JOURNAL OF BACTERIOLOGY, July 2001, p. 3931–3938 Vol. 183, No. 13
0021-9193/01/$04.00+0 DOI: 10.1128/JB.183.13.3931–3938.2001

Intra- and Interspecies Signaling between *Streptococcus salivarius* and *Streptococcus pyogenes* Mediated by SalA and SalA1 Lantibiotic Peptides

M. UPTON,[1,2]† J. R. TAGG,[2] P. WESCOMBE,[2] AND H. F. JENKINSON[1]*

Department of Oral and Dental Science, University of Bristol Dental School, Bristol, BS1 2LY, United Kingdom,[1] and Department of Microbiology, University of Otago, Dunedin, New Zealand[2]

Received 28 November 2000/Accepted 10 April 2001

***Streptococcus salivarius* 20P3 produces a 22-amino-acid residue lantibiotic, designated salivaricin A (SalA), that inhibits the growth of a range of streptococci, including all strains of *Streptococcus pyogenes*. Lantibiotic production is associated with the *sal* genetic locus comprising *salA*, the lantibiotic structural gene; *salBCTX* genes encoding peptide modification and export machinery proteins; and *salYKR* genes encoding a putative immunity protein and two-component sensor-regulator system. Insertional inactivation of *salB* in *S. salivarius* 20P3 resulted in abrogation of SalA peptide production, of immunity to SalA, and of *salA* transcription. Addition of exogenous SalA peptide to *salB* mutant cultures induced dose-dependent expression of *salA* mRNA (0.2 kb), demonstrating that SalA production was normally autoregulated. Inactivation of *salR* encoding the response regulator of the SalKR two-component system led to reduced production of, and immunity to, SalA. The *sal* genetic locus was also present in *S. pyogenes* SF370 (M type 1), but because of a deletion across the *salBCT* genes, the corresponding lantibiotic peptide, designated SalA1, was not produced. However, in *S. pyogenes* T11 (M type 4) the *sal* locus gene complement was apparently complete, and active SalA1 peptide was synthesized. Exogenously added SalA1 peptide from *S. pyogenes* T11 induced *salA1* transcription in *S. pyogenes* SF370 and in an isogenic *S. pyogenes* T11 *salB* mutant and *salA* transcription in *S. salivarius* 20P3 *salB*. Thus, SalA and SalA1 are examples of streptococcal lantibiotics whose production is autoregulated. These peptides act as intra- and interspecies signaling molecules, modulating lantibiotic production and possibly influencing streptococcal population ecology in the oral cavity.**

Streptococcus salivarius is a primary colonizer of neonatal oral mucosal surfaces and a predominant component of the human adult oral microbiota and is not associated with disease in healthy individuals (39). *Streptococcus pyogenes*, on the other hand, persists in the pharynx in a carrier state in approximately 10% of the population, is a common cause of pharyngeal infections, especially in school-aged children, and is usually present in high numbers only during acute infection (1). All *S. pyogenes* strains tested have been found to be susceptible to growth inhibition by salivaricin A (SalA), a lantibiotic peptide produced by *S. salivarius* (34, 37), and it has been suggested that growth of *S. pyogenes* in vivo may be modulated by indigenous SalA-producing *S. salivarius*.

Lantibiotics are antimicrobial peptides that are produced by, and are active against, closely related gram-positive organisms. These peptides are ribosomally synthesized and then undergo posttranslational modifications, including amino acid dehydration (38) and thioether bridge formation (20). Lantibiotics form two families; type A lantibiotics are linear, and type B lantibiotics have more globular conformations. The peptides are synthesized as prepropeptides consisting of an inhibitor propeptide and a leader region, the features of which have been utilized to group the type A lantibiotics into three subclasses (36). In the members of one of these subclasses, subclass AII, the amino acid (aa) residues Gly-Gly, Gly-Ser, or Gly-Ala immediately precede the site of leader cleavage. These Gly-Gly type cleavage sites are more commonly found in nonmodified bacteriocins or pheromones, such as the ComC peptide responsible for competence induction in *Streptococcus pneumoniae* (15). The streptococcal pheromones are quorum-sensing molecules, analogous to the *N*-acyl homoserine lactones produced by gram-negative bacteria (35), that regulate microbial community responses.

SalA (22 aa residues) is a subclass AII lantibiotic produced by *S. salivarius* 20P3 via processing of a 48-aa prepropeptide encoded by the *salA* gene (34). By utilizing *salA* as a DNA hybridization probe it was shown that all SalA peptide-producing strains of *S. salivarius* contained *salA* sequences and that, somewhat surprisingly, 63 of 65 *S. pyogenes* strains of different M types contained a *salA* gene homolog designated *salA1* (37). We hypothesized that *S. pyogenes* failed to produce SalA1 inhibitor and was sensitive to inhibition by SalA from *S. salivarius* because the genetic locus for lantibiotic production and immunity was incomplete or transcriptionally inactive. In the present study, the structure of the *sal* genetic locus in *S. salivarius* 20P3 was determined and the genes necessary for regulation of lantibiotic production were identified. In *S. pyogenes* SF370 (M type 1), the corresponding *sal* genetic locus had a deletion spanning three genes encoding peptide modification and export proteins, and the *salA1* gene was transcriptionally inactive, thus accounting for the lack of SalA1 production. However, the *salA* gene was actively transcribed in *S. pyogenes*

* Corresponding author. Mailing address: Department of Oral and Dental Science, University of Bristol Dental School, Lower Maudlin Street, Bristol, BS1 2LY, United Kingdom. Phone: 44 117 928 4358. Fax: 44 117 928 4313. E-mail: howard.jenkinson@bristol.ac.uk.

† Present address: Department of Medical Microbiology, Manchester Royal Infirmary, Manchester M13 9WL, United Kingdom.

T11 (M type 4), which contained a complete *sal* genetic locus, and active SalA1 inhibitor peptide was produced. To the best of our knowledge, this is the first report of an autoregulatory lantibiotic produced by streptococci, and the evidence presented here suggests that SalA-like peptides form a family of signaling factors recognized by different species of streptococci.

MATERIALS AND METHODS

Bacterial strains and media. *S. salivarius* 20P3 (wild type, SalA$^+$) (34), *S. pyogenes* SF340 (M type 1, SalA$^-$) (41), and *S. pyogenes* T11 (M type 4, SalA$^+$ clinical isolate) were maintained on Columbia agar plates (Life Technologies Ltd., Paisley, United Kingdom) supplemented with human blood (5%, vol/vol) and $CaCO_3$ (0.1%, wt/vol) in a 5% CO_2–air atmosphere at 37°C. Streptococcal strains were routinely cultured in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) or in M17 medium (Difco) supplemented with $CaCO_3$ (10 mM) and sucrose (10 mM) (M17CS medium) for maximal SalA peptide production. *Escherichia coli* DH5α (9) and the highly electrocompetent derivative strain DH10B (14) were maintained on Luria-Bertani (LB) agar (Difco) or were grown in LB broth with aeration at 37°C. The following antibiotic concentrations were used (where appropriate): ampicillin, 100 μg/ml (*E. coli*); and spectinomycin, 100 μg/ml (*E. coli*), 60 μg/ml (*S. pyogenes*), or 600 μg/ml (*S. salivarius*).

PCR and sequence analysis. Primers for PCR amplification of *salA* and the regions downstream of *salA* in *S. salivarius* 20P3 were designed on the basis of the corresponding *sal* locus sequence in *S. pyogenes* SF370 (University of Oklahoma Advanced Center for Genome Technology; http://www.genome.ou.edu/strep.html). The major primers utilized (and their corresponding target sites within the *S. salivarius* 20P3 *sal* locus [10,610 nucleotides; GenBank accession number AY005472]) were SalAF (positions 604 to 629; 5′GATATTTTGAACAATGCT ATCGAAGA), DsintF (positions 856 to 876; 5′CAACATCAGTTTTACGAA TAC), ORFIR (positions 2340 to 2320; 5′GTGAACTTCAATCTTTCATCG), SalYF (positions 6665 to 6684; 5′GGTCAAGCTCTAGTTATCGC), SalYR (positions 8413 to 8393; 5′GATTATGGATGTCACTAACCG), and SalRterm (positions 10610 to 10590; 5′TCAGAAATCCATAAAATACCC). PCRs were carried out with *Taq* polymerase (Roche Diagnostics Ltd., Lewes, England) for 30 cycles consisting of denaturation at 95°C for 30 s, annealing at between 55 and 64°C (as appropriate) for 30 s, and extension at 72°C for 1 min per kb of DNA to be amplified; this was followed by a final elongation step at 72°C for 5 min. PCR products were ligated into pGEM-T (Promega, Madison, Wis.) and sequenced with a Perkin-Elmer ABI 377A sequencer. Primary sequence data were collated with SeqEd sequencer software, and sequence alignment, translation, and general analyses were performed by using DNAMAN (Lynnon BioSoft, Vaudreuil, Canada). Deduced amino acid sequences for open reading frames were compared with sequences in protein sequence databases by using the BLAST facilities on the National Center for Biotechnology Information server (www.ncbi.nlm.nih.gov) and the University of Oklahoma server.

DNA manipulation and transformation. Streptococcal genomic DNA was isolated as described by Upton et al. (43). Briefly, confluent growth from one-half of an agar plate culture was collected in TE buffer (10 mM Tris-HCl [pH 8.0] containing 10 mM EDTA), the cells were harvested by centrifugation (8,000 × *g* for 10 min) and suspended in 0.3 ml of TE buffer, and 0.3 ml of a lysis mixture (50 mM Tris-HCl [pH 8.0] containing 10 mM EDTA and 2% [vol/vol] Triton X-100) was added. Lysis was achieved by adding 30 U of mutanolysin (Sigma) and then 0.3 mg of pronase (Sigma) and incubating the preparation at 37°C for 2 h. The suspension was extracted once with 0.6 ml of phenol and once with 0.6 ml of phenol-chloroform (0.3 ml of phenol plus 0.3 ml of chloroform-isoamyl alcohol [24:1]). Nucleic acids were precipitated from the aqueous phase with 2 volumes of 100% ethanol at −70°C and collected by centrifugation (12,000 × *g* for 20 min), and the pellet was washed with 70% (vol/vol) ethanol, air dried, and dissolved in TE buffer. In a number of experiments the solutions were then incubated with 50 μg of RNase A (Sigma)/ml at 37°C for 30 min and extracted with phenol-chloroform, and the DNA was precipitated, washed, and dissolved in TE buffer as described above.

Plasmid DNA was isolated from *E. coli* by using Qantum Prep (Bio-Rad Laboratories, Hercules, Calif.). For insertional inactivation of streptococcal genes, target fragments were generated by PCR amplification, cloned into pGEM-T, and recovered by restriction digestion with *Nco*I and *Nsi*I endonucleases. Fragments were gel purified and ligated into vector pFW5 (29) digested with *Nco*I and *Nsi*I. *E. coli* strains were transformed with plasmid DNA by using a TransPorator (BTX, San Diego, Calif.) in 0.1-cm cuvettes (Bio-Rad Laboratories, Richmond, Calif.). Following electroporation, bacteria were incubated for 1 h in 1 ml of 2 × YT broth (16 g of Bacto Tryptone per liter, 10 g of Bacto Yeast Extract per liter, 5 g of NaCl per liter; pH 7.0) at 37°C with shaking at 200 rpm before being spread onto LB agar plates containing the appropriate antibiotics (9). *S. salivarius* and *S. pyogenes* strains were prepared for electrotransformation by the method of Chen et al. (4); DL-threonine was included in the media at final concentrations of 0.6 and 0.4 M, respectively. Electroporation was carried out in 0.1-cm cuvettes by using a Bio-Rad Gene Pulser II set at 1.8 kV, 25 μF, and 200 Ω, and transformants (frequency, 50 to 100 transformants per μg of DNA) were selected on agar containing spectinomycin.

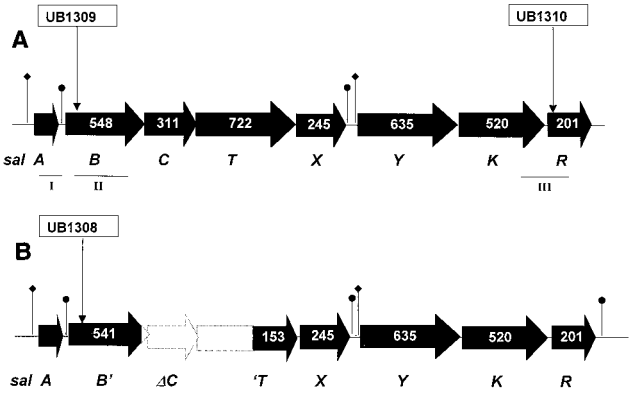

FIG. 1. Genetic structures of the *sal* loci in *S. salivarius* 20P3 (A) and *S. pyogenes* SF370 (M type 1) (B). Open reading frames are depicted by arrows which show the numbers of aa residues in the deduced polypeptides. The sites of insertional inactivation with plasmid pFW5 constructs used to generate strains UB1309, UB1310, and UB1308 are shown. PCR-amplified segments of DNA used for hybridization probes or cloning in pFW5 are indicated as follows: I, 0.3-kb *salA* probe; II, 1.48-kb *salB* fragment; III, 1.16-kb *salKR* fragment. Symbols: ◆, putative promoter; ●, inverted repeat.

Generation of mutants. To generate *S. salivarius* UB1309 *salB*::pMU1011 Sp^r, an internal 1.48-kb *salB* fragment (Fig. 1), PCR amplified from 20P3 DNA by using primers DsintF and ORF1R, was cloned into pFW5 to produce pMU1001, and this plasmid was then transformed onto the *S. salivarius* 20P3 chromosome. *S. pyogenes* UB1308 *salB*::pMU1016 Sp^r was generated in the same way by transforming pMU1016 (pFW5 carrying a 1.48-kb *salB* fragment from T11) onto the *S. pyogenes* T11 chromosome. *S. salivarius* UB1310 *salR*::pMU1002 Sp^r was generated by PCR amplifying a 1.16-kb fragment from the *salKR* gene region (Fig. 1) of 20P3 DNA with primers SalKF (positions 9249 to 9269; 5′GTTGG ATTGTACTCATGAAGG) and SalRR (positions 10411 to 10391; 5′TCAACA TAATCCTGAGATTCG), cloning this fragment into pFW5 to produce pMU1002, and transforming this plasmid into wild-type strain 20P3 as described above. Integration of pFW5 plasmid constructs onto the streptococcal chromosome was confirmed by PCR amplification with pFW5-specific primers pFW5F (5′GATCAGGAGTTGAGAGTGGAC) and pFW5R (5′TGGAGAAGATTCA GCCACTGC).

RNA analysis. To prepare total RNA from streptococci, cells were harvested by centrifugation from M17CS medium, suspended in 0.2 ml of spheroplasting buffer (20 mM Tris-HCl [pH 6.8] containing 10 mM $MgCl_2$ and 26% [wt/vol] raffinose) containing 500 U of mutanolysin/ml and 0.1 mg of spectinomycin/ml, and incubated 37°C for 30 min. Spheroplasts were collected by centrifugation, and RNA was extracted by using a Qiagen RNAeasy kit and a Qiashredder column (Qiagen Ltd., Crawley, England) as recommended by the manufacturer. RNA samples (10 μg per lane) were subjected to electrophoresis through 1% (wt/vol) agarose in 1× MOPS (morpholinepropanesulfonic acid) buffer (0.2 M MOPS containing 50 mM sodium acetate and 10 mM EDTA; pH 7.0) supplemented with 1% (wt/vol) formaldehyde at 80 V for 3 h and were vacuum blotted onto a nylon membrane. For Northern analysis of *sal* transcripts, a PCR product generated with primers SalAF and SalAR (positions 901 to 883; 5′AGAAGTA TCTAGTATGTCG) was radioactively labeled with [^{32}P]dATP by using Prime-a-Gene (Promega) as directed by the manufacturer. Hybridization reactions were carried out at 65°C for 18 h as described elsewhere (5). For reverse transcription (RT)-PCR analysis, DNase-treated RNA that had been extracted from exponentially growing cells was utilized for cDNA synthesis by using the SalRterm oligonuclotide primer and avian myeloblastosis virus reverse transcrip-

TABLE 1. Levels of homology of *S. salivarius sal* gene product sequences to peptide database sequences and to corresponding *S. pyogenes* SF370 *sal* gene product sequences

S. salivarius 20P3 gene	Size of precursor (aa)	Most similar sequence				% Identity to corresponding *sal* gene product in *S. pyogenes* SF370[a]
		Molecule (accession no.)	No. of aa residues	Region of homology to *sal* gene product (residues)	% Identity	
salA	48	Salivaricin A, *S. salivarius* (P36500)	48	1–48	100	92
salB	548	Lacticin 481 biosynthesis protein, *L. lactis* (P37609)	922	29–470	26	83
salC	311	Lacticin 481 biosynthesis protein, *L. lactis* (P37609)	922	539–908	25	
salT	722	ComA transporter, *S. pneumoniae* (Q03727)[b]	717	11–697	24	92
salX	245	ABC transporter, *B. subtilis* (P42423)	257	5–246	44	99
salY	635	No significant database match				91
salK	520	ComP sensor kinase, *B. subtilis* (Q99027)	769	564–754	21	85
salR	201	DegU transcriptional regulator, *B. brevis* (P54662)	236	10–232	27	92

[a] *salC* is not present in SF370, while the *salB* and *salT* genes encode truncated proteins in SF370.
[b] The N-terminal 100 aa of the *salT* product shows significant sequence identity with the cysteine protease activity regions of several bacteriocin transporters (16) and includes the fully conserved Cys, His, and Asp residues involved in catalysis (36)

tase (First Strand cDNA synthesis kit; Roche Diagnostics). PCR amplifications were then performed by utilizing the Expand Long Template PCR system (Roche Diagnostics) with primers SalAF and SalXR (positions 6510 to 6490; 5′CTCTCCTCTATCGGATAAAGC), primers SalY2S (positions 6571 to 6591; 5′CTATTTTCTAGCTACGATCGG) and SalRterm, or primers SalAF and SalRR.

SalA production and expression. SalA peptide was purified from *S. salivarius* 20P3 M17CS broth culture supernatant as previously described (34). To test for SalA induction of transcription, streptococcal strains were grown for 18 h in M17CS medium with or without SalA peptide. Cells were harvested by centrifugation (16,000 × *g* for 20 min at 4°C), and the cell-free supernatants were collected in sterile tubes. For medium swap experiments the bacterial pellets were suspended in the appropriate spent culture supernatant and incubated at 37°C for 4 h before RNA was extracted as described above. Levels of inhibitor production and of immunity to inhibition were determined by using a deferred antagonism assay performed on Columbia blood agar plates supplemented with 0.1% (wt/vol) $CaCO_3$ as described elsewhere (42). Levels of lantibiotic production by producer strains and growth inhibition of test strains were scored as 0 (no inhibition zone), 1 (narrow inhibition zone that was 1 to 2 mm wide), 2 (medium zone of inhibition that was 2 to 5 mm wide), 3 (larger zone of inhibition that was 5 to 8 mm wide), or 4 (inhibition zone that was more than 8 mm wide).

Nucleotide sequence accession number. The nucleotide sequence data for the *S. salivarius* 20P3 *sal* locus have been deposited in the GenBank database under accession number AY005472.

RESULTS

Structure of the *sal* locus in *S. salivarius* 20P3. To determine the sequence and genetic structure of the *S. salivarius* 20P3 *sal* gene locus, PCR primers were synthesized based on the nucleotide sequence of the approximately 7-kb region downstream of the *salA1* gene in *S. pyogenes* SF370 (Fig. 1). These primers were utilized to PCR amplify segments that comprised the complete *sal* genetic locus in *S. salivarius* 20P3. The genetic locus comprised eight open reading frames designated *salABCTXYKR* (Fig. 1A). These open reading frame designations were assigned on the basis of database homology searches and were in accordance with conventional lantibiotic gene cluster nomenclature (7, 36).

The first gene (*salA*), encoding the 48-aa precursor lantibiotic peptide, was preceded by a potential ribosome binding sequence (GGGAG) and an AT-rich region containing putative promoter sequences. Downstream of *salA*, the *salB* and *salC* genes (Fig. 1A) are predicted to encode peptides with significant identities to the N-terminal and C-terminal sequences, respectively, of lactococcin biosynthesis protein produced by *Lactococcus lactis* (32) (Table 1). We identified *salB* and *salC* as separate genes because there are multiple stop codons present in all three reading frames in the putative intergenic sequence. SalB contains a number of motifs that are conserved in LanM modification peptides (36), while SalC is predicted to contain at least five membrane-spanning sequences and is probably responsible for the formation of thioether bridges in mature SalA peptide (12, 27). The deduced aa sequences encoded by the next two genes, *salT* and *salX*, exhibit significant identities with ATP binding cassette (ABC) transporters (16, 46) (Table 1). For lantibiotics and unmodified bacteriocins with a GG cleavage site, the leader is removed by a cysteine protease activity in the N-terminal region of the LanT polypeptide (16). Retention of the leader peptide might have an immunity function (45). Motifs characteristic of LanT protease regions (36) are present in the N-terminal region of the SalT polypeptide. Collectively, these data suggest that leader peptide cleavage and export of modified SalA are carried out by a SalTX protein complex located within the cell membrane.

The *salY* gene, situated upstream of the *salKR* genes at the distal end of the locus, encodes a 635-aa polypeptide for which there were no significant database matches. It is proposed that the products of the *salKR* genes form a two-component sensor kinase-response regulator system (40) based on significant sequence identities between SalK and *Bacillus subtilis* ComP (47) and between SalR and the *Bacillus brevis* DegU transcriptional regulator (24) (Table 1). Other features of the *sal* locus that have relevance for transcriptional regulation include putative promoter regions upstream of the *salA* and *salY* genes (Fig. 1A) and inverted repeats with the potential to form stem-loop structures immediately downstream of the *salA* and *salX* genes (Fig. 1A). An inverted repeat downstream of the inhibitor peptide structural gene is present in several lantibiotic gene clusters (13, 18, 23, 33). The potential stem-loop structure that is formed functions as a transcriptional attenuator that allows only partial readthrough transcription of the downstream genes.

Structure of the *sal* locus in *S. pyogenes*. *S. pyogenes* SF370 (M type 1) contains the *salA*-like gene designated *salA1* (37) but does not produce detectable levels of lantibiotic inhibitor (Table 2). While the deduced aa sequence of the mature SalA1 peptide from strain SF370 has two conserved changes, residue

TABLE 2. SalA production and immunity profiles for *S. salivarius* and *S. pyogenes* strains as determined by deferred cross-antagonism testing

Indicator organism	Activities with the following producers[a]:					
	S. salivarius strains			*S. pyogenes* strains		
	20P3	UB1309 *salB*	UB1310 *salR*	SF370	T11	UB1308 *salB*
S. salivarius strains						
20P3 wild type	0	0	0	0	0	0
UB1309 *salB*	4	0	2	0	2	0
UB1310 *salR*	3	0	0	0	1	0
S. pyogenes strains						
SF370 wild type	4	1	4	0	3	0
T11 wild type	3	0	3	0	0	0
UB1308 *salB*	4	0	3	0	3	0

[a] The activities of SalA in producer strains against indicator strains were scored from 4 (strongest activity) to 0 (no activity) as described in Materials and Methods.

R_2 to K and residue I_7 to F (Fig. 2), it is predicted that these changes do affect the hydrophobicity of the mature peptide or the maturation process. Indeed, the deduced aa sequence of SalA1 from the lantibiotic inhibitor-producing strain *S. pyogenes* T11 (M type 4) is identical to that of SF370 SalA1. In deferred antagonism tests the inhibitory profile of *S. pyogenes* T11 was similar to that of *S. salivarius* 20P3 (Table 2).

A comparison of the *sal* locus sequence present in *S. pyogenes* SF370 with that in *S. salivarius* 20P3 showed that a 3.2-kb deletion occurred in strain SF370, which was predicted to result in truncation of the *salB* gene product and abrogation of SalC and SalT production (Fig. 1B). Thus, if the *salA1* gene was to be transcribed and translated normally, the prepropeptide could not be modified or exported. Apart from the deleted region, the genetic structure of the *sal* locus in *S. pyogenes* SF370 (Fig. 1B) was similar to that in *S. salivarius* 20P3 (Fig. 1A), and the corresponding polypeptide sequence identities ranged from 85 to 99% (Table 1). These comparisons were based on the available GAS genomic sequence (University of Oklahoma), and the results were confirmed by sequencing several PCR products generated for the *salYKR* genes that allowed minor corrections to be made to the SF370 genomic sequence in this region. By utilizing PCR amplification with primers DsintF and SalYR to screen a number of *S. pyogenes* strains, we have shown that only M type 4 (including *S. pyogenes* T11) and M type 57 strains carry the complete *salBCT* coding region.

Transcriptional analysis of the *sal* locus in *S. pyogenes* and *S. salivarius*. For Northern analysis of *salA* mRNA transcript levels, nitrocellulose blots of streptococcal RNAs were incubated with a PCR-generated ^{32}P-labeled 0.3-kb DNA fragment (*salA*) (Fig. 1). The *salA* fragment probe reacted strongly with a single mRNA transcript approximately 200 bases long in *S. salivarius* 20P3 and less strongly with a band of similar size in *S. pyogenes* T11; no significant signal was obtained with mRNA extracts of *S. pyogenes* SF370 (Fig. 3 and 4). The relative *salA* mRNA levels corresponded well to the levels of inhibitor produced by these strains, as assayed by deferred antagonism on agar (Table 2). To confirm the identities of the other predicted transcripts of the locus, mRNA from *S. salivarius* 20P3 cells was subjected to RT-PCR analysis. A 5.9-kb product was generated with primers SalAF and SalXR (Fig. 5A), supporting the suggestion that the *salABCTX* genes were cotranscribed. In addition, amplification of a 4-kb fragment with primers SalYS and SalRterm demonstrated that there was transcriptional linkage of the *salYKR* genes (Fig. 5A). By utilizing primers SalAF and SalRR we were also able to demonstrate the presence of a 9.3-kb mRNA transcript corresponding to *salABCTXYKR* (Fig. 5B). In all these experiments no PCR products were obtained from RNA samples that had not been incubated with reverse transcriptase (Fig. 5).

Transcription of *salA* depends upon SalA modification and export. In order to test the hypothesis that the *salBCT* gene products were essential for production of SalA inhibitor, the *salB* gene in *S. salivarius* 20P3 was inactivated by inserting integrational plasmid pMU1011 in the *salB* coding region (Fig. 1A). A Northern blot analysis of mRNA extracted from UB1309 *salB*::pMU1011 in which the *salA* probe was used demonstrated that transcription of the *salA* gene was abrogated (Fig. 6, lane 1). *S. salivarius* UB1309 was phenotypically

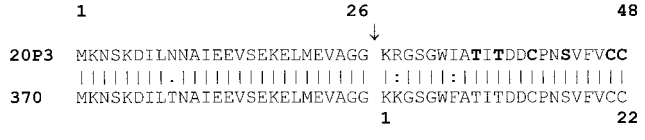

FIG. 2. Comparison of the inferred aa sequences of the precursor SalA peptide in *S. salivarius* 20P3 and SalA1 peptide in *S. pyogenes* SF370. The arrow indicates the site of proteolytic cleavage (after residue 26) to generate the 22-aa SalA or SalA1 propeptides. The residues in boldface type are those likely to be involved in thioether bridge formation.

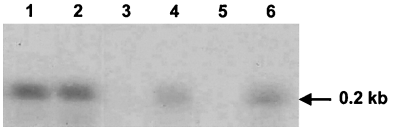

FIG. 3. Northern analysis of *SalA1* gene transcription in *S. pyogenes* probed with a *salA1* fragment corresponding to *salA* fragment I (Fig. 1B). Lane 1, *S. pyogenes* T11 grown in M17CS medium; lane 2, *S. pyogenes* T11 grown in *S. pyogenes* UB1308 *salB* culture medium; lane 3, *S. pyogenes* SF370 grown in M17CS medium; lane 4, *S. pyogenes* SF370 grown in strain T11 culture medium; lane 5, *S. pyogenes* UB1308 *salB* grown in M17CS medium; lane 6, *S. pyogenes* UB1308 *salB* grown in strain T11 culture medium. The position of the *salA1* mRNA transcript (0.2 kb) is indicated by an arrow. The lanes contained equivalent amounts of total RNA (10 μg).

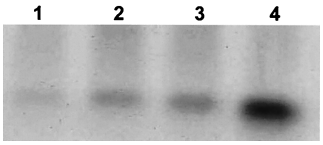

FIG. 4. Autoinduction of SalA in *S. salivarius* 20P3: Northern analysis of *salA* mRNA transcripts from streptococci grown at 37°C for 30 min in M17CS medium (lane 1) and in M17CS medium containing purified SalA peptide at concentrations of 0.05 pmol/ml (lane 2), 0.5 pmol/ml (lane 3), and 5 pmol/ml (lane 4). The lanes contained equivalent amounts of total RNA (10 μg).

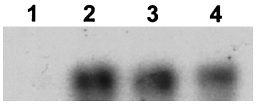

FIG. 6. Northern analysis of *salA* gene transcription in *S. salivarius* UB1309 *salB* probed with *salA* fragment I (Fig. 1A). mRNAs were extracted from *S. salivarius* UB1309 cells grown at 37°C for 4 h. Lane 1, cells grown in their own cell-free spent culture medium; lane 2, cells grown in fresh M17CS medium containing 5 pmol of purified SalA/ml; lane 3, cells grown in cell-free *S. salivarius* 20P3 spent culture medium; lane 4, cells grown in cell-free *S. pyogenes* T11 spent culture medium.

SalA$^-$ as determined by an agar inhibition assay and did not significantly inhibit growth of *S. pyogenes* (Table 2). The analogous *salB* gene inactivation experiment was then carried out with *S. pyogenes* T11 by integrating plasmid pMU1016 in the *salB* chromosomal gene (Fig. 1B). The resulting isogenic mutant, UB1308, did not produce a 0.2-kb *salA1* transcript (Fig. 3, lane 5) and was also inhibitor production negative (SalA1$^-$) as determined by the agar inhibition assay (Table 2). These results confirmed that the *salBCT* gene products were essential for production of SalA and SalA1 peptides and suggested that SalA and SalA1 lantibiotics modulated *salA* and *salA1* gene transcription.

Expression of SalA is autoregulated. To test the hypothesis that *salA* expression depended upon the presence of active SalA, *S. salivarius* 20P3 cells were removed from a late-exponential-phase culture in M17CS medium. The spent medium was inoculated with *S. salivarius* UB1309 *salB* cells; the control consisted of spent medium from a UB1309 culture. The cultures were incubated at 37°C for 4 h, and mRNAs were then extracted, used for Northern analysis, and probed with the *salA* gene fragment as described above. Following incubation in the *S. salivarius* 20P3 spent culture medium, the UB1309 *salB* cells produced a 0.2-kb *salA* mRNA transcript (Fig. 6, lane 3) that was absent from the control cells (Fig. 6, lane 1).

In similar experiments, induction of *salA1* transcription was observed in *S. pyogenes* UB1308 *salB* cells when these cells were grown in spent culture supernatant from *S. pyogenes* T11 cultures (SalA1$^+$) (Fig. 3, lane 6) but not when they were grown in fresh broth or in spent culture supernatant from their own cultures (Fig. 3, lane 5). In addition, *S. pyogenes* SF370 cells, which did not normally express *salA1* mRNA (Fig. 3, lane 3), were induced to express the *salA1* mRNA transcript when they were grown in T11 culture supernatant (Fig. 3, lane 4). These data strongly suggested that production of SalA and production of SalA1 were autoregulated in *S. salivarius* 20P3 and *S. pyogenes* T11, respectively. To confirm this, *S. salivarius* 20P3 cells were grown to the mid-exponential phase in M17CS medium, harvested, washed, and suspended in fresh medium containing 0 to 5 pmol of purified SalA peptide per ml. After incubation of the cultures at 37°C for 4 h, mRNAs were extracted and subjected to Northern analysis. In the absence of exogenous SalA, no *salA* mRNA transcript was detected (Fig. 4, lane 1), but there was a dose-dependent increase in *salA* mRNA levels as the SalA concentration increased up to 5 pmol/ml (Fig. 4). Purified SalA peptide was also effective in inducing *salA* transcription in *S. salivarius* UB1309 *salB* (Fig. 6, lane 2).

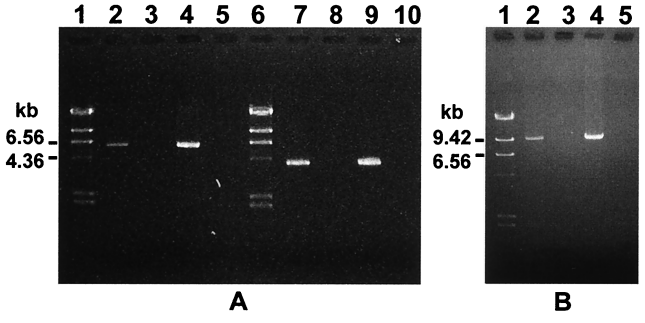

FIG. 5. RT-PCR analysis of *sal* locus transcripts from *S. salivarius* 20P3. cDNA was generated from mRNA by using the oligonucleotide SalRterm. (A) Lanes 2 to 5 PCRs performed with primers SalAF and SalXR; lanes 7 to 10, PCRs performed with primers SalY2S and SalRterm; lanes 2 and 7, cDNA template generated by RT; lanes 3 and 8, RNA controls (no RT); lanes 4 and 9, chromosomal DNA template; lanes 5 and 10, no-template controls. (B) PCRs performed with primers SalAF and SalRR. Lane 2, cDNA template generated by RT; lane 3, RNA control; lane 4, chromosomal DNA; lane 5, no template. The molecular mass markers (panel A, lanes 1 and 6; panel B, lane 1) were λ DNA *Hin*dIII fragments (23.1, 9.41, 6.56, 4.36, 2.32, and 2.02 kb).

SalA and SalA1 are intra- and interspecies signaling molecules. To investigate the specificity of autoinduction by SalA and SalA1, medium swap experiments were carried out with the SalA1-producing organism *S. pyogenes* T11 and the non-SalA-producing organism *S. salivarius* UB1309 *salB*. In the latter strain, *salA* mRNA transcription was clearly induced by the *S. pyogenes* SalA1 peptide present in T11 culture medium (Fig. 6, lane 4), and the level was similar to that induced by the *S. salivarius* peptide SalA (Fig. 6, lane 3).

Since gene regulatory activities of other lantibiotics and peptide pheromones are mediated through two-component systems, it seemed likely that SalA and SalA1 function through the *salKR* gene products, sensor kinase and response regulator proteins. To test this hypothesis, we attempted to inactivate the *salK* gene in *S. salivarius* 20P3, but we were not successful. Attempts to inactivate the *salY* gene in *S. salivarius* 20P3 or the *salK* and *salY* genes in *S. pyogenes* SF370 also were unsuccessful, but we were able to obtain an insertion of pMU1022 in the *salR* gene of *S. salivarius* 20P3 (Fig. 1A). Strain UB1310 expressed *salA* mRNA at a level that was about 50% of the level present in wild-type 20P3 (data not shown). In deferred antagonism assays strain UB1310 was much less inhibitory to growth of *S. salivarius* UB1309 (Table 2), providing evidence that SalR played a role in expression of SalA.

The inhibitory activities of the various strains and their cross-immunity profiles are shown in Table 2. *S. salivarius* 20P3

produced the highest SalA inhibitor activity of all the strains, and growth of wild-type 20P3 cells was not inhibited by any of the other strains. *S. pyogenes* T11 had an inhibitory and immunity profile similar to that of *S. salivarius* 20P3, but it was weaker. By contrast, *S. pyogenes* SF370 and UB1309 and *S. salivarius* UB1308 did not produce inhibitory activities (Table 2). These strains were also sensitive to growth inhibition by SalA and SalA1 peptides produced by *S. salivarius* 20P3 and UB1310 *salR* and *S. pyogenes* T11. *S. salivarius* UB1310 *salR*, which exhibited a moderate level of inhibitory activity against *S. pyogenes* T11 and UB1308, was sensitive only to inhibition by *S. salivarius* 20P3 and *S. pyogenes* T11. Thus, streptococci that produce SalA or SalA1 peptides have increased immunity to both lantibiotics compared with SalA-negative or SalA1-negative streptococci.

DISCUSSION

It is now well established that gram-positive bacteria, especially lactic acid bacteria, produce an array of extracellular peptides with diverse functions. These molecules may act as highly specific intercellular signals for controlling competence development, mating responses, and virulence factor production in bacterial communities or may act as bacteriocin-like inhibitors of the growth of other neighboring species (10). Salivaricin A was one of the first bacteriocin-like inhibitors that were purified, sequenced, and characterized (34). This lantibiotic is produced by *S. salivarius*, and homologous SalA1 peptides are produced by M type 4 strains of *S. pyogenes* (37). In this study we extended our understanding of the production and function of this lantibiotic through comparative genetic analysis of the *sal* genetic loci in *S. salivarius* and *S. pyogenes*.

The genetic organization of the *sal* locus in strain 20P3 resembles that of other lantibiotic synthesis gene clusters (36). Transcriptional analyses suggested that there are at least two *sal* mRNA transcripts, a major (inducible) *salA* mRNA transcript approximately 200 bases long and a *salABCTXYKR* readthrough transcript. RT-PCR data are consistent with the proposal that the *salBCTX* genes are cotranscribed with *salA*, but the presence of an inverted repeat sequence downstream of the *salA* structural gene would be predicted to form a hairpin loop and attenuate transcriptional readthrough from *salA* into *salBCTX*. This would allow production of higher levels of SalA compared with the levels of SalBCTX, the modification and export machinery, and is a control feature found in a number of other lantibiotic synthesis gene operons (18, 23, 33). From the sequence analysis, we predicted that transcription of *salYKR* may also be initiated from a promoter immediately upstream of *salY*, but transcriptional start point mapping experiments would be necessary to confirm this. The significance of the different transcripts to lantibiotic production and control is not yet understood, but it is worth noting that among the transcripts from the *L. lactis* nisin lantibiotic gene locus, a transcript corresponding to the entire locus has been reported (31).

Comparison of the polypeptide sequences encoded by the *sal* gene loci in *S. salivarius* and *S. pyogenes* with the sequences in protein sequence databases allowed us to assign polypeptide functions. One unusual feature of the locus was the presence of two genes (*salB* and *salC*) encoding lantibiotic modification enzymes, since subclass AII lantibiotics with the characteristic Gly-Gly motif are usually modified by the product of a single gene (*lanM*) (36). The only gene for which a possible function of the product could not be inferred from a database match was *salY*. Since most lantibiotic gene clusters carry a self-immunity protein gene (36), it is possible that the 635-aa SalY protein is associated with self-protection against SalA. However, it is also possible that genetic determinants required for immunity are located elsewhere on the chromosome (2, 11).

Inactivation of the *salB* gene in *S. salivarius* 20P3 or *S. pyogenes* T11 led to a lantibiotic-negative ($SalA^-$ or $SalA1^-$) phenotype, confirming that the *salB* gene product plays an essential role in lantibiotic production. However, inactivation of *salB* also resulted in abrogation of *salA* and *salA1* transcription. This transcription was restored by exogenous addition of subinhibitory levels of SalA or SalA1 peptides, demonstrating that production of these lantibiotics is autoregulated, like production of nisin and subtilin (21, 23). Interestingly, *salB* mutants were also rendered sensitive to growth inhibition by SalA or SalA1 peptides, so lantibiotic production and immunity to the lantibiotic are coregulated. These results explain clearly why *S. pyogenes* strains which contain a deletion across the *salBCT* genes, such as SF370 (M type 1), are not producers of SalA1 lantibiotic and are also very sensitive to growth inhibition by SalA or SalA1 peptides.

A model for autoregulation of SalA production in *S. salivarius* is presented in Fig. 7; this model is based on experimental evidence presented here and comparative data for other lantibiotic synthesis systems (7, 8, 20, 21, 24). Transcription of the *salABCTX* genes is initiated at a promoter upstream of *salA*. The primary translation products include the 48-aa precursor pro-SalA and the SalB, SalC, SalT, and SalX protein modification and transport machinery. Modification of the precursor peptide occurs with a membrane-associated SalB-SalC enzyme complex, and the membrane-integral SalT-SalX polypeptides then mediate translocation of SalA across the cytoplasmic membrane (Fig. 7). Cleavage of the precursor SalA leader peptide results from cysteine protease activity located in the N-terminal region of SalT, which generates extracellular mature SalA peptide. The fate of the precursor SalA leader peptide is not known.

The gene clusters encoding production of nisin (12), streptococcin SA-FF22 (26), and subtilin (22) each contain two genes for a two-component (sensor-regulator) system. It has been shown that in the nisin and subtilin systems the mature inhibitor peptide is recognized by the sensor kinase (21, 23, 30). We propose that mature SalA peptide is sensed by the sensor histidine kinase of a two-component system (putatively SalKR), which leads to activation of the response regulator (SalR) and induction of transcription from the *salA* promoter (Fig. 7). Although we were unable to generate a *salK* mutant and therefore were unable to formally test the notion that SalK is the cognate sensor for SalA, we generated a *salR* mutant that contained reduced *salA* mRNA transcript levels and exhibited reduced SalA inhibitor production. This is consistent with the proposed role for SalR in autoregulation of SalA, but since *salA* mRNA transcription was not shut down in this mutant, other regulatory pathways must also be operative.

An interesting finding is that SalA production by one streptococcal species may be induced by sensing of the homologous

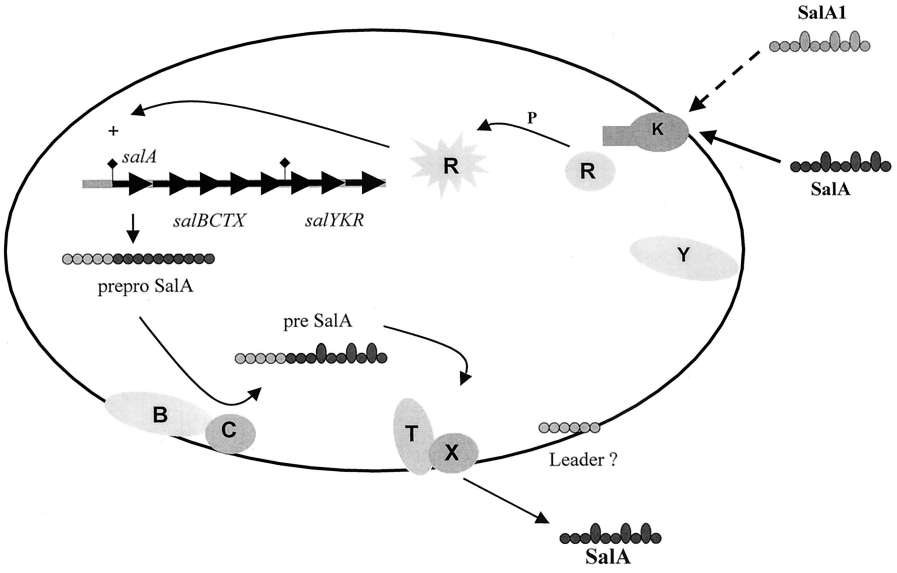

FIG. 7. Model for autoregulation of SalA production in *S. salivarius* and *S. pyogenes*. The product of the *salA* gene, preproSalA peptide, is modified by the membrane-associated SalBC polypeptides and then exported with leader peptide cleavage by the membrane-integral SalTX polypeptides. Extracellular lantibiotic peptide from *S. salivarius* (SalA) or from *S. pyogenes* (SalA1) is sensed at the cell surface, possibly by the two-component SalKR system, and transcription of the *salA* promoter is upregulated via a phosphorylated (P) regulatory protein (R). Only the bacteria that respond to extracellular SalA peptide by producing active SalA are immune to the inhibitory effects of the lantibiotic peptide, but the mechanism of immunity is unknown.

peptide from another streptococcal species. Interspecies signaling between taxonomically diverse streptococcal species has not been demonstrated previously. Most peptide signaling molecules are specific for their cognate sensor-response systems in streptococci (6, 10), but the SalA peptide sensing system apparently does not discriminate between SalA and SalA1 (Fig. 2). Minor modifications to other inhibitor peptides, such as epilancin K7 (44), streptococcin SA-FF22 (19), and subtilin (3), are sufficient to affect their biological activities, but the two conservative changes in SalA1 do not significantly affect the inhibitor activity or profile. Interspecies community sensing could regulate relative population levels of streptococci. The ability of SalA1-producing *S. pyogenes* strains to respond to SalA from *S. salivarius* (and vice versa) could provide a selective mechanism for cocolonization of the mucosal epithelium by pathogen and commensal cell populations. For example, SalA1 produced by rapidly multiplying *S. pyogenes* cells might stimulate production of SalA by *S. salivarius* strains, leading to modulation of the number of *S. pyogenes* cells. In this way, the number of *S. pyogenes* cells may be self-limiting, thus promoting maintenance of the population in a carrier state. An alternative scenario is that sensing of SalA by *S. pyogenes* may influence expression of other genes, including genes related to virulence. In this regard it has recently been demonstrated that a bacteriocin-like peptide (BlpC) in *S. pneumoniae* induces a set of 16 genes following activation of its cognate two-component sensor-regulator system (6). We are currently investigating the possibility that SalR, like CsrR (17) or Mga (25) proteins, may have a global regulatory function in *S. pyogenes* and modulate production of virulence factors, such as streptolysin S (28).

In summary, here we describe the genetic locus encoding the polypeptides necessary for autoregulated production of SalA and SalA1 lantibiotics by *S. salivarius* and *S. pyogenes*. The SalA and SalA1 peptides act as inhibitors of sensitive streptococcal strains which do not themselves produce these peptides, but they are also sensed by other SalA-producing or SalA1-producing strains and there is concomitant induction of lantibiotic synthesis. It could be envisaged, therefore, that SalA production and SalA1 production have a role in modulating the coexistence of streptococcal populations in vivo, where it is becoming apparent that signaling peptides are likely to have a major influence on oral microbial community structure.

ACKNOWLEDGMENTS

We thank Nancy Ragland for expert technical assistance, K. Ross and K. Dierksen for helpful discussions, R. Burne for advice on electroporation, A. Podbielski for kindly supplying plasmids, J. Ferretti for providing strains, and N. Jakubovics for help with preparation of the manuscript. We gratefully acknowledge the BLAST search facilities at the National Center for Biotechnology Information (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 UO0605 from the Marsden Fund, Royal Society of New Zealand.

REFERENCES

1. **Bisno, A. L.** 1991. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. **325:**783–793.
2. **Brurberg, M. B., I. F. Nes, and V. G. H. Eijsink.** 1997. Pheromone-induced production of antimicrobial peptides in *Lactobacillus*. Mol. Microbiol. **26:** 347–360.
3. **Chan, W. C., B. W. Bycroft, M. L. Leyland, L. Y. Lian, and G. C. Roberts.** 1993. A novel post-translational modification of the peptide antibiotic subtilin: isolation and characterization of a natural variant from *Bacillus subtilis* ATCC 6633. Biochem. J. **291:**23–27.
4. **Chen, Y. Y., C. A. Weaver, D. R. Mendelsohn, and R. A. Burne.** 1998. Transcriptional regulation of the *Streptococcus salivarius* 57.I urease operon. J. Bacteriol. **180:**5769–5775.

5. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:**1991–1995.
6. **de Saizieu, A., C. Gardes, N. Flint, C. Wagner, M. Kamber, T. J. Mitchell, W. Keck, K. E. Amrein, and R. Lange.** 2000. Microarray-based indentification of a novel *Streptococcus pneumoniae* regulon controlled by an autoinduced peptide. J. Bacteriol. **182:**4696–4703.
7. **de Vos, W. M., G. Jung, and H.-G. Sahl.** 1991. Appendix: definitions and nomenclature of lantibiotics, p. 457–464. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. Escom Publishers, Leiden, The Netherlands.
8. **de Vos, W. M., O. P. Kuipers, J. R. van der Meer, and R. J. Siezen.** 1995. Maturation pathway of nisin and other lantibiotics: post-translationally modified antimicrobial peptides exported by gram positive bacteria. Mol. Microbiol. **17:**427–437.
9. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:**6127–6145.
10. **Dunny, G. M., and B. A. B. Leonard.** 1997. Cell-cell communication in gram-positive bacteria. Annu. Rev. Microbiol. **51:**527–564.
11. **Eijsink, V. G. H., M. Skeie, H. Middelhove, M. B. Brurberg, and I. F. Nes.** 1998. Comparative studies of class IIa bacteriocins of lactic acid bacteria. Appl. Environ. Microbiol. **64:**3275–3281.
12. **Engelke, G., Z. Gutowski-Eckel, M. Hammelmann, and K.-D. Entian.** 1992. Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the NisB protein. Appl. Environ. Microbiol. **58:**3730–3743.
13. **Engelke, G., Z. Gutowski-Eckel, P. Kiesau, K. Siegers, M. Hammelmann, and K.-D. Entian.** 1994. Regulation of nisin biosynthesis and immunity in *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. **60:**814–825.
14. **Hanahan, D., J. Jessee, and F. R. Bloom.** 1991. Plasmid transformation of *Escherichia coli* and other bacteria. Methods Enzymol. **204:**63–113.
15. **Havarstein, L. S., G. Coomaraswamy, and D. A. Morrison.** 1995. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. Proc. Natl. Acad. Sci. USA **92:**11140–11144.
16. **Havarstein, L. S., D. B. Diep, and I. F. Nes.** 1995. A family of bacteriocin ABC transporters carry out proteolytic processing of their substrates comcomitant with export. Mol. Microbiol. **16:**229–240.
17. **Heath, A., V. J. Di Rita, N. L. Barg, and N. C. Engleberg.** 1999. A two-component regulatory system, CsrR-CsrS, represses expression of three *Streptococcus pyogenes* virulence factors, hyaluronic acid capsule, streptolysin S, and pyogenic extoxin B. Infect. Immun. **67:**5298–5305.
18. **Hynes, W. L., J. J. Ferretti, and J. R. Tagg.** 1993. Cloning of the gene encoding Streptococcin A-FF22, a novel lantibiotic produced by *Streptococcus pyogenes*, and determination of its nucleotide sequence. Appl. Environ. Microbiol. **59:**1969–1971.
19. **Jack, R. W., and J. R. Tagg.** 1991. Isolation and partial structure of streptococcin SA-FF22, p. 171–179. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. Escom Publishers, Leiden, The Netherlands.
20. **Jung, G.** 1991. Lantibiotics: a survey, p. 1–34. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. Escom Publishers, Leiden, The Netherlands.
21. **Kleerebezem, M., W. M. de Vos, and O. P. Kuipers.** 1999. The lantibiotics nisin and subtilin act as extracellular regulators of their own biosynthesis, p. 159–174. *In* G. M. Dunny and S. C. Winans (ed.), Cell-cell signaling in bacteria. American Society for Microbiology, Washington, D.C.
22. **Klein, C., C. Kaletta, and K.-D. Entian.** 1993. Biosynthesis of the lantibiotic subtilin is regulated by a histidine kinase/response regulator system. Appl. Environ. Microbiol. **59:**296–303.
23. **Kuipers, O. P., M. M. Beerthuyzen, P. G. G. A. de Reuyter, E. J. Luesink, and W. M. de Vos.** 1995. Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal transduction. J. Biol. Chem. **270:**27299–27304.
24. **Louw, M. E., S. J. Reid, D. M. James, and T. G. Watson.** 1994. Cloning and sequencing the *degS-degU* operon from an alkalophilic *Bacillus brevis*. Appl. Microbiol. Biotechnol. **42:**78–84.
25. **McIver, K., A. S. Thurman, and J. R. Scott.** 1999. Regulation of *mga* transcription in the group A streptococcus: specific binding of Mga within its own promoter and evidence for a negative regulator. J. Bacteriol. **181:**5373–5383.
26. **McLaughlin, R. E., J. J. Ferretti, and W. L. Hynes.** 1999. Nucleotide sequence of the streptococcin A-FF22 lantibiotic regulon: model for the production of the lantibiotic SA-FF22 by strain of *Streptococcus pyogenes*. FEMS Microbiol. Lett. **175:**171–177.
27. **Meyer, C., G. Bierbaum, C. Heidrich, M. Reis, J. Suling, M. I. Iglesias-Wind, C. Kempter, E. Molitor, and H.-G. Sahl.** 1995. Nucleotide sequence of the lantibiotic Pep5 biosynthetic gene cluster and functional analysis of PepP and PepC. Evidence for a role of PepC in thiether formation. Eur. J. Biochem. **232:**478–489.
28. **Nizet, V., B. Beall, D. J. Bast, V. Datta, L. Kilburn, D. E. Low, and J. C. S. de Azavedo.** 2000. Genetic locus for streptolysin S production by group A streptococcus. Infect. Immun. **68:**4245–4254.
29. **Podbielski, A., B. Spellberg, M. Woschnik, B. Pohl, and R. Lütticken.** 1996. Novel series of plasmid vectors for gene inactivation and expression analysis in group A streptococci (GAS). Gene **177:**137–147.
30. **Ra, S. R., M. Qiao, T. Immonen, I. Pujana, and P. E. J. Saris.** 1996. Genes responsible for nisin synthesis, regulation and immunity form a regulon of two operons and are induced by nisin in *Lactococcus lactis* N8. Microbiology **142:**1281–1288.
31. **Ra, S. R., M. M. Beerthuyzen, W. M. de Vos, P. E. J. Saris, and O. P. Kuipers.** 1999. Effects of gene disruptions in the nisin gene cluster of *Lactococcus lactis* on nisin production and producer immunity. Microbiology **145:**1227–1233.
32. **Rince, A., A. Dufour, S. Le Pogam, D. Thuault, C. M. Bourgeois, and J. Le Pennec.** 1994. Cloning, expression, and nucleotide sequence of genes involved in production of lactococcin DR, a bacteriocin from *Lactococcus lactis* subsp. *lactis*. Appl. Environ. Microbiol. **60:**1652–1657.
33. **Rince, A., A. Dufour, P. Uguen, J. Le Pennec, and D. Haras.** 1997. Characterization of the lacticin 481 operon: the *Lactococcus lactis* genes *lctF, lctE*, and *lctG* encode a putative ABC transporter involved in bacteriocin immunity. Appl. Environ. Microbiol. **63:**4252–4260.
34. **Ross, K. F., C. W. Ronson, and J. R. Tagg.** 1993. Isolation and characterization of the lantibiotic salivaricin A and its structural gene *salA* from *Streptococcus salivarius* 20P3. Appl. Environ. Microbiol. **59:**2014–2021.
35. **Salmond, G. P., B. W. Bycroft, G. S. Stewart, and P. Williams.** 1995. The bacterial enigma: cracking the code of cell-cell communication. Mol. Microbiol. **16:**615–624.
36. **Siezen, R. J., O. P. Kuipers, and W. M. De Vos.** 1996. Comparison of lantibiotic gene clusters and encoded proteins. Antonie Leeuwenhoek **69:** 171–184.
37. **Simpson, W. J., N. L. Ragland, C. W. Ronson, and J. R. Tagg.** 1995. A lantibiotic gene family widely distributed in *Streptococcus salivarius* and *Streptococcus pyogenes*. Dev. Biol. Stand. **85:**639–643. Karger, Basel.
38. **Skaugen, M., J. Nissen-Meyer, G. Jung, S. Stevanovic, K. Sletten, C. I. M. Abildgaard, and I. F. Nes.** 1994. In vivo conversion of L-serine to D-alanine in a ribosomally synthesised polypeptide. J. Biol. Chem. **269:**27183–27185.
39. **Smith, D. J., J. M. Anderson, W. F. King, J. van Houte, and M. A. Taubman.** 1993. Oral streptococcal colonization of infants. Oral Microbiol. Immunol. **8:**1–4.
40. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53:**450–490.
41. **Suvrov, A. N., and J. J. Ferretti.** 1996. Physical and genetic map of an M type 1 strain of *Streptococcus pyogenes*. J. Bacteriol. **178:**5546–5549.
42. **Tagg, J. R., and L. V. Bannister.** 1979. "Fingerprinting" β haemolytic streptococci by their production of and sensitivity to bacteriocin-like inhibitors. J. Med. Microbiol. **12:**379–411.
43. **Upton, M., P. E. Carter, M. Morgan, G. F. S. Edwards, and T. H. Pennington.** 1995. Clonal structure of invasive *Streptococcus pyogenes* strains in Northern Scotland. Epidemiol. Infect. **115:**231–241.
44. **van de Kamp, M., L. M. Horstink, H. W. van den Hooven, R. N. Konings, C. W. Hilbers, A. Frey, H.-G. Sahl, J. W. Metzger, and F. J. van de Ven.** 1995. Sequence analysis by NMR spectroscopy of the peptide lantibiotic epilancin K7 from *Staphylococcus epidermidis*. Eur. J. Biochem. **227:**757–771.
45. **van der Meer, J. R., J. Polman, M. M. Beerthuyzen, R. J. Siezen, O. P. Kuipers, and W. M. De Vos.** 1993. Characterization of the *Lactococcus lactis* nisin A operon genes *nisP*, encoding a subtilisin-like serine protease involved in precursor processing, and *nisR*, encoding a regulatory protein involved in nisin biosynthesis. J. Bacteriol. **175:**2578–2588.
46. **Walker, J. E., M. Sarste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinase and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1:**945–951.
47. **Weinrauch, Y., R. Penchev, E. Dubnau, I. Smith, and D. Dubnau.** 1990. A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. Genes Dev. **4:**860–872.