A bacteriocin “fingerprinting” scheme for the typing of beta-hemolytic streptococci uses a set of nine standard indicator strains, each representing a different M serotype, only 10 of these srtA-positive strains produced active streptin. The failure of some strains to express streptin was attributed to an ~4.5-kb deletion in their streptin loci, encompassing genes putatively encoding proteins involved in streptin processing (srtB and srtC) and transport (srtT). In other strains, srtA transcription appeared to be defective. No direct association could be detected between the production of streptin and the production of the lantibiotic-like hemolysin streptolysin S in strain M25.

Bacteriocin production by *Streptococcus pyogenes* was first documented in 1971 (30). Streptococcin A-FF22 (SA-FF22), the inhibitory product of *S. pyogenes* strain FF22, was later found to have characteristics similar to those of nisin, the inhibitory product of *S. pyogenes* strain FF22, was later found to have characteristics similar to those of nisin, the “prototype” of the lantibiotic class of bacteriocins produced by certain strains of *Lactococcus lactis* (32).

A bacteriocin “fingerprinting” scheme for the typing of beta-hemolytic streptococci uses a set of nine standard indicator bacteria (I₁ to I₉) to detect patterns of inhibitory activity produced in a deferred antagonism test on blood agar medium (29). In practice, these patterns are converted to numerical code designations called bacteriocin production (P) types. For example, *S. pyogenes* strain FF22 is P-type 436 (29). Testing of a set of 54 *S. pyogenes* strains, each strain representing a different M protein serotype, indicated that P-type patterns 655, 614, and 777 were given only by the M-type 4, 57, and 60 strains, respectively (29). Subsequent studies have shown that a homologue of the *S. salivarius* lantibiotic salivaricin A (21) is produced uniquely in *S. pyogenes* by M-type 4 strains (unpublished data). Similarly, all tested M-type 57 strains of *S. pyogenes* appear to produce a unique bacteriocin-like inhibitory substance (BLIS), in this case a plasmid-encoded, heat-labile protein (26).

More recent testing of 73 M-prototype *S. pyogenes* strains showed 10 of these (types 11, 12, 25, 28, 60, 66, 67, 71, and 76) to be P-type 777 (28). There was no apparent correlation detected between the production of P-type 777 activity and the disease association of the *S. pyogenes*. It was noted, however, that except for M-types 12, 67, and 71, all of the P-type 777-producing strains were opacity factor positive (13). The inhibitory spectrum of the P-type 777 strains was shown to include a wide variety of streptococci and, surprisingly, one strain of the gram-negative species *Bacteroides intermedius* (13) (now *Prevotella intermedia* [24]). Hynes and Tagg (14) partially purified the P-type 777 BLIS from the serotype M25 *S. pyogenes* strain M25 and showed that there was no apparent link between its production and the production of streptolysin S (SLS) (14). Furthermore, in all of nine Tn916-derived BLIS-negative mutants of strain M25 the production of SLS did not appear to have been affected (14). In other experiments, all of six protease-negative variants derived after treatment of strain M25 with nitrosoguanidine, although remaining unchanged in their production of SLS, were found to have become BLIS negative (14). This indicated a potential involvement of streptococcal proteinase in production of P-type 777 activity.

Recently, a novel locus consisting of 10 open reading frames was identified in an *S. pyogenes* strain of undefined M-type (16). It was noted by the authors that the structural gene (*srtA*) for the putative lantibiotic (named streptin) had “significant homology with *nisA*.” Associated with the loss of streptin production after Tn916 mutagenesis, there appeared to be an associated loss of SLS activity, causing the authors of that study to speculate that streptin gene products may have a role in SLS synthesis, modification, maturation, or secretion (16).

In the present study the purification of streptin is documented. We further demonstrate that streptin is responsible for P-type 777 BLIS activity in *S. pyogenes* and reaffirm our previous observation that its production is not directly linked to that of SLS.
**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** The *S. pyogenes* isolates used in the present study were strains M25 (wild type, streptin+ [P-type 77]) (14), Tn16 (streptin− derivative of strain M25 generated by Tn916 mutagenesis [P-type 000]) (14), SF570 (M-type 1, streptin+ [P-type 000]) (8), Blackmore (SLS− SLO+ and C203U [SLS− SLO−]) (31), M-type 60 strains 76068 (P-type 000) and ?3220 (P-type 77), M-type 11 strains 74823 (P-type 000) and ?71468 (P-type 77), M-type 4 strain 148 (P-type 655), and M-type 57 prototype strain (P-type 614) were from the laboratory culture collection. The standard indicator strains (I1 to I4) and the set of 73 prototype *S. pyogenes* (M-types 1 to 81) have been described previously (28). *S. equisubsp. zoopneumoniae* strain 4881 was the source of the murahtic BLIS zoonin A (25). Routine culture was at 37°C in 5% CO2 in air on BaCa (Columbia agar base [CAB; Life Technologies, Ltd., Paisley, United Kingdom], supplemented with 5% [vol/vol] human blood and 0.1% [wt/vol] CaCO3). The agar plate culture was collected in TE buffer (10 mM Tris-HCl [pH 8] containing 1 mM EDTA and 2% [vol/vol] Triton X-100) was added. Lysis was done by comparison with lanthionine peaks in the standard run. Mass spectrometry was done with a matrix-assisted laser desorption ionization–time-of-flight mass analyzer (Finnigan Lasermat 2000; Thermo Bioanalysis), and N-terminal amino acid sequencing were done by the Protein Microchemistry Facility, Department of Biochemistry, University of Otago. The amino acid compositions of phenyliiocarbamyl derivatives were determined by using a narrow-bore binary reversed-phase HPLC system (10). Detection of lantionine was done by comparison with lantionine peaks in the standard run. Mass spectrometry was done with a matrix-assisted laser desorption ionization–time-of-flight mass analyzer (Finnigan Lasermat 2000; Thermo Bioanalysis), and N-terminal sequencing was done by automated Edman degradation on a 470A pulsed liquid protein sequencer (Applied Biosystems, Inc.) (11).

**Purification of streptin.** Crude and purified streptin preparations were digested with an equal volume of trypsin (1 g/liter) in 0.1 M phosphate buffer (pH 7). Each preparation was incubated at 37°C for 3 h, followed by heating at 80°C for 30 min to inactivate the enzyme prior to assaying the preparation against indicator I1.

**srT4 detection by using colony and dot blots.** The distribution of *srT4* among M-prototype *S. pyogenes* and various known BLIS-producing bacteria was determined by dot blotting. The DNA applied to these membranes was derived by a method based on that of Upton et al. (34), but with the use of only a single phenoilohloroform extraction of each DNA sample. A 5-μl portion of each DNA sample was applied to a nylon membrane (Hybond-N; Amersham Pharmacia Biotech, Inc.) via a vacuum manifold, followed by 100 μl of XSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Denaturation of the DNA was done by exposure to two 2-min washes of 0.4 M NaOH, followed by two 2-min washes with 1 M Tris-HCl. The membrane was then exposed to UV light for ca. 5 min and probed with a digoxigenin (DIG)-DUTP (Roche Diagnostics, Ltd., Lewes, England)-labeled *srT4* probe, derived with the PCR primers srtAF (positions 916 to 946; 5′-AACGTTCAGCTTCAGTTGAA-3′) and srtAR (positions 3020 to 2998; 5′-GAATACATTTCACACAGAAAC-3′) corresponding to srt of the streptin locus (12,040 nucleotides; GenBank accession no. AB080381). The PCR was carried out by using *Taq* polymerase (Roche) for 30 cycles, with denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s.

**Production of streptin.** A biphasic culture system was used to obtain streptin in a liquid form. This consisted of 50 ml of BaCa base in a 500-ml Schott bottle on a lawn culture of *S. pyogenes* strain FF22 (indicator I2), followed by incubation for 18 h at room temperature in a sealed container. The membranes were then layered onto filter papers

**Peptide analysis.** Amino acid composition analysis, mass spectrometry, and N-terminal amino acid sequencing were done by the Protein Microchemistry Facility, Department of Biochemistry, University of Otago. The amino acid compositions of phenyliiocarbamyl derivatives were determined by using a narrow-bore binary reversed-phase HPLC system (10). Detection of lantionine was done by comparison with lantionine peaks in the standard run. Mass spectrometry was done with a matrix-assisted laser desorption ionization–time-of-flight mass analyzer (Finnigan Lasermat 2000; Thermo Bioanalysis), and N-terminal sequencing was done by automated Edman degradation on a 470A pulsed liquid protein sequencer (Applied Biosystems, Inc.) (11).

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soaked in 10% sodium dodecyl sulfate and left for 45 min to allow cell lysis. The released DNA was fixed by exposure to 0.5 M NaOH in 1.5 M NaCl (fix solution) for 20 min (colony side up) on soaked Whatman paper, followed by flooding with fix solution for 20 min. Each membrane was then boiled twice for 20 min in 500 ml of 0.5% sodium dodecyl sulfate, rinsed in 2× SSC, and probed with an ort1 probe as described above. The membranes were subsequently stripped by exposure to 0.4 M NaOH solution for 30 min at room temperature and then reprobed by using a DIG-labeled salA PCR amiplon. This probe was generated with the primers salAF (positions 604 to 629; 5'-GATATTTTGAACAAAGTATCAGA AGA) and salAR (positions 897 to 921; 5'-ACTAATAGAATCTAGATAGT GTCC) based on the S. salivarius 20P5 sal locus (10,610 nucleotides; GenBank accession no. AY085472).

Long template PCR. PCR amplification of the entire streptin locus was done with the PCR primer pair strIF (positions 352 to 369; 5'-GAGAACCCCGGGTT TGGCC) and strGR (positions 10740 to 10720; 5'-GTAACCCGACCGGTGA CTCC) based on the S. pyogenes strain BL-T streptin locus (12,040 nucleotides; GenBank accession no. AB030831) with an annealing temperature of 60°C and by using the Expand Long Template PCR kit (Roche) according to the manufacturer’s instructions.

RNA extraction and analysis. RNA was extracted by a modification of the methods of Lunsford (18) and Chomczynski and Sacchi (4). The cells used as the RNA source were in each case harvested from a 10- to 16-h (30°C) lawn culture of the test strain on BaCa in 9-cm-diameter petri dishes. Approximately one-quarter of the cellular growth was collected on a cotton swab and resuspended into 1 ml of 0.85% NaCl. After centrifugation (5,000 × g for 5 min at 4°C), the cell pellet was resuspended in 200 μl of spheroplasting buffer (20 mM Tris-HCl [pH 6.8] plus 10 mM MgCl₂ and 26% [wt/vol] raf solution) containing 500 U of mutanolysin and 0.1 mg of spectinomycin/ml and then incubated at 37°C for 30 min. Spheroplasts were collected by centrifugation (5,000 × g for 5 min at 4°C), and the RNA was extracted by using a Qiagen RNEasy kit and a Quiaexrodder column (Qiagen, Ltd., Crawley, England) as recommended by the manufacturer. RNA was quantitated by measuring the absorbance at 260 nm.

RNA samples (15 μg per lane) were subjected to electrophoresis (80 V for 2 to 3 h) through 1% agarose containing 0.5 M NaOH in 1.5 M NaCl (fixation was achieved by HPLC by using a combination of C₈ and C₁₈ resins (Fig. 2). Three different forms of streptin were sequentially achieved by HPLC by using a combination of C₈ and C₁₈ resins (Fig. 2). Three different forms of streptin were sequentially eluted from C₈ resins by using 32% isocratic acetonitrile: streptin 1 (2,424 Da), streptin 1 (2,426 Da), and streptin 2 (2,809 Da). Trace amounts of several other molecular species were also detected in mass spectrophotographs of purified streptin fractions, and these appear to correspond to various less-dehydrated forms of either streptin 1 (2,494, 2,476, and 2,460 Da) or streptin 2 (2,840 and 2,821 Da). N-terminal amino acid sequencing showed that the first six amino acid residues of each streptin 1 and streptin 1  were VGXRYL, where X indicates no recognizable amino acid. Streptin 2, however, had three additional amino acid residues at the N terminus, its sequence being TPYVGXRYL. The amino acid composition analysis of streptin 1 matched almost exactly the numbers of each amino acid residues of both streptin 1 and streptin 1 , and no recognizable amino acid. Streptin 2, however, had three additional amino acid residues at the N terminus, its sequence being TPYVGXRYL. The amino acid composition analysis of streptin 1 matched almost exactly the numbers of each amino acid residues of both streptin 1 and streptin 1 .
FIG. 2. (A) C₈ reversed-phase HPLC elution profile of a partially purified streptin preparation that had been obtained by SepPak C₁₈ chromatography of a 95% acidified methanol cell extract of strain M25. Protein material eluted in an acetonitrile gradient was detected by A₂₁₄ measurement, and the inhibitory activity of each fraction was tested against indicator I₁. Solid bar A represents a fraction further purified by C₁₈.
TABLE 1. Amino acid composition of streptin 1

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>Estimated no. of residues(\text{mol ratio})</th>
<th>Expected no. of residues(\text{mol ratio})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp/Asn</td>
<td>1 (0.65)</td>
<td>0</td>
</tr>
<tr>
<td>Glu/Gln</td>
<td>0 (0.31)</td>
<td>0</td>
</tr>
<tr>
<td>Ser</td>
<td>0 (0.20)</td>
<td>0</td>
</tr>
<tr>
<td>Gly</td>
<td>2 (1.97)</td>
<td>2</td>
</tr>
<tr>
<td>His</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Arg</td>
<td>1 (0.82)</td>
<td>1</td>
</tr>
<tr>
<td>Thr</td>
<td>0 (0.49)</td>
<td>0</td>
</tr>
<tr>
<td>Ala</td>
<td>0 (0.48)</td>
<td>0</td>
</tr>
<tr>
<td>Pro</td>
<td>1 (1.30)</td>
<td>1</td>
</tr>
<tr>
<td>Tyr</td>
<td>1 (0.81)</td>
<td>1</td>
</tr>
<tr>
<td>Lanthionine</td>
<td>2 (1.77)</td>
<td>2</td>
</tr>
<tr>
<td>Val</td>
<td>3 (2.98)</td>
<td>3</td>
</tr>
<tr>
<td>Met</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Cys</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Ile</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Leu</td>
<td>2 (1.82)</td>
<td>2</td>
</tr>
<tr>
<td>Phe</td>
<td>1 (0.89)</td>
<td>1</td>
</tr>
<tr>
<td>Lys</td>
<td>2 (2.12)</td>
<td>2</td>
</tr>
<tr>
<td>Trp</td>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>Unknown(e)</td>
<td>2 (2.02)</td>
<td>4f</td>
</tr>
</tbody>
</table>

**a** The number of residues was estimated from the mole ratio (shown in parentheses).

**b** Expected number of amino acid residues in the streptin propeptide resulting from translation of \(srtA\). Each thioether ring is taken to be one amino acid.

**c** ND, not determined.

**d** This amino acid could not be determined.

**e** This amino acid could not be determined.

**f** This value is not accurate due to lack of knowledge about the nature of the unknown peak.

**g** This value includes three dehydrated Thr and one 3-methylanthionine.

(Table 1). Two lanthionine residues were detected, as was an unidentified peak that may correspond to 3-methylanthionine.

Crude streptin preparations showed no loss of activity when stored for three months at either 4 or 20 °C. These preparations were also stable in response to heating at 100 °C for 10 min and were relatively more stable under alkaline conditions (pH 10), in which 50% of the activity was lost within 24 h. Streptin 1 and crude streptin preparations were inactivated upon treatment with trypsin.

**Evaluation of the distribution and composition of the streptin locus in \(S. pyogenes\).** The lantibiotic purified in the present study from strain M25 appears to be consistent with the predicted product of the streptin gene cluster described by Karaya et al. (16). The streptin structural gene \(srtA\) was used as a probe to test a variety of streptococci in dot and colony blot hybridizations. Of 58 tested \(S. pyogenes\) isolates, each representing a different M-type, 41 hybridized with the \(srtA\) probe (Table 2). Interestingly, only 10 of these strains appeared to express biologically active (inhibitory) streptin. None of 75 \(S. salivarius\), 8 \(S. mutans\), and 9 \(S. uberis\) strains tested were found to be positive for the streptin structural gene.

The nucleotide sequence of \(srtA\) in 10 \(S. pyogenes\) isolates was found to be identical, regardless of whether these strains expressed streptin (strains 73220 [M-type 60], 71948 [M-type 11], M-12 [M-type 12], M25 [M-type 25], P5 [T-type 25], and 79009 [M-type 2]) or did not express streptin (strains 76068 [M-type 60], 74823 [M-type 11], and SF370 [M-type 1]). In each case the sequence was identical to that published for \(S. pyogenes\) strain BL-T (16).

A direct comparison of the streptin loci in the P-type 777 (streptin-expressing) strains M25 and BL-T with that of the P-type 000 (streptin-nonexpressing) strain SF370 revealed that the strain SF370 locus contains three deletions within the \(srtT\), \(srtC\), and \(srtB\) region (Fig. 3).

**PCR amplification of the entire streptin locus in a variety of \(S. pyogenes\) strains by using the primers \(srtIF\) and \(srtGR\) indicated that some other inhibitor-negative \(srtA\)-positive strains such as 76068 (M-type 60) also contained detectable deletions within the streptin locus. However, other \(srtA\)-positive, streptin-negative strains such as 148 (P-type 655) and 74823 (P-type 000) had \(srt\) amplicons that appeared to be the same size as in strain M25.

**Northern analysis of \(srtA\) transcription and autoinduction.** The levels of \(srtA\) mRNA were estimated in cells from 10 to 14 h strain M25 cultures (Fig. 4). No \(srtA\) transcript was detected in the mRNA extracted from 10-h cultures, but both the 12- and the 14-h cultures contained 0.25-kb \(srtA\) transcripts.

Pure preparations of either streptin 1 or streptin 2 induced streptin production in strain M25 but failed to cross-induce the production of the heterologous lantibiotics SA-FF22, nisin Z, and salivaricin A in \(S. pyogenes\) strain FF22, \(L. lactis\) strain A5, and \(S. salivaricus\) strain 20P3, respectively (Table 3).

**Northern analysis of \(srtA\) transcripts was carried out on strains M25, Tn2, SF370, 148, and M-57 (Fig. 5). Samples were obtained from 14-h cultures on BaCa that had either been presupplemented with a subinhibitory amount of streptin (induced culture) or not supplemented (control culture). A 0.25-kb \(srtA\) transcript was detected in the total mRNA extracted from streptin-induced strain Tn216 but appeared to be absent in the corresponding (uninduced) strain Tn216 mRNA. An \(srtA\) transcript was also evident in the total mRNA from both induced and uninduced strain M25 cultures. However, the induced strain M25 mRNA contained substantially increased levels of the transcript. The total mRNA from strain SF370 cultures contained only very low levels of the \(srtA\) transcript, irrespective of whether or not the cells had been grown on streptin-supplemented medium. Similarly, neither induced nor uninduced cultures of strains 148 or M-57 appeared to contain \(srtA\) transcripts.

**Association between the production of streptin and of SLS.** Previously, Hynes and Tagg had derived nine inhibitor-negative derivatives of strain M25 by Tn916 mutagenesis and concluded that there was no apparent direct link between the production of the P-type 777 inhibitor and SLS in this strain (14). In contrast, Karaya et al. (16) suggested that the produc-
tion of SLS and streptin may be interdependent in strain BL-T (16). Therefore, in the present study the nine inhibitor-negative mutants of strain M25 originally described by Hynes and Tagg (14) were reevaluated, and one of these (strain Tn216) was found to contain only a single Tn\textsuperscript{916} insertion. This insertion was found to be within \textit{srtB} (base pair position 7463) of the streptin locus, only 4 bp upstream of the Tn\textsuperscript{916} insertion site in the strain BL-T mutant described by Karaya et al. (base pair position 7467) (16). SLS production by strains M25 and Tn\textsubscript{216} was compared, and no difference in the level of hemolysis was observed on Ba between the two strains. The hemolysis associated with strains of \textit{S. pyogenes} is typically due to the action of both SLS and SLO. SLO-associated hemolysis is reduced in the presence of cholesterol. Similar levels of hemolysis were given by strains Tn\textsubscript{216}, M25, and Blackmore (SLS\textsuperscript{−}/SLO\textsuperscript{+}) on Ba plus cholesterol medium. In contrast, the hemolytic activity of strain C203U (SLS\textsuperscript{−}/SLO\textsuperscript{−}) was much reduced (Fig. 6).

DISCUSSION

In the present study, the agent responsible for production of P-type 777 inhibitory activity on blood agar medium by \textit{S. pyogenes} strain M25 was shown to be the lantibiotic streptin. Our previous finding (14) that the inhibitory agent is not produced in liquid cultures of P-type 777 \textit{S. pyogenes} was confirmed. Sufficient starting material for purification purposes could, however, be derived from cultures of strain M25 in a biphasic growth medium. Hynes had previously observed that, when relatively dilute suspensions of strain M25 cells were used as the inocula in deferred antagonism tests, there was greatly reduced production of inhibitory activity (12). These observations are consistent with streptin production being under quorum-sensing control. In the present study it was indeed demonstrated that streptin production by strain M25 on BaCa is specifically enhanced in the presence of biologically active

![FIG. 3. Structures of the srt loci in S. pyogenes BL-T (A) and S. pyogenes SF370 (B). Open reading frames are depicted by solid arrows that show the numbers of amino acid residues in the deduced polypeptides. Unfilled regions represent areas of the streptin locus that are absent in strain SF370. Open reading frame srt4 consists of a deduced polypeptide of 46 amino acids. The sites of insertion of Tn\textsuperscript{916} into \textit{srtB} of strain M25 (the present study) and BL-T (16) are shown as Tn\textsubscript{16} and N22, respectively. The position of the PCR-amplified segment of DNA used to probe for the presence of \textit{srt4} is also shown (I).]
(inhibitory) streptin. It seems likely that the formation of inducing levels of biologically active streptin must occur too late in conventional liquid cultures to effect significant amplification of production before cell metabolism ceases. In the dually incubated biphasic cultures, streptin produced during the initial growth of strain M25 on the agar surface presumably diffuses into the fluid phase of the secondary culture sufficiently quickly to effect induced lantibiotic production. Apparently, other factors are also involved, since it was also found (results not shown) that presupplementation of THB cultures of strain M25 with various concentrations of crude streptin did not lead to enhanced streptin production.

Washed cells from these biphasic cultures provided excellent starting material for the subsequent extraction and purification of cell-associated streptin. A number of other bacteriocins have been shown to exist in both cell-associated and extracellular forms in cultures (6, 15). Two markedly different molecular forms of streptin were detected in acidified 95% methanol extracts of the producer cells. The relatively stronger hydrophobic nature of streptin 2 compared to streptin 1 was shown by its delayed elution from C8 resins in acetonitrile gradients. Streptin 1 and streptin 2 appeared, however, to have similar inhibitory and autoinducing characteristics. Streptin 2 is thought to represent an incompletely processed form of the molecule. Streptin 1 lacks the residues TPY present at the N terminus of streptin 2 and was confirmed to be present in the cell extracts in either a relatively more (streptin 1, M₂, 2,424) or less (streptin 1', M₂, 2,442) dehydrated form. Several other molecular species apparently corresponding to differing degrees of dehydration of streptin 1 and streptin 2 were also detected by mass spectroscopy but were not present in sufficient quantities or degrees of purification for N-terminal sequencing. The lantibiotic Pep5 has also been shown to exist in multiple Mᵢ forms representing differing degrees of dehydration of its six serine or threonine residues (22). Interestingly, the incompletely dehydrated forms of Pep5 were only detected in lysates of the producer cells, with only the fully dehydrated

![Image](http://aem.asm.org/)

**FIG. 4.** Northern analysis of srtA transcription in *S. pyogenes* strain M25 probed with a srtA fragment corresponding to bar I (see Fig. 3). *S. pyogenes* strain M25 was grown on BaCa at 30°C in air for 10 h (lane 10), 12 h (lane 12), and 14 h (lane 14). The srtA mRNA transcript (0.25 kb) is indicated by an arrow. The lanes contained equivalent amounts of total RNA (15 μg). Shown directly underneath each lane is the streptin inhibitory activity against indicator I₁ produced by cultures of strain M25 under the corresponding incubation conditions. Aliquots (20 μl) of strain M25 from an 18-h THB culture were incubated on BaCa in adjacent positions for either 10, 12, or 14 h prior to removing the growth, sterilizing the agar surface with chloroform vapor, and then applying the indicator I₁ lawn. After incubation, the level of streptin activity is indicated by the size of the inhibition zone.

![Image](http://aem.asm.org/)

peptide detected in the culture supernatant. Since the processing of lantibiotics appears commonly to be completed during the final stages of secretion through the cell envelope, it may be speculated that the various incompletely processed forms of streptin are relatively unlikely to be recovered from the extracellular fluids of streptin-producing cultures.

The strong homology between srtA and the corresponding nisin and subtilin structural genes indicates that streptin should also be classified as a class A1 lantibiotic. On this basis, a comparison of the nisin and streptin propeptides (Fig. 7) leads us to predict that the Ser residue encoded in position 3 of streptin 1 is probably involved in lanthionine ring formation with the Cys residue in position 7. The failure to detect any Cys in the streptin amino acid composition analysis indicates that all three Cys residues predicted by the srtA sequence are likely to be incorporated within either lanthionine or 3-methyllanthionine ring structures in the mature peptide. Streptin differs from nisin and subtilin in that it contains a Ser residue between the Gly and Cys residues of the otherwise highly conserved LCTPGC propeptide motif. One implication of this is that involvement of this Ser in lanthionine bridge formation would be expected to constrain the flexibility of the streptin molecule [Fig. 7, streptin 1 (a)]. This relatively flexible hinge region in other type A1 lantibiotics has been shown to be important for their membrane pore-forming capability (36). Thus, pore formation may not be the major mechanism of the killing action of streptin. Alternatively, a 3-methyllanthionine ring may form between Cys-17 and one of the Thr residues downstream at either position 19, 20, or 21 in streptin 1 [Fig. 7, streptin 1 (b)]. This would allow retention of a flexible hinge region in streptin 1 and facilitate pore formation as a potential mechanism of cell killing. It appears that the total activity of streptin is not dependent upon the complete modification of the propeptide since variant, incompletely modified forms such as streptin 1' appear to retain inhibitory activity against M. luteus.

The arrangement of the streptin locus in strain M25 is identical to that reported by Karaya et al. (16) for S. pyogenes strain BL-T and is composed of 10 open reading frames encoding putative proteins involved in streptin immunity (SrtI) and regulation (SrtR and SrtK), followed by open reading frames for the streptin propeptide (SrtA), transport (SrtT), prepeptide modification (SrtC and SrtB), and self-protection (SrtF, SrtE, and SrtG) (Fig. 8). Although these proteins are broadly similar to those described for nisin and subtilin biosynthesis, the arrangements of the individual open reading frames in the operons are quite different. The subtilin and streptin operons differ also from the nisin operon in that they do not encode a dedicated proteinase for processing of the lantibiotic propeptide. It is most unusual for lantibiotic operons to not include a gene specifying a specific proteinase.

Interestingly, srtA appears to be highly conserved in 67% of the 58 S. pyogenes strains of different M-types tested. However, only 10 of these strains appear to produce biologically active streptin. There may be various reasons for the lack of streptin production in srtA-positive S. pyogenes. For example, S. pyogenes strains SF370 and 76068 have been demonstrated to have incomplete srt loci. The missing regions appear to be confined to srtT, srtC, and srtB. Inadequate expression of these genes is likely to result in the loss of active streptin production through an inability to modify the propeptide and to export it through

FIG. 6. Hemolytic activity of four S. pyogenes strains on Ba plus cholesterol medium after 18 h of incubation at 37°C in 5% CO₂ in air. Strains C203U (SLS² SLO¹), Blackmore (SLS¹ SLO²), M25 (streptin producer), and Tn216 (Tn916 inhibitor negative derivative of strain M25) are indicated.
the cell membrane. Other \textit{srtA}-positive streptin-negative strains such as \textit{S. pyogenes} strains 148 and 74823 seem, however, to have intact streptin loci. Strain 148 does not appear to be capable of sensing and upregulating the expression of \textit{srtA} in response to the presence of external streptin in the culture media. Therefore, the reason for the lack of streptin production in this strain may be a nonfunctional promoter sequence upstream of \textit{srtA}. Interestingly, both the salivaricin A (\textit{salA}) (35) and nisin (\textit{nisA}) (19) structural genes have also been identified in strains that do not appear to produce the active lantibiotics. Upton et al. (35) have demonstrated that the lack of salivaricin A production in some \textit{salA}-positive \textit{S. pyogenes} is due to a deletion within the region of the \textit{sal} locus containing the \textit{salT}, \textit{salC}, and \textit{salB} open reading frames. \textit{nisA}-positive

\[ \begin{array}{c}
\text{Streptin 1 (a)} \\
\text{Streptin 1 (b)} \\
\text{Nisin A} \\
\text{Streptin 2 Streptin 1} \\
\end{array} \]

\[ \begin{array}{c}
\text{FIG. 7. (A) Two possible bridging structures of the streptin propeptide, streptin 1 (a) and streptin 1 (b), based on the known nisin propeptide structure. Thioether bridges are indicated by "—S—". Modified residues are shaded: Ala-S-Ala, lanthionine; Abu-S-Ala, 3-methylanthionine; Dha, didehydroalanine; Dhb, didehydrobutyrine. In streptin 1 (b), 3-methylanthionine could form between any one of the three dehydrobutyrine residues (as indicated by the dotted lines). (B) Schematic representation of the streptin 1 and streptin 2 prepeptides. L-20 indicates the leader peptide consisting of 20 amino acid residues. Labeled arrows indicate cleavage sites of the prepeptide to release either streptin 1 or streptin 2. Residues that are believed to be modified in the propeptide are shaded.}\n\end{array} \]

\[ \begin{array}{c}
\text{Streptin 29.8\% G+C} \\
\text{Nisin A 32\% G+C} \\
\text{Subtilin 37\% G+C} \\
\end{array} \]

\[ \begin{array}{c}
\text{FIG. 8. Organization of the streptin, nisin A, and subtilin gene clusters. Structural genes (not drawn to scale) are black, and genes with similar proposed functions have the same patterns. The GC content of each locus is presented as a percentage. Gene designations are according to de Vos et al. (7).}\n\end{array} \]
nisin-nonproducing *L. lactis* strains have been identified that do not transcribe *nisA*, whereas others have a *nisA* transcript but do not produce active nisin (19).

The site of insertion of Tn916 within *srtB* of strain Tn216 was just 4 bp upstream of the corresponding Tn916 insertion site reported by Karaya et al. (16) in strain BL-T. Tn916 insertion “hot spots” have been identified to be regions that contain an A-rich sequence separated by about six bases from a T-rich sequence (23). The Tn916 insertion site within *srtB* conforms to this criterion. Northern analysis showed that strain Tn216, unlike the parent strain M25, failed to produce *srtA* transcripts unless exposed to preformed streptin. This finding supports the model of control of streptin production through a response regulator system similar to that controlling nisin production (17).

Application of the plate induction assay showed that both streptin 1 and streptin 2 are inducer molecules for streptin production in strain M25. Northern analysis also indicated enhanced production of streptin mRNA in induced cultures. In spite of this enhanced messenger formation, the production of biologically active streptin in THB cultures is poor, and so other factors are clearly involved in the effective expression of streptin. One such factor may be the need for the expression and activation of a host cell proteinase capable of processing the streptin prepeptide. In the case of subtilin, prepeptide processing is effected by *B. subtilis* host-encoded proteinases (27). Blocking of proteinase activity with the serine proteinase inhibitor phenylmethylsulfonyl fluoride led to the accumulation of biologically inactive subtilin precursor peptides with leader peptide moieties of differing lengths still attached (27). Incubation of these species with culture media of different subtilin-nonproducing *B. subtilis* strains yielded active subtilin. By analogy, our previous studies demonstrated that production of P-type 777 inhibitory activity by *S. pyogenes* appeared to be dependent on the concomitant expression of streptococcal proteinase (SpeB) by the producer strain (14). Evidence included the elimination of inhibitor production when cultures were grown under conditions inimical to proteinase production, such as low temperature and alkaline pH, or upon addition to the medium of substances known to have anti-proteinase activity such as glucose, iodoacetic acid, lincomycin, and trypsin blue. Moreover, all of six proteinase-negative mutants of *S. pyogenes* strain M25 were found also to have become inhibitor negative (14). This apparent dependence of biologically active streptin production on functional SpeB implicates this molecule in the processing of the streptin prepeptide. Furthermore, SpeB has been shown previously not to degrade the mature form of streptin (14) but could be capable of effecting cleavage of the streptin leader. *S. pyogenes* proteinase activity (possibly SpeB) has also been shown to enhance the release of SLS (1), now known to be encoded by an operon closely resembling those characterizing the lantibiotics (20). Evidence for the role of proteinase in the release of SLS includes the interference with SLS production by protease inhibitors and reversal of this effect on addition of trypsin (1). Interestingly, the formation of both SLS and streptin occurs principally during the stationary-growth phase of *S. pyogenes* cultures, at a time when SpeB expression is also maximal (3).

In the study by Karaya et al. (16) it was noted that inactivation of the streptin locus by insertion of Tn916 into *srtB* appeared to be associated with a concomitant loss of SLS production in the producer strain, and it was speculated that some of the gene products from the streptin locus may also be essential for the production of SLS. In the present study, however, we have found that a single Tn916 insert in a position just 4 bp upstream of that in the Karaya et al. (16) study did not affect the production of SLS in strain M25. Further evidence for the lack of a direct association between the expression of streptin and of SLS is our current finding that 18 of 58 SLS-positive *S. pyogenes* strains of different M types are negative for *srtA*.

**ACKNOWLEDGMENTS**

We thank M. Upton, K. Dierksen, A. Carne, C. Ronson, and G. Cooke for helpful discussions and advice. We gratefully acknowledge the BLAST search facilities at the National Library of Medicine, Washington, D.C., and the University of Oklahoma Streptococcal (GAS) Genome Sequencing Project, funded by a USPHS/NIH grant to B. A. Roe, S. P. Linn, L. Song, X. Yuan, S. Clifton, R. E. McLaughlin, M. McShan, and J. Ferretti.

This work was supported by grant U00605 from the Marsden Fund, Royal Society of New Zealand, and by the Health Research Council of New Zealand.

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