sciuri carries three resistance genes different from those commonly found in staphylococci may suggest that S. sciuri, which constitutes part of the physiological skin flora of animals, may promote the distribution of novel resistance genes into the staphylococcal gene pool. In this regard, it should be noted that co-selection of the resistance genes cfr, erm(33), spc and lsa(B) located on plasmid pSCFS1 may occur under fold. Immediately upstream of the coding region, two ORFs for small peptides of 26 (position 4008–4088) and seven amino acids (position 4094–4117) were found. Within the ORF of the 26 amino acid peptide two perfect inverted repeats of 14 bp were detected (IR1: position 4036–4049; IR2: position 4072–4085). Another set of imperfect inverted repeats of 12 bp was identified with IR3 (position 4103–4114) being located within the ORF of the seven amino acid peptide and IR4 (position 4131–4142) comprising the ribosome-binding site of the gene for the ABC transporter. These structures closely resemble translational attenuators which play a role in the expression of various exporter genes involved in the antimicrobial resistance of Gram-positive bacteria.

The novel ABC transporter from plasmid pSCFS1 shows a G+C content of 34.3% which is in the range of that of staphylococcal genomes (32–36%). This protein is highly similar (82% identity and 90% similarity) to an ABC transporter with unknown function found during whole-genome sequencing of Bacillus anthracis strain Ames (accession no. NP_844899). Moreover, a lesser degree of 41% identity and 64% similarity to the ABC transporter Lsa from Enterococcus faecalis was observed. Lsa was shown to be responsible for the intrinsic resistance of E. faecalis to clindamycin and streptogramin A compounds. To determine whether the substrate spectrum of the ABC transporter from plasmid pSCFS1 also comprises antimicrobial agents, a 3034 bp EcoRV–HpaI fragment, which did not include any of the aforementioned three resistance genes, cfr, erm(33) or spc, was cloned into plasmid pT181MCS. The resulting recombinant plasmid pT181MCS-ABC was introduced into S. aureus RN4220. The transformant carrying pT181MCS-ABC did not differ from the original S. aureus RN4220 and the S. aureus RN4220:pT181MCS exhibited MICs of <_0.12 mg/L, that for the transformant carrying the 3 kb fragment with the ABC transporter gene was at 2 mg/L. This corresponded to at least a 16-fold increase in the MIC of clindamycin. Since A and B compounds of the streptogramins were not commercially available separately, we were only able to test the combination quinupristin/dalfopristin. The MIC of 0.5 mg/L for the transformant was only slightly higher compared with the other strains for which the MICs were 0.25 mg/L. From these data we conclude that the ABC transporter confers low-level clindamycin resistance. Based on the similarities in structure and substrate spectrum, the novel ABC transporter gene received the designation lsa(B) from the Nomenclature Center for MLS Resistance Genes (http://faculty.washington.edu/marilynr/).

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<table>
<thead>
<tr>
<th>Open reading frame</th>
<th>Position</th>
<th>Size</th>
<th>Gene</th>
<th>Function/similarity</th>
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<tr>
<td>ORF1</td>
<td>718–224</td>
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<td>ORF4</td>
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<td></td>
<td>Similarity to the N-terminus of recombinase/mobilization proteins of Gram-positive bacteria</td>
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<td>9898–10629</td>
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<td>ORF9</td>
<td>12065–11688</td>
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<td>Transposase C of Tn554 from S. aureus</td>
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<td>13869–12508</td>
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<td>Similarity to the C-terminus of recombinase/mobilization proteins of Gram-positive bacteria</td>
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<td>Similarity to the N-terminus of recombinase/mobilization proteins from Lactobacillus spp.</td>
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<td>ORF14</td>
<td>16888–15872</td>
<td>338</td>
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<td>Similarity to the plasmid replication gene from M. varigena</td>
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</table>

*The singular BstEII site was considered as start (position 1) in pSCFS1. 
*Given in amino acids. 
*Only gene designations accepted by databases were used.
selective pressure imposed by the use of either macrolides, spectinomycin, chloramphenicol, florfenicol or lincosamides. Although plasmids such as pSCFS1 have been detected very rarely to date, the occurrence of a plasmid that was indistinguishable from pSCFS1 in its restriction map and resistance phenotype/genotype, in a bovine *Staphylococcus simulans* isolate indicates the potential of this plasmid to be spread horizontally to other staphylococcal species.

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References