

# Characterization of a Novel Plasmid-Borne Thiopeptide Gene Cluster in *Staphylococcus epidermidis* Strain 115

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**Thiopeptides are small (12- to 17-amino-acid), heavily modified peptides of bacterial origin. This antibiotic family, with more than 100 known members, is characterized by the presence of sulfur-containing heterocyclic rings and dehydrated residues within a macrocyclic peptide structure. Thiopeptides, including micrococcin P1, have garnered significant attention in recent years for their potent antimicrobial activity against bacteria, fungi, and even protozoa. Micrococcin P1 is known to target the ribosome; however, like those of other thiopeptides, its biosynthesis and mechanisms of self-immunity are poorly characterized. We have discovered an isolate of *Staphylococcus epidermidis* harboring the genes for thiopeptide production and self-protection on a 24-kb plasmid. Here we report the characterization of this plasmid, identify the antimicrobial peptide that it encodes, and provide evidence of a target replacement-mediated mechanism of self-immunity.**

Thiopeptides are an intriguing class of peptide antibiotics that have been investigated for more than 6 decades for their antimicrobial activity. Widely produced by bacteria in nature, thiopeptides possess complex structures that arise from extensive posttranslational tailoring (Fig. 1). A hallmark of thiopeptide structure is the presence of numerous thiazolyl groups arranged around a macrocyclic structure with a pyridine, piperidine, or dehydropiperidine core (1). It is also common for thiopeptides to possess dehydro-amino acids (Dha and Dhb) and other modifications directed by specialized biosynthetic enzymes. Detailed reviews of thiopeptide biosynthesis can be found elsewhere (2–5). These alterations make thiopeptides some of the most densely posttranslationally modified peptides known (6).

Thiopeptides are potent inhibitors of protein synthesis, particularly among Gram-positive bacteria. In general, thiopeptides have been shown to act by two distinct mechanisms, depending on the size of the macrocycle; 26-membered macrocycles (e.g., micrococcin P1 [MP1] and thiostrepton) (Fig. 1) bind at a cleft between ribosomal protein L11 and the 23S rRNA known as the GTPase-associated center (7–9). This region is a critical binding site for elongation factor G (EF-G), which is necessary for ribosome translocation along template mRNA. When thiopeptides are bound here, EF-G is prevented from binding and protein synthesis is halted (10–13). Thiopeptides with 29-membered rings (e.g., GE2270A) (Fig. 1) bind to elongation factor Tu (EF-Tu), blocking its aminoacyl-tRNA binding site, thus preventing peptide elongation (14–16). Beyond their intrinsic antibacterial properties, thiopeptides (especially thiostrepton and MP1) have been investigated for a variety of other applications, including antifungal (17), antiplasmidial (18–21), and anticancer properties (22–24).

Mechanisms of thiopeptide self-immunity are poorly understood. The majority of thiopeptide-producing bacteria have been shown to antagonize closely related species or even members of the same species, implying that mechanisms of immunity are encoded alongside the genes for biosynthesis. Methylation of 23S rRNA has been shown to confer self-immunity in thiostrepton-producing strains of *Streptomyces azureus* and nosiheptide-producing strains of *Streptomyces actuosus* (25). However, for many

other thiopeptide producers, the precise self-immunity mechanisms remain unknown or untested.

Here we report the characterization of a novel thiopeptide gene cluster and mechanism of self-immunity in an MP1-producing isolate of *Staphylococcus epidermidis*, strain 115. First discovered in 1983, this avian isolate was reported to produce an interfering compound that inhibited virulent strains of *S. aureus* (26). In subsequent studies, researchers devised means to extract the compound in order to evaluate certain physical characteristics (27). However, they were unable to identify the compound or map the genes required for antimicrobial production. Using this strain, we have identified the compound as MP1, defined the MP1 biosynthetic gene cluster, and addressed the mechanism by which strain 115 protects itself from MP1 toxicity.

## MATERIALS AND METHODS

**Target range assays.** The *S. epidermidis* 115 target spectrum was assessed by using either the flanking-patch assay or the spot-on-lawn assay. Precise medium types varied according to the strain being tested; for details, see Table S1 in the supplemental material. Flanking-patch assays were performed by growing *S. epidermidis* 115 (source patch) for 18 h at 37°C before patching indicator strains 5 mm from the source patch (see Fig. 2A). Strains were incubated for a further 12 to 24 h before assessment of resistance or sensitivity. For assays with purified MP1, spot-on-lawn assays were performed. These assays were conducted by spreading 150 µl of a 1:50-diluted overnight culture of the test strain on a compatible growth medium. Plates were allowed to dry before applying 5-µl spots of MP1 dilutions. Spots were allowed to dry before incubation of the plates for 18 to 24 h at 37°C. Strains were deemed sensitive when a clear zone of inhi-

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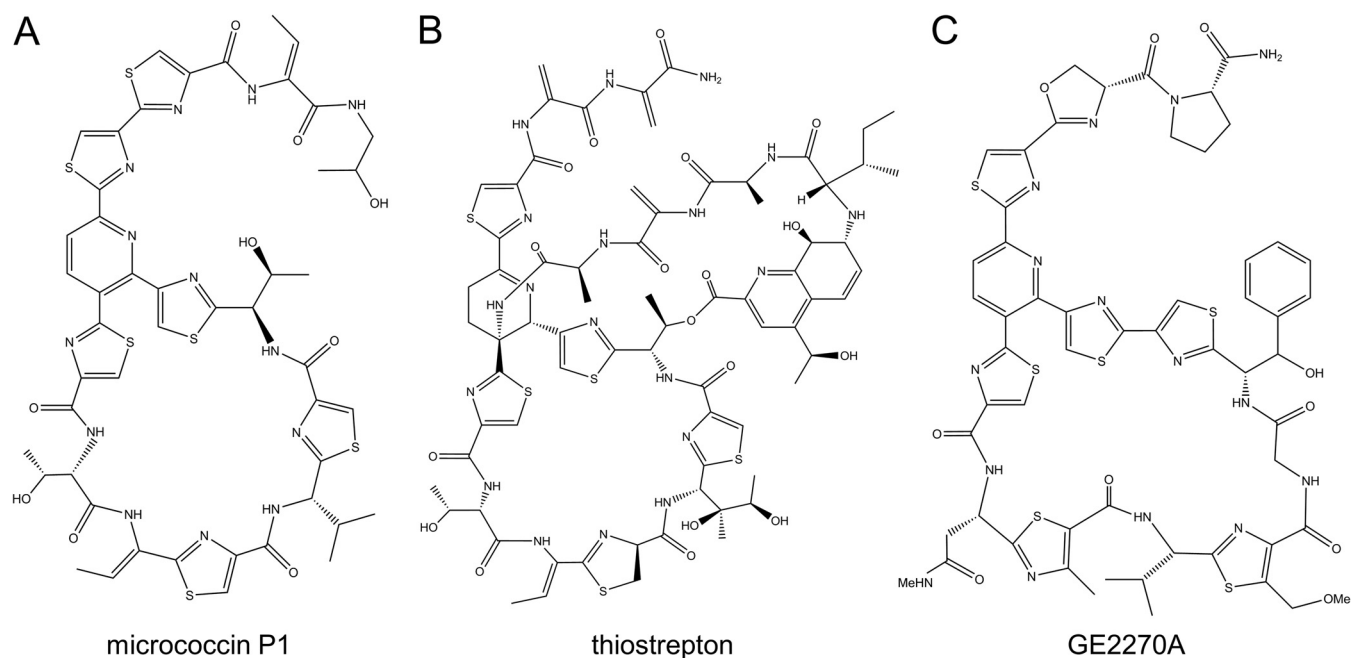


FIG 1 Structures of various thiopeptide antibiotics. Panels: A, MP1; B, thiostrepton; C, GE2270A.

bition was present surrounding MP1 spots but absent around vehicle-only spots.

**MP1 purification.** *S. epidermidis* strain 115 was cultured on 2×YT agar and then harvested by scraping plates into 0.9% NaCl. Harvested cells were pelleted in 50-ml Oak Ridge centrifuge tubes (Nalgene), weighed, and then resuspended in 7 M urea (4 ml/1-g cell pellet). The suspension was incubated at 25°C for 5 min with intermittent vortexing and then centrifuged at 12,000 × *g* for 10 min. MP1-containing supernatant was transferred to a new tube and recentrifuged to remove remaining particulate material. The final supernatant was heat treated to kill residual cells (65°C, 20 min) and then tested for activity by spot-on-lawn assay.

Urea-extracted MP1 was further purified with a Sep-Pak C<sub>18</sub> Plus Long cartridge (Waters). The cartridge was equilibrated with 50% and then 20% acetonitrile (ACN). Approximately 30 ml of extract was applied to the cartridge before it was washed with 30% ACN. Elution was accomplished with 50% ACN. All solvents were acidified with 0.1% formic acid (FA).

Final purification was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) on an Agilent 300SB-C<sub>8</sub> column. MP1 was eluted isocratically with 35% (vol/vol) ACN–0.1% (vol/vol) FA over 16 min at a flow rate of 0.8 ml/min. The active fraction (retention time, 10 to 12.5 min) was collected for further analysis. All purification steps were performed at room temperature. In parallel, the same steps were followed for pBac115-cured *S. epidermidis* strain 115C. This isogenic control strain is incapable of antimicrobial compound production (see below).

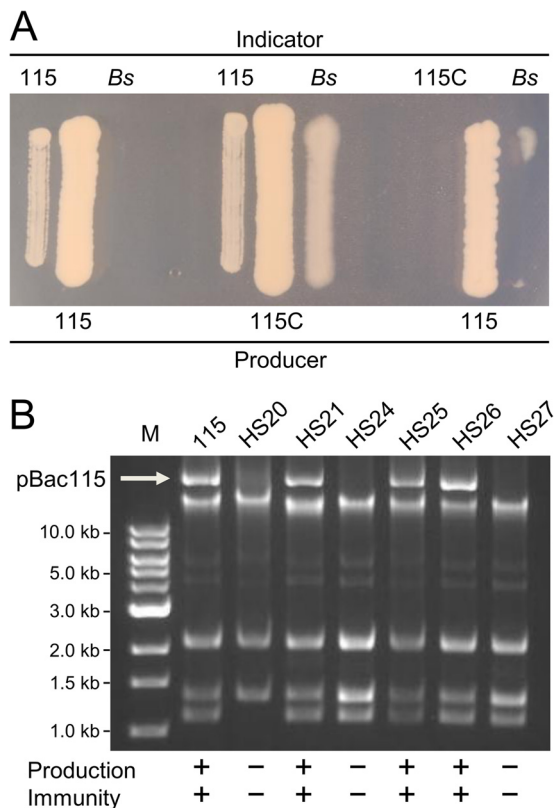
**Plasmid curing.** To generate plasmid-cured derivatives, overnight cultures of *S. epidermidis* 115 were diluted 4 × 10<sup>4</sup>-fold into fresh tryptic soy broth containing 0.004% SDS and cultured with shaking (225 rpm) at 45°C overnight. Cultures were plated on tryptic soy agar and incubated for 18 to 24 h at 37°C. Heat/SDS-surviving (HS) strains were tested by flanking-patch assay for MP1 production and sensitivity. The plasmid profiles of HS strains were evaluated by standard alkaline lysis miniprep after treatment of cells with lysostaphin (Sigma) at a concentration of 120 μg/ml for 30 min at 37°C. The resulting DNA was analyzed on a 1% agarose gel.

**Plasmid sequencing and annotation.** Plasmid minipreps of strain 115 were generated as described above and submitted for 454 shotgun se-

quencing (Roche) at the Brigham Young University DNA Sequencing Center. Sequence assembly was accomplished with GS *De Novo* Assembler (Roche) with localized PCR and Sanger sequencing implemented to resolve ambiguous areas. Contigs representing portions of pBac115 were identified on the basis of successful PCR amplification from strain 115 and no amplification from pBac115-cured strain 115C. These tests are summarized in Table S2 in the supplemental material. Annotation of pBac115 was accomplished by using GeneMark.hmm prokaryotic gene prediction software (28) to find open reading frames (ORFs) and BLAST analysis (<http://www.ncbi.nlm.nih.gov/blast>) to assign predicted functions. We confirmed the sequence of the MP1 biosynthetic gene cluster by resequencing the entire region by Sanger sequencing.

**MS and NMR analysis.** The identity of purified MP1 was determined by a combination of high-resolution electrospray ionization mass spectrometry (ESI-MS) and high-resolution nuclear magnetic resonance (NMR) spectroscopy. ESI-MS was performed at the MS Core Facility (Brigham Young University) with an Agilent MSD time-of-flight instrument with ESI. Theoretical MP1 masses were calculated by mMass (29). One- and two-dimensional NMR spectra were collected on a Varian NMR System 500-MHz spectrometer equipped with a OneNMR probe and Pro-Tune (NMR Facility, Brigham Young University). All NMR experiments are summarized in Table S3 in the supplemental material. The sample used for NMR analysis was composed of approximately 14 mg of MP1 dissolved in 0.8 ml of hexadeuterodimethyl sulfoxide in a 5-mm NMR tube (~10 mM). Both the <sup>13</sup>C and <sup>15</sup>N spectra were acquired at natural abundance. The spectra were analyzed with VNMRJ 3.2 software.

**Genetic manipulation of *Bacillus subtilis*.** Plasmid pRB014 (see Fig. S1A in the supplemental material) was used to generate the *B. subtilis* strain expressing *tlcQ* in *trans* at the *amyE* locus. A similarly constructed plasmid (pRB015) (see Fig. S1B) was used to introduce the *tlcWX* cassette into the *amyE* locus. These preliminary experiments were performed with *B. subtilis* strain SCK6, which is modified for xylose-inducible competence. The more definitive allelic exchange strains depicted in Fig. 6 were created with *B. subtilis* strain 168. Strains PB213, PB230, PB214, and PB215 resulted from *rplK* allelic replacement with plasmids pRB029, pRB031, pRB030, and pRB024, respectively. For the full plasmid sequences, see Fig. S1. The allelic replacements were designed to maintain



**FIG 2** The antimicrobial activity of *S. epidermidis* strain 115 is plasmid encoded. (A) Examples of flanking-patch assays for production and immunity. In each triplet, the central patch was applied to the agar 18 h prior to the flanking patches. 115C is a pBac115-cured derivative. *Bs* corresponds to *B. subtilis* 168, a susceptible indicator strain. (B) Plasmid preparations from *S. epidermidis* 115 and HS derivatives. Lane M, molecular size markers.

the native overall transcriptional unit so that the expression of introduced alleles and downstream genes would be left unperturbed.

Plasmids were constructed by standard techniques with enzymes purchased from New England BioLabs. The high-fidelity polymerases Q5 (New England BioLabs) and *Pfx50* (Invitrogen) were used for insert amplification. All constructs and genetic insertions or replacements were confirmed by Sanger sequencing. Custom oligonucleotides were purchased from Invitrogen.

**Spot dilution assays.** Overnight liquid cultures of *B. subtilis* mutants were normalized to an optical density at 600 nm of 0.1 before 10-fold serial dilutions were performed. Five-microliter spots were made on LB agar supplemented with chloramphenicol (5  $\mu$ g/ml), with or without MP1 (18  $\mu$ g/ml). The spots were allowed to dry before the plates were incubated at 37°C for 24 h.

**Nucleotide sequence accession number.** The complete sequence of pBac115 has been deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/GenBank/>) under accession number KM613043.

## RESULTS

***S. epidermidis* 115 produces a potent, broad-spectrum antimicrobial compound.** In order to test the target range of strain 115, we devised a flanking-patch assay (Fig. 2A), which we used on a broad panel of bacterial isolates in our possession. We observed that strain 115 secretes a diffusible compound that inhibits the growth of diverse Gram-positive bacteria (Table 1). Of particular interest was the observation that many clinically relevant patho-

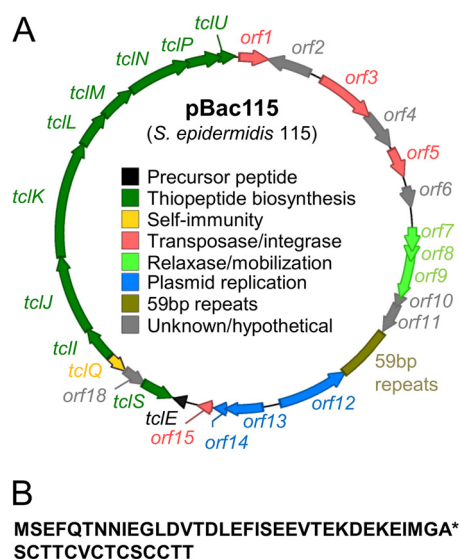
gens were inhibited, including vancomycin-resistant *Enterococcus faecalis* (VRE), methicillin-resistant *S. aureus* (MRSA), *Clostridium difficile*, *Streptococcus agalactiae*, *Bacillus anthracis*, *Listeria monocytogenes*, and *Streptococcus pyogenes*. Interestingly, none of the Gram-negative bacteria tested were sensitive. This activity profile is consistent with that of many known thiopeptides (4).

**The antimicrobial activity of *S. epidermidis* 115 is plasmid encoded.** Earlier speculation suggested that antimicrobial production by strain 115 may be plasmid mediated (27). We investigated this hypothesis by screening for derivatives of *S. epidermidis* 115 that lose antimicrobial production under conditions that promote plasmid curing. Following growth at elevated temperatures and in the presence of low concentrations of SDS, we successfully screened for HS derivative strains that had lost the ability to inhibit the growth of *B. subtilis* 168. The loss of antimicrobial pro-

**TABLE 1** Inhibitory spectrum of strain 115<sup>a</sup>

Species	No. of strains	
	Sensitive	Resistant
Gram-positive bacteria		
<i>Staphylococcus</i> spp.		
<i>S. aureus</i>	2	0
<i>S. aureus</i> (MRSA)	6	0
<i>S. epidermidis</i>	2	0
<i>Enterococcus</i> spp.		
<i>E. faecalis</i>	1	0
<i>E. faecalis</i> (VRE)	1	0
<i>Streptococcus</i> spp.		
<i>S. agalactiae</i>	1	0
<i>S. pyogenes</i>	2	0
<i>S. pneumoniae</i>	1	0
<i>Bacillus</i> spp.		
<i>B. anthracis</i>	1	0
<i>B. brevis</i>	1	0
<i>B. cereus</i>	0	1
<i>B. licheniformis</i>	1	0
<i>B. megaterium</i>	1	0
<i>B. mycoides</i>	1	0
<i>B. sphaericus</i>	1	0
<i>B. subtilis</i>	4	1
<i>B. thuringiensis</i>	2	0
<i>Clostridium</i> spp.		
<i>C. difficile</i>	1	0
<i>C. perfringens</i>	1	0
<i>Listeria monocytogenes</i>		
<i>Paenibacillus polymyxa</i>	1	0
Gram-negative bacteria		
<i>Escherichia coli</i>	0	2
<i>Yersinia pseudotuberculosis</i>	0	1
<i>Burkholderia thailandensis</i>	0	1
Acid-fast bacterium <i>Mycobacterium smegmatis</i>		
	1	0

<sup>a</sup> For detailed strain information, see Table S1 in the supplemental material.



**FIG 3** pBac115 contains thiopeptide biosynthetic (*tcl*) genes. (A) Plasmid map of pBac115 with annotation based upon results from BLASTx and direct alignments with previously identified *tcl* genes. (B) Amino acid sequence of the TclE precursor peptide. The asterisk indicates the predicted cleavage site; the core peptide, which undergoes modification to produce the mature thiopeptide, is underlined.

duction always coincided with loss of resistance to the compound (strain 115C in Fig. 2). Plasmid preparations from HS strains revealed that derivatives deficient in both the production and immunity phenotypes were devoid of a high-molecular-weight plasmid that we have named pBac115 (Fig. 2B). We were able to replicate this phenomenon on repeated occasions; in every instance, the loss of antimicrobial production and immunity was perfectly correlated with the absence of pBac115.

**pBac115 contains thiopeptide biosynthetic genes.** In order to identify the plasmid-encoded biosynthetic genes, we sequenced DNA from an alkaline-lysis plasmid preparation derived from *S. epidermidis* 115. This preparation appeared to contain at least three low-molecular-weight plasmids, as well as pBac115. A second high-molecular-weight band was attributed to chromosomal DNA fragments (Fig. 2B). The resulting 454 pyrosequencing data allowed us to discern a circular 24-kb plasmid consistent with the properties of pBac115. First, in our panel of HS strains (Fig. 2B), those harboring the pBac115 band also tested positive for the 24-kb molecule by PCR, whereas those lacking the pBac115 band tested negative for the 24-kb molecule. Second, annotation of the 24-kb plasmid revealed 27 putative ORFs, including an 11-gene cluster predicted to encode thiopeptide biosynthetic functions (*tcl* genes) (Fig. 3A). The *tcl* designations are according to reference 6. For a detailed report of predicted pBac115 gene functions, see Table S4 in the supplemental material.

Thiopeptide biosynthesis consists of extensive posttranslational tailoring of a structural peptide by multiple biosynthetic enzymes. Although the biosynthesis of thiopeptides is incompletely understood, thiopeptide maturation is thought to occur on a precursor peptide scaffold from which the mature modified peptide is cleaved by a final macrocyclization reaction (30). Peptide modification steps include dehydration of Ser and Thr residues, Cys cyclization to give thiazolyl groups, and the joining of Dha

pairs to form the central macrocycle, consequently cleaving the leader peptide (1, 31). On pBac115, the short ORF *tclE* drew our attention as potentially encoding a precursor peptide (Fig. 3B) because the C-terminal 14 amino acids are identical to the core peptide from which a certain family of thiopeptides (thiocillins) are derived in other organisms (6).

**Purification of the antimicrobial activity of *S. epidermidis* 115.** According to previous reports, the antimicrobial compound produced by strain 115 can be extracted by suspending cell pellets in 7 M urea (27). We used this approach to obtain high yields of a urea-extracted compound with high biological activity. We further enriched this extract by reversed-phase cartridge chromatography. We hypothesized that this purified activity would be found in strain 115 but not in pBac115-cured strain 115C. To test this, we performed parallel purification procedures with both strains. As expected, the strain 115 extract retained antimicrobial activity in a spot-on-lawn assay even when diluted 1,000-fold, whereas the strain 115C sample exhibited no detectable activity. We analyzed the strain 115 and 115C samples by HPLC and observed a very prominent peak present in the strain 115 sample that was absent from the strain 115C sample (Fig. 4A). In spot-on-lawn assays, fractions corresponding to this peak exhibited antimicrobial activity while all other fractions were devoid of activity.

**Structural analysis identifies the unknown compound as MP1.** The antimicrobial compound was analyzed by MS and NMR. By high-resolution ESI-MS, we observed dominant peaks at *m/z* ratios of 572.61, 1,144.22, 1,161.24, and 1,166.20 (Fig. 4B). These peaks are remarkably consistent with the thiopeptide MP1 in various ionization states: the dually protonated form (572.61), the singly protonated form (1,144.22), the ammonium adduct (1,161.24), and the sodium adduct (1,166.20) (Fig. 4C). MP1, also produced by *Bacillus cereus* strain ATCC 14579 (6), is known to be synthesized on the peptide scaffold SCTTCVCTCSCCTT, which we noted above is encoded by *tclE* on pBac115. The pBac115-dependent compound was finally analyzed by NMR, which confirmed its identity as MP1 (see Tables S3 and S5 and Fig. S2 in the supplemental material). The structure of MP1 is shown in Fig. 1A.

**The plasmid pBac115 harbors only a single immunity gene.** Having identified the antimicrobial compound known as MP1, we compared the putative MP1-encoding gene cluster on pBac115 with the only other empirically tested MP1 biosynthetic gene cluster, the *tcl* cluster from *B. cereus* ATCC 14579 (6). Composed of 24 *tcl* genes within a 22-kb region of the *B. cereus* chromosome, the *tcl* cluster reportedly gives rise to eight different thiopeptides (including MP1) from the SCTTCVCTCSCCTT scaffold. Genetic comparisons of pBac115 and the *B. cereus* gene cluster identified 11 genes clustered within an 11-kb region of pBac115 with homology to *tcl* genes (Fig. 5; see Table S4 in the supplemental material). Of these 11, only one copy of the precursor peptide was identified (*tclE*), in contrast to the four tandem copies (*tclE* to *-H*) in *B. cereus*. Additionally, of the four genes presumed to encode immunity functions in *B. cereus* (*tclQ*, *tclT*, *tclW*, and *tclX*), the pBac115 cluster contains only a single gene; a homolog of *tclQ* and *tclT* (*tclQ* and *tclT* are identical copies of the same gene in *B. cereus*). This observation led us to hypothesize that a single gene (here referred to as *tclQ*) may be sufficient to confer self-immunity in *S. epidermidis* 115.

**TclQ confers resistance to MP1 in *B. subtilis*.** TclQ is a homolog of 50S ribosomal protein L11, which is located near the ribosomal GTPase center (32, 33) and is known to be involved in

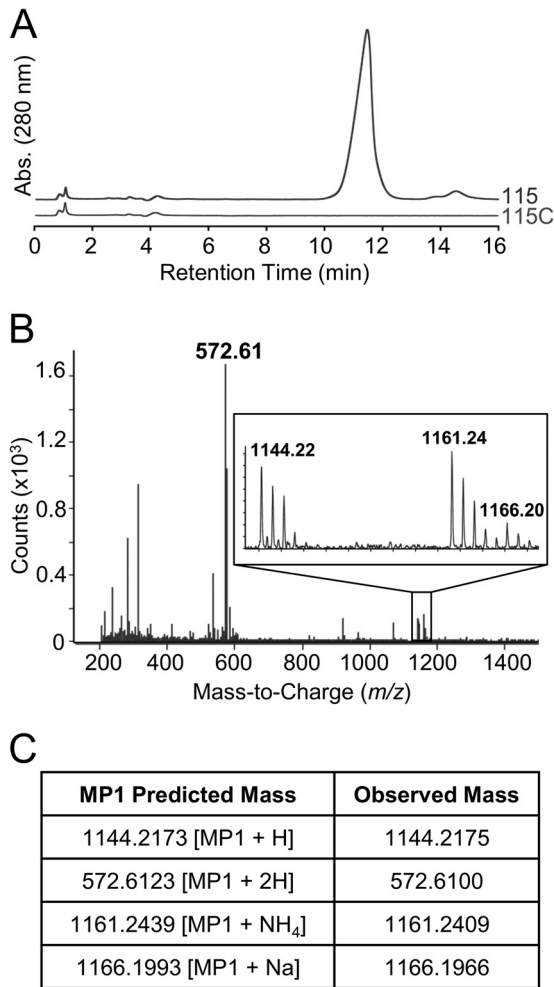


FIG 4 Biochemical analysis of *S. epidermidis* 115 antimicrobial activity. (A) RP-HPLC analysis of purified material from strain 115 and material similarly prepared from pBac115-cured strain 115C. Abs, absorbance. (B) ESI-MS fragmentation of the major HPLC peak from strain 115. The inset shows a closeup of the region from  $m/z$  1,142 to  $m/z$  1,168. (C) Comparison of observed  $m/z$  values with those predicted for MP1.

the mechanism of action of MP1 (9). We hypothesized that TcQ confers immunity by replacing native L11 to protect ribosomes from MP1. To test our “target replacement” hypothesis, we evaluated whether TcQ could confer MP1 resistance in the model *B. subtilis* strain 168. This strain is sensitive to MP1, and we have

determined that ribosomal protein L11 is a major target, since spontaneous resistance is invariably associated with mutations in the L11-encoding gene *rplK* (data not shown). In multiple independent MP1-resistant isolates, we see the same *rplK* mutation that results in deletion of Pro-22 (referred to here as the  $\Delta P22$  allele).

As an initial test of our target replacement hypothesis, the *tclQ* gene from pBac115 was expressed in *trans* from its native promoter at the *B. subtilis amyE* locus. This strain was modestly (10-fold) more resistant to MP1 than the wild type was. In a similar experiment, *tclWX*, the putative efflux system from *B. cereus*, did not confer detectable resistance (data not shown). We reasoned that TcQ expression in *trans* may not confer very high resistance in *B. subtilis* because of competition for ribosomal incorporation with the native L11 protein. According to this logic, replacement of *rplK* (the L11-encoding gene) with *tclQ* would lead to much higher levels of resistance, as competition for ribosomal incorporation is eliminated. As shown in Fig. 6, replacement of *rplK* with *tclQ* allows nearly normal growth and high-level resistance to MP1. MP1 dilution experiments show that the replacement strain was  $\geq 200$ -fold more resistant than the wild type. This increased resistance suggests that TcQ substitutes for L11 on the ribosome, but we could not rule out the possibility that removal of L11 would lead to high resistance regardless of what replaces it. To address this, we constructed an isogenic strain in which the *rplK* ORF was simply removed from the chromosome and not replaced with *tclQ*. This strain was viable but very slow growing. It was, however, noticeably resistant to MP1 (Fig. 6B). The robust growth observed in the *tclQ* replacement strain shows that *tclQ* functionally complements the *rplK* mutant for normal protein synthesis and growth. This strongly suggests that the TcQ protein is incorporated into the ribosome in place of L11 and also confers resistance to MP1.

## DISCUSSION

We have shown that *S. epidermidis* 115 produces the broad-spectrum antibiotic MP1. This is the first reported instance of MP1 being produced by a member of this species and the second reported example of thiopeptide production in *Staphylococcus* spp. (34). *S. epidermidis* is an animal-derived bacterial isolate, whereas the majority of thiopeptide-producing bacteria isolated to date were derived from environmental samples (31). In their early work, Jensen and colleagues (26, 27, 35) demonstrated that strain 115 confers protective benefits on host animals, suggesting that thiopeptide-producing microbiota may play an important role in shaping microbial communities in animal hosts.

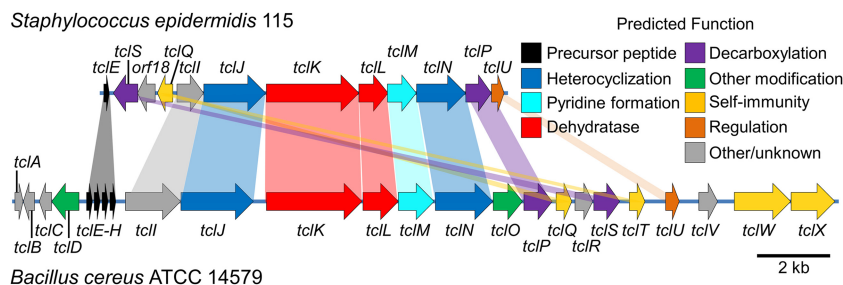
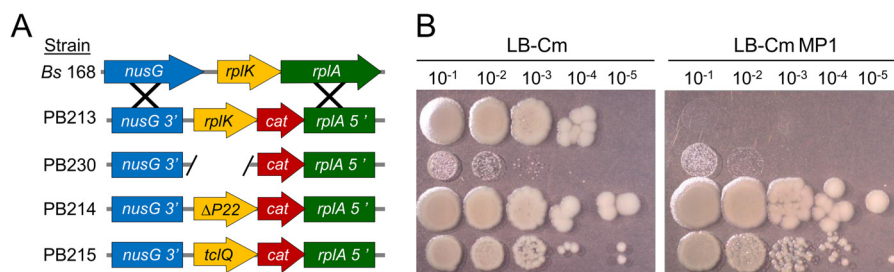


FIG 5 Comparisons of the *tcl* gene clusters from *S. epidermidis* 115 and *B. cereus* ATCC 14579. Connectors indicate homologous genes based on BLASTp comparisons of their predicted protein products. Predicted functions are shown in the color-coded key.



**FIG 6** Allelic-exchange experiments at the L11-encoding *rplK* locus. (A) Map of *rplK* alterations of the *B. subtilis* 168 (*Bs*) chromosome, with strain names indicated.  $\Delta P22$  is an abbreviation for the *B. subtilis* *rplK* $\Delta P22$  allele, which is known to give resistance to MP1. The *tclQ* allele was amplified from *S. epidermidis* 115. (B) Spot dilution assays showing growth at 24 h on LB agar with or without MP1 (18  $\mu$ g/ml). The rows correspond to the genotypes indicated in panel A. Cm, chloramphenicol.

We have also provided evidence for a remarkably simple yet efficient mechanism of self-immunity in *S. epidermidis* 115: TcQ replaces L11 at the ribosome and prevents the action of MP1. In the annotation of the thiopeptide gene cluster from *B. cereus*, the authors predicted that *tclWX*, as well as *tclWX*, might contribute to self-immunity (6). In our *B. subtilis* bioassay, we did not observe *tclWX*-based immunity. With this observation and the fact that *tclWX* is not found in the *S. epidermidis* MP1 gene cluster, it appears that *tclQ* may provide the dominant mechanism of self-immunity in both organisms.

The plasmid-encoded nature of the *S. epidermidis* MP1 biosynthetic gene cluster is also of interest. Beyond pBac115, only one other plasmid-encoded thiopeptide gene cluster has been reported to date (36). pBac115 shows strong sequence similarity to plasmid pMCCL1 from *Micrococcus caseolyticus* JCSC5402 (37). These plasmids show more than 94% sequence identity through an 8.7-kb stretch of DNA that includes replication- and mobilization-associated genes. However, all of the *tcl* genes on pBac115 are absent from pMCCL1, suggesting that they are gained and lost as a single functional unit.

With few exceptions, the biosynthetic functions of many thiopeptide-modifying enzymes remain largely undemonstrated. The *B. cereus* TcM enzyme has been implicated in what is thought to be the culminating macrocyclization step of thiopeptide maturation (38). In a few other cases, researchers have modified the gene encoding the precursor peptide and detected resultant biochemical changes in the mature thiopeptide, including expansion of macrocyclic ring size (39–41). The *tcl* gene cluster on pBac115 provides a unique platform for continuing these kinds of studies because it is half the size of the homologous gene cluster from *B. cereus* and it controls the biosynthesis of a single thiopeptide product.

Access to a simpler thiopeptide gene cluster may also facilitate pharmaceutical development of these potent antibiotics. At present, poor pharmacokinetics and poor aqueous solubility have limited the translation of thiopeptide technology from laboratory to clinic. Thiostrepton and nosiheptide remain the only successfully commercialized thiopeptides to date; both are used in veterinary applications (31). Some attempts have been made to improve thiopeptide solubility and generate synthetic derivatives with improved pharmacokinetics (42–44), but this has been difficult. The discovery of this streamlined MP1 gene cluster opens the door to effectively addressing these issues and improving synthetic strategies.

MP1 is considered the optimal platform to explore the medic-

inal chemistry of thiopeptides for a number of reasons: its mechanism of action has been well addressed, it is produced by a variety of bacterial species, and its structure is relatively simple compared with those of other thiopeptides, yet it retains all of the attractive biochemical properties (potency, stability, etc.) of this chemical family (4). Furthermore, the availability of a simple system of self-immunity, which we have demonstrated here, enables further development with model bacterial hosts such as *B. subtilis*.

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