Bioactive specialized metabolites from *Staphylococcus*: diversity, biosynthesis and biotechnological potential

Ruben Maes[1,2,*], Mahmoud Naser Aldine[3,*], Hans Gerstmans[1,2], Chris Michiels[3,†], Joleen Masschelein[1,2,†]

AFFILIATIONS

[1]Department of Biology, KU Leuven, 3001 Leuven, Belgium

[2]VIB-KU Leuven Center for Microbiology, Flanders Institute for Biotechnology, 3001 Leuven, Belgium

[3]Department of Microbial and Molecular Systems, KU Leuven, 3001 Leuven, Belgium

[*]These authors contributed equally

[†]Corresponding authors: joleen.masschelein@kuleuven.be, chris.michiels@kuleuven.be

ABSTRACT

Staphylococci are a heterogeneous group of bacteria capable of colonizing diverse ecological niches and adopting a wide variety of lifestyles. While several strains are known as notorious, multidrug-resistant human pathogens, others are harmless inhabitants of soil, water and food products, or beneficial members of the skin microbiota. To survive and remain competitive under challenging environmental conditions, staphylococci have evolved the ability to assemble and secrete a diverse range of ribosomally synthesized and post-translationally modified peptides, nonribosomal peptides, terpenes, siderophores and other specialized metabolites with antibiotic, immunomodulating and metal chelating activities. In this review, we provide an overview of the bioactive metabolite arsenal of staphylococci with a focus on their biosynthetic pathway, mode of action and industrial application potential. We also highlight unexplored natural product biosynthetic pathways in staphylococci, along with strategies to access this hidden potential.

INTRODUCTION

Staphylococci are a genus of Gram-positive, non-sporulating bacteria that comprises over 80 different species and 30 subspecies. They are spherical in shape and form grape-like clusters. *Staphylococcus* species are ubiquitous environmental bacteria, capable of thriving in diverse ecological niches, ranging from the skin of humans and animals, and the gastrointestinal tract of fish and caterpillars, to soil and marine environments, air, food products and even nectar.[1–4] They can tolerate a wide temperature (6.5 – 45 °C) and pH (4.2 and 9.3) range and are even capable of surviving at 60 °C for up to 30 min.[2] The overwhelming majority of *Staphylococcus* species are catalase-positive and facultatively anaerobic bacteria. Based on their ability to produce the coagulase enzyme, which promotes blood plasma clotting, they are divided into coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS).[5] The major pathogenic species (e.g. *S. aureus*) are coagulase-positive, while those lacking the coagulase enzyme, such as *S. epidermidis* and *S. lugdunensis*, are mostly commensal inhabitants of the skin and mucous membranes of humans and

animals. However, CNS are increasingly recognised as a global public health threat due to their ability to cause opportunistic infections in immunocompromised hosts. Indeed, while many staphylococci have a beneficial or symbiotic relationship with their host, they can switch to a pathogenic lifestyle if they gain access to host tissues through (surgical) wounds or abrasions.[6] Staphylococcal infections can result in a range of mild and life-threatening illnesses, including skin and urinary tract infections, bacteremia, endocarditis, osteomyelitis, toxic shock syndrome, and septic arthritis. Other staphylococci are known to cause food poisoning by releasing enterotoxins in contaminated food products, resulting in high fever, nausea, vomiting, muscle aches, stomach pain, and diarrhea.[7] Initially, staphylococcal infections were treated with penicillin.[8] However, most clinical isolates, including methicillin-resistant *Staphylococcus aureus* (MRSA) strains and isolates of *S. lentus, S. urealyticus, S. haemolyticus,* and *S. sciuri*, are now resistant to the commonly used antibiotics.[9] Multidrug-resistant staphylococcal infections are acquired both inside hospitals via contaminated surfaces or medical instruments, or outside through community-acquired infections[9]. In light of this looming health crisis, there is a strong global effort to develop vaccines and find new antimicrobials with the potential to prevent and combat multidrug-resistant *Staphylococcus* infections.[10]

Staphylococci have evolved a variety of mechanisms to support their remarkable fitness in the challenging environments and competitive microbial communities they live in. One of these is their ability to produce a diverse range of specialized metabolites with potent biological activities, including ribosomally synthesized and post-translationally modified peptides (RiPPs), polyketides, nonribosomal peptides, terpenes and siderophores.[1] Specialized metabolites, or natural products, serve a multitude of ecological functions, ranging from host protection and elimination of competing species, to nutrient scavenging, signalling and protection against predation.[11] The exponentially growing number of whole genome sequences, along with the increasing availability of bioinformatics tools, has greatly facilitated the discovery of novel antimicrobials and other bioactive metabolites from staphylococci over the past few years.[12,13] The genes responsible for the biosynthesis and export of these natural products are typically located adjacent to each other in bacterial genomes, making such biosynthetic gene clusters (BGCs) relatively easy to identify using bioinformatic approaches. In this review, we highlight the biosynthetic ingenuity of *Staphylococcus* spp. by providing an overview of their natural product repertoire. We discuss the chemical diversity of these bioactive metabolites, along with their biosynthetic pathway, mode of action and industrial application potential. We also explore cryptic natural product biosynthetic pathways in staphylococci and offer some perspectives on how this hidden biocatalytic potential may be unlocked.

RIBOSOMALLY SYNTHETIZED AND POST-TRANSLATIONALLY MODIFIED PEPTIDES (RiPPs)

The majority of bioactive metabolites produced by staphylococci are RiPPs, a rapidly expanding and structurally diverse class of natural products. Most RiPPs, also referred to as class I bacteriocins, are first synthesized as inactive precursor peptides, composed of a leader peptide and a core region. The leader peptide often serves as a recognition and binding site for enzymes that post-translationally modify the core peptide. Many RiPP clusters also contain additional, leader-independent tailoring enzymes which further modify the core peptide backbone, adding to the chemical complexity of this natural product family. Ultimately, the unmodified leader peptide is proteolytically removed and the mature, biologically active RiPP is exported out of the cell by dedicated export proteins. Upon secretion, RiPPs have been reported to perform a broad range of biological functions, e.g. as

antimicrobial, anti-cancer, antiviral or immunomodulatory agents, enzyme inhibitors or biosurfactants. The genes that encode the RiPP precursor peptide and the post-translational modification enzymes are typically located together in the genome with genes involved in regulation, export and self-immunity. Based on their biosynthetic pathway and structural features, RiPPs are further divided into different subclasses.[14,15]

Lantibiotics

Lantibiotics are antimicrobial lanthipeptides, a group of RiPPs that contain intramolecular ring structures formed by thioether-containing (methyl)lanthionine ((Me)Lan) residues.[15,16] Like other RiPPs, lanthipeptides are initially synthesized as inactive precursor peptides, consisting of an N-terminal leader peptide and a C-terminal core peptide.[15] The leader sequence is recognized by a dehydratase that converts multiple serine and/or threonine residues in the core peptide into dehydroalanine (Dha) and dehydrobutyrine (Dhb) residues, respectively.[15] Subsequent intramolecular Michael-type addition of cysteine thiols onto these dehydroamino acids forms the characteristic β-thioether crosslinks (**Figure 1**).[16]

Depending on the nature of the biosynthetic enzymes, lanthipeptides are divided into five classes.[17] In class I lanthipeptides, the dehydration and cyclization reactions are catalyzed by a separate aminoacyl-tRNA-dependent dehydratase (LanB) and cyclase (LanC), respectively, which together form a multienzyme complex.[17] Class II lanthipeptides rely on a multifunctional modification enzyme (LanM) that harbors an N-terminal ATP-dependent dehydratase and a C-terminal cyclase domain.[17] Class III and IV lanthipeptides are synthesized by LanKC and LanL modification enzymes, respectively. Both enzymes are organized in three functional domains: an N-terminal phosphoSer/Thr lyase domain, a central Ser/Thr kinase domain and a C-terminal cyclase domain.[18,19] LanL and LanKC are highly homologous, but differ in their cyclase domain. In LanL, this domain contains a zinc-binding motif (Cys-Cys-His/Cys), while the corresponding domain in LanKC is Zn^{2+}-independent.[17] Lastly, class V lanthipeptides employ a stand-alone LanK kinase and LanY phosphoSer/Thr lyase, which together form a heterodimeric dehydratase complex.[20–24] Cyclization of these peptides may occur spontaneously or through the action of a LanC-type lanthipeptide cyclase.[21] Lanthipeptides may also undergo other, less common post-translational modifications, such as hydroxylations, oxidations, decarboxylations, acylations and amino acid conversions.[25] Ultimately, the leader peptide is cleaved off by a dedicated protease (LanP) and the mature lantibiotic is secreted out of the cell via an ATP-binding cassette (ABC) transporter (LanT).[16,26] The genes encoding the lantibiotic precursor peptide, modification enzymes and export proteins are typically clustered in the genome together with genes involved in regulation (*lanR*), self-resistance (*lanEFG*) and additional immunity (*lanI*).[16,26]

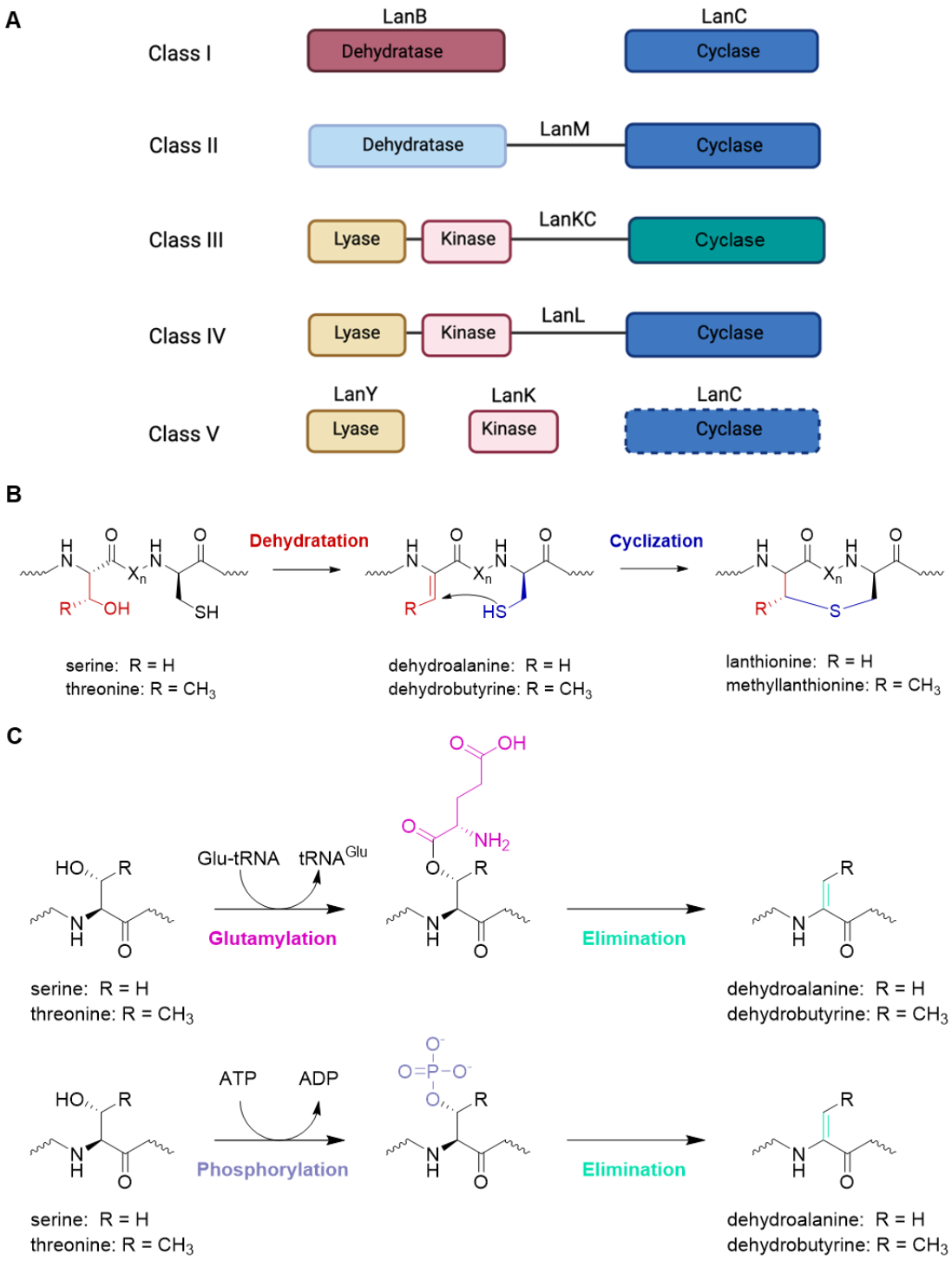


Figure 1. Overview of lanthipeptide classes and lanthionine installation. **A**. Domain organization of lanthionine-installing biosynthetic enzymes across the five classes of lanthipeptides. Class I lantibiotics rely on separate aminoacyl-tRNA-dependent dehydratase (LanB) and cyclase (LanC) enzymes. Class II lanthipeptides employ a dual-function LanM that contains an ATP-dependent dehydratase and cyclase domain, while classes III and IV utilize trifunctional lanthionine synthases, called LanKC and LanL, respectively. Class V lanthipeptide biosynthesis involves a three-component lanthionine synthetase formed by a standalone monofunctional kinase, phosphoSer/Thr lyase and cyclase. **B.** A two-step reaction process involving Ser/Thr dehydration followed by cyclization leads to the formation of lanthionine residues. **C.** Mechanisms of Ser/Thr dehydration. LanB enzymes catalyse dehydration by glutamylating the side chains of Ser/Thr residues using glutamyl-tRNA, followed by the elimination of glutamate to generate Dha/Dhb (top). In contrast, class II-V lanthionine synthetases initiate dehydration through phosphorylation of the Ser/Thr hydroxyl groups via ATP-dependent kinase domains. The subsequent elimination step differs among these classes: it occurs within the kinase active site for LanM enzymes, while it is catalyzed by specialized phosphoSer/Thr lyase domains in LanKC, LanK and LanL enzymes (bottom).

Epidermin and gallidermin

Epidermin and its variant gallidermin are class I lantibiotics produced by commensal *S. epidermidis* and *S. gallinarum* strains, respectively (**Table 1**).[27,28] Both lantibiotics are identical in sequence, except for one amino acid in the core peptide (Leu_6Ile in gallidermin).[29] The epidermin and gallidermin BGCs consist of 11 genes (*Epi/GdmGDFHTABCDQP*) and are both located on plasmids. The dehydratase and cyclase enzymes Epi/GdmBC catalyze the formation of one MeLan and two Lan residues, which introduces three thioether rings in the core peptide. The flavin mononucleotide (FMN)-containing enzyme Epi/GdmD then catalyzes the oxidative decarboxylation of the C-terminal cysteine of the peptides. Experiments with truncated variants of the EpiA precursor peptide have demonstrated that EpiD does not interact with the leader peptide and only requires the final four amino acids of EpiA (SYCC) for recognition.[30] The X-ray co-crystal structure of the enzyme bound to a pentapeptide substrate analog indicates that the FMN cofactor first oxidizes the terminal cysteine to a thioaldehyde, which is then converted into a reactive thioenolate intermediate by spontaneous decarboxylation. Subsequent addition of a dehydroalanine residue, likely catalyzed by the Epi/GdmC cyclase, yields an unusual *S*-((*Z*)-2-aminovinyl)-*D*-cysteine residue and a fourth thioether ring (**Figure 2**).[31,32] The fully processed peptide is secreted from the cell by the ABC transporter Epi/GdmHT, followed by extracellular removal of the leader peptide by the Epi/GdmP serine protease.[29,33,34] The production of epidermin/gallidermin is controlled by the transcriptional activators Epi/GdmQ. These proteins bind to palindromic sequences upstream of the -35 box in the promoter regions of the different transcription units, including *Epi/GdmABCD, Epi/GdmFEG, Epi/GdmT, and Epi/GdmH.*[29,33,34] Additionally, the *agr* quorum sensing system controls the expression of the protease gene *Epi/GdmP.*[35]

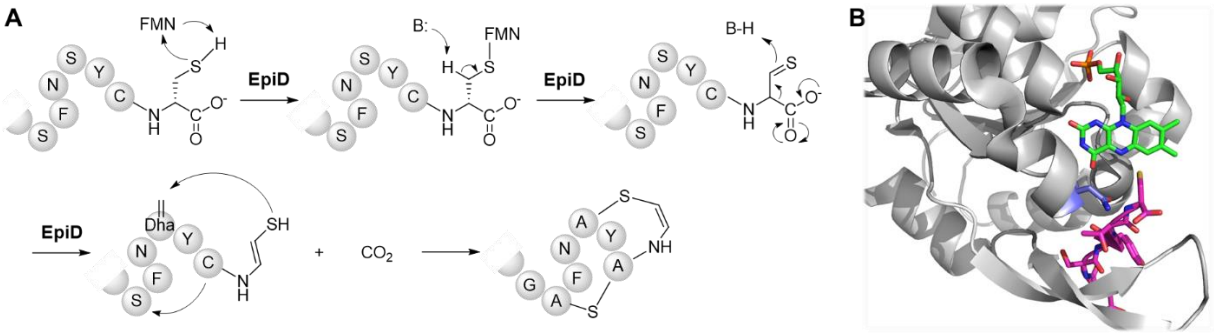


Figure 2. Oxidative decarboxylation of the C-terminal Cys residue yields an unusual aminovinyl cysteine moiety and a fourth thioether ring in in epidermin and gallidermin. **A.** Mechanism of EpiD-catalyzed aminovinyl cysteine formation, involving flavin-mediation oxidation of the cystine thiol to the corresponding thioaldehyde and subsequent decarboxylation. The resulting thioenolate performs a nucleophilic attack onto the β-carbon of a Dha residue to afford the *S*-((*Z*)-2-aminovinyl)-*D*-cysteine moiety. **B.** X-ray co-crystal structure of an EpiD monomer in complex with its FMN cofactor (green) and a pentapeptide substrate mimic (pink) (PDB ID 1G5Q). The Asn residue at position 117 that is proposed to act as a base during catalysis is highlighted in blue.

To protect themselves against the harmful effects of epidermin/gallidermin, the producer strains expel the lanthipeptides to the extracellular environment through the combined action of the immunity and export proteins Epi/GdmFEG and Epi/GdmHT, respectively.[29,34] The active lantibiotics are composed of 22 residues and adopt a screw-like structure in solution with lipotropic and aromatic side chains on one side and hydrophilic residues on the other side.[29,36] Both peptides carry a net positive charge of three and have been shown to interact with lipid I, II, III, as well as IV, thereby inhibiting both murein and cell wall teichoic acids biosynthesis.[29,37,38] Due to their amphiphilic and cationic nature, epidermin and gallidermin induce pore formation in the membrane of sensitive bacteria, which includes related staphylococci, streptococci, and other skin bacteria, such as *Propionibacterium acnes*.[39,40] However, the peptides are not long enough to span the entire

cytoplasmic membrane. It has therefore been suggested that pore formation is dependent on the thickness of the target cell membrane and that the interaction with lipid I and II is more important for antibacterial activity.[29] Additionally, there is evidence suggesting that gallidermin inhibits the expression of two genes that are associated with biofilm formation, namely *ica* (intercellular adhesion) and *atl* (major autolysin).[41]

Pep5, epicidin 280 and homicorcin

Pep5 is a 34-residue class I lantibiotic produced by *S. epidermidis* that exhibits antimicrobial activity against *S. aureus,* coagulase-negative staphylococci, and *M. luteus* (**Table 1**). Its BGC comprises six genes (*pepTIAPBC)* and is located on a small 20 kb plasmid, named pED503.[42,43] The mature peptide has a molecular mass of 3488 Da following post-translational modifications (PTMs) and features one MeLan, two Lan, two central Dhb and an N-terminal 2-oxobutyryl residue.[44] The latter is formed by dehydration of the first threonine residue in the core peptide, resulting in Dhb formation.[34,44] After leader peptide removal, the N-terminal Dhb becomes unstable and spontaneously deaminates through the addition of a water molecule to form the 2-oxobutyryl residue.[33,44]

To elucidate the function of the modified residues in Pep5, site-directed mutagenesis was performed.[45] The deletion of individual modified residues resulted in a notable reduction in antimicrobial activity.[45] Replacing the cysteine residues that form the characteristic thioether crosslinks reduced the stability of the peptide by rendering it susceptible to proteolytic digestion.[45] These findings underscore the importance of the ring structures for maintaining peptide stability and bioactivity.[45] Furthermore, introducing potential modification sites in the central region of the precursor peptide resulted in the formation of novel modified residues, but this also reduced the antimicrobial activity of Pep5.[45] The lethal effect of Pep5 is linked to its ability to form pores in the cell membranes of target cells.[46] Pore formation is believed to be induced by ionic interactions between the cationic peptide and the anionic phospholipid head groups.[46] Pep5 induces the formation of pores with diameters of up to 1 nm. Pore formation results in the cellular efflux of small molecules, ATP, and K^+, ultimately leading to membrane depolarization and cell lysis.[46] Unlike epidermin and gallidermin, the Pep5 BCG encodes only a single immunity gene, *pepI.*[43] The PepI peptide, composed of 69 aa, protects the producing strain against the harmful effect of Pep5, presumably by shielding its target on the cell membrane.[47] The production of *pepI* is strictly coupled to that of Pep5.[48] This is achieved at the transcriptional level through a mechanism involving stabilization of the mRNA transcripts[48]. Biochemical analysis of PepI revealed that the N-terminus of the peptide, which facilitates the transport of PepI out of the cell, is nonpolar and hydrophobic, while the C-terminus, which confers the immunity phenotype, is polar and carries a net positive charge.[48]

Epicidin 280 is a variant of pep5 produced by *S. epidermidis* BN 280[49]. Its BGC (*eciOIAPBC*) is located on a plasmid called pCH01 and is similar to that of Pep5, except for the absence of one gene (*pepT*) and the addition of another (*eciO*).[49] Epicidin 280 is shorter than Pep5, comprising 30 amino acid residues in total. The overall sequence similarity between both lantibiotics is 75, with a notable loss of 4 aa residues in the flexible central part of the epicidin 280 core peptide.[33] The same region also harbors a proline residue, suggesting that epicidin 280 is structurally more rigid than Pep5. Compared to Pep5, epicidin 280 shows reduced antimicrobial activity.[50] Further comparisons indicated that it has a different charge distribution, carrying a net charge of +4 instead of +7 in the case of Pep5.[49] The net charge of the C-terminus of epicidin 280 has a net neutral charge, primarily due to the presence of an aspartic acid residue at position 28. Unlike in the case of epidermin, transport of epicidin 280 out of the cell is likely carried out by a non-dedicated host transport system encoded elsewhere in the genome.[49] The BCG also encodes an immunity gene (*eciI)* that produces a peptide

with 62 amino acid residues.[49] Comparative analysis between PepI and EciI showed that EciI is six amino acids shorter, with an overall similarity of 74.2%.[33] To test whether EciI and PepI can block the activity of Pep5 and epicidin 280, respectively, a deferred antagonism test was performed. The results demonstrated that both strains exhibited cross-immunity, indicating that both peptides can block the activity of both lantibiotics. Therefore, it was proposed that EciI shares the same mechanism of immunity as PepI.[49] Compared to Pep5, the epicidin 280 BGC harbors an additional gene, named *eciO,* which encodes an oxidoreductase. This enzyme has been shown to modify the N-terminus of epicidin 280[49]. Similar to Pep5, the N-terminal amino acid of the epicidin 280 core peptide is converted into Dha. Since Dha is not stable following removal of the leader peptide, it undergoes spontaneous deamination to form an oxopropionyl residue. In epicidin 280, EciO can reduce this residue to form a 2-hydroxypropionyl group, generating a mixture of peptides with oxopropionyl and 2-hydroxypropionyl groups at their N termini (**Figure 3**).[49] Heterologous expression of the *eciOIAPBC in S. carnosus* TM 300 showed that EciO is not essential for the antimicrobial activity of epicidin 280.[49]

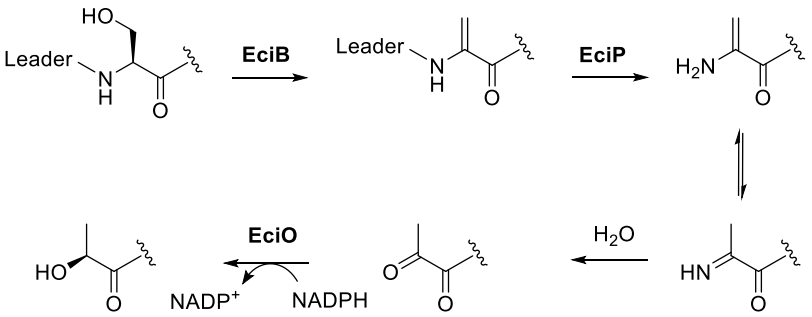


Figure 3. Mechanism of 2-hydroxypropionyl formation in epicidin. EciB functions as a dehydratase to convert a Ser residue into Dha. Following removal of the leader peptide by EciP, the N-terminal Dha becomes unstable and spontaneously deaminates through the addition of a water molecule. The resulting 2-oxopropionyl residue can be reduced by the oxidoreductase EciO, yielding a 2-hydroxypropionyl moiety.

Recently, a novel class I lanthipeptide named homicorcin was discovered in *S. hominis* strain MBL_AB63, which was isolated from jute seeds.[51] Homicorcin shares 82% sequence similarity with epicidin 280, with both core peptides differing only at seven amino acid positions[51]. The homicorcin BGC (*homOIAPBC*) is highly similar to that of epicidin 280, exhibiting 81-94% sequence identity.[51] Like epicidin 280, homicorcin is produced as a mixture of peptides due to the action of the oxidoreductase HomO, which can reduce the N-terminal oxopropionyl residue into a 2-hydroxypropionate unit.[51] While the structure of the peptide has not yet been elucidated, predictions using the RiPPMiner tool suggest that it has the same ring patterns as epicidin 280.[51] Homicorcin is active against a number of Gram-positive bacteria, such as *M. luteus,* exerting its bactericidal effect by forming pores in the cell membrane, resulting in membrane disruption and cell lysis.[51]

Epilancin K7, Epilancin 15X and SWLP1

Epilancin K7 is a class I lanthipeptide produced by *S. epidermidis* K7 (**Table 1**).[52] Unlike Pep5, epicidin 280, epidermin, and other plasmid-encoded lantibiotics from *S. epidermidis*, epilancin K7 is chromosomally encoded.[52] However, only a subset of genes responsible for its biosynthesis have been identified[52]. Among the genes that have been discovered are *elkT, elkA*, and *elkP*, which encode the transporter, precursor peptide, and peptidase, respectively. The precursor peptide is composed of 55 amino acids, 26 of which form the leader peptide and 31 the core peptide.[34,52] The structure of epilancin K7, elucidated by Van de Kamp *et al.*, features 3 ring structures, six lysine residues, a free C-terminal carboxylic acid group and an N-terminal hydroxypropionyl residue.[52] While the mechanism for the formation of the 2-hydroxypropionyl group has not yet been elucidated, it is believed to be

installed in a similar manner to that in epicidin 280.[52] Given that leader peptide cleavage occurs intracellularly, it has been suggested that the N-terminal 2-oxopropionyl intermediate may serve as an unintended substrate for a cytoplasmic enzyme, such as lactate dehydrogenase, leading to its conversion into a 2-hydroxypropionyl residue.[52] To evaluate whether this group influences the antimicrobial activity of the peptide, the first two residues were removed, and the modified peptide was subsequently tested for antimicrobial activity. The results showed that the peptide retained 75% of its original activity, indicating that the N-terminal 2-hydroxypropionyl residue is not crucial for the antimicrobial activity of the peptide.[52]

Epilancin 15X is a variant of epilancin K7 produced by *S. epidermidis* 15X154 (**Table 1**).[53] The active form of this lanthipeptide comprises 31 amino acid residues, including one Lan, two MeLan, one Dha, three Dhb and an N-terminal 2-hydroxypropionyl residue[53]. Structural comparisons have indicated that both peptides are highly positively charged with very similar charge distributions.[33] The epilancin 15X BGC consists of 11 genes (*elxOTAPBCI*$_1$*I*$_2$*I*$_3$), along with two coding sequences of unknown function. The functions of the biosynthetic genes are similar as described in the previous sections, with *I*$_1$*I*$_2$*I*$_3$ corresponding to immunity genes.[54] To determine the role of the N-terminal 2-hydroxypropionyl group, epilancin 15X peptides with and without this residue were incubated with commercial aminopeptidases.[54] These experiments revealed that the hydroxypropionyl group effectively protects epilancin 15X from degradation by bacterial aminopeptidases.[54] Self-immunity against epilancin 15X is mediated by three immunity peptides encoded by *elxI*$_1$*I*$_2$*I*$_3$, though their detailed mechanism of action has not yet been elucidated.[54] E*lxI*$_1$ and *I*$_3$ encode two peptides of 72 and 71 amino acids, respectively, with no sequence homology to any known proteins[54]. Analysis using TMHMM 2.0 revealed that both peptides possess highly hydrophobic α-helical domains and hydrophilic C-termini.[54] The same structural pattern is observed in the immunity peptides encoded by the Pep5 and epicidin 280 BGCs, indicating that ElxI$_1$ and I$_3$ protect the producer strain in a similar way.[42,49,54] *ElxI*$_2$ encodes a putative transmembrane protein with high sequence similarity to membrane-bound metalloproteases.[54] Such proteases have previously been shown to mediate self-resistance to certain RiPPs, like streptolysin S, plantaricin EF, and JK. ElxI$_2$ is therefore believed to provide protection from epilancin 15X by direct degradation of the peptide.[54]

Finally, SWLP1 (*Staphylococcus warneri* lantibiotic peptide 1) is a lanthipeptide isolated from *S. warneri* DSM 16081 that is closely related to epilancin K7 (**Table 1**).[55] Sequence alignment of the active peptide with epilancin K7 revealed 80% identity.[55] SWLP1 shows antimicrobial activity against multiple Gram-positive bacteria, including *S. epidermidis.*[55] Its BGC remains to be identified and characterized.[1]

Nukacin ISK-1 and its variants

Nukacin ISK-1 is a class II lanthipeptide produced by *S. warneri* ISK-1 (**Table 1**).[56] The nukacin BGC is located on the plasmid pPI-1 and comprises eight genes: *nukAMTFEGH* and *orf1.*[57] A notable feature of nukacin ISK-1 biosynthesis is that all PTMs are installed by the modification enzyme NukM which forms a multimeric, membrane-associated complex with NukT. NukT mediates cleavage of the leader peptide and subsequent secretion.[58,59] The mature peptide has a linear N-terminus and a globular C-terminal region, and consists of 27 amino acids, including two Lan, one MeLan, and one Dhb residue.[33,56,57] Similar to epidermin, self-resistance is mediated by an ABC transporter-like complex encoded by *nukFEG* and *nukH.*[57,60] Regulation of the nukacin ISK-1 BGC is not fully understood.[53,56] It has been suggested that the BGC is regulated by a two-component system, as it encodes a putative response regulator protein (Orf1), but no histidine kinase. Heterologous expression of the BGC in *S. carnosus* TM300 and *L. plantarum* ATCC 14917 did not lead to precursor

peptide production, suggesting that the cluster is regulated by Orf1 and a histidine kinase encoded elsewhere on the host chromosome.[61]

Depending on the strain and cell membrane composition, nukacin ISK-1 can exhibit both bactericidal and bacteriostatic properties.[62,63] The bactericidal effect is mediated by binding to lipid II, which causes disruption of the membrane potential and pore formation, ultimately resulting in loss of membrane integrity and cell lysis.[62] Site-directed mutagenesis experiments have provided further insight into the structure-activity relationship of nukacin ISK-1[64]. Deletion of three N-terminal lysine residues (nukacin$_{4-27}$) or their substitution with alanine residues impaired the antimicrobial activity of the lantibiotic.[64] In contrast, the addition of two lysine residues at the N-terminus did not enhance antimicrobial activity.[64] The chemically synthesized tail (nukacin$_{1-7}$) and ring regions (nukacin$_{7-27}$) of nukacin ISK-1 showed no antimicrobial activity, indicating that the full-length peptide is required for activity. It has also been demonstrated that the N-terminal tail region of nukacin ISK-1 has a high affinity for anionic membranes.[64] The addition of the two lysine residues at the N-terminus further increased this binding affinity, while no binding was observed in the absence of the tail lysine residues.[64] These experiments suggested that nukacin ISK-1 likely binds to target membranes through its N-terminal lysine residues.[64] Subsequent NMR titration experiments revealed that nukacin ISK-1 exists in two structural states in solution. In the active state, lanthionine ring A (nukacin$_{9-14}$) binds to the pyrophosphate moiety of lipid II through hydrogen bond interactions, while ring C (nukacin$_{19-26}$) binds to the isoprene chain via hydrophobic interactions.[65] The latter interaction is proposed to induce insertion of nukacin ISK-1 into the membrane.

Over the past few years, multiple variants of nukacin ISK-1 have been discovered, including nukacin 3299 produced by S. *simulans* 3299.[66] The nukacin 3299 BGC has an identical organization to that of nukacin ISK-1, except for the presence of an insertional element (IS257) between the *orf1* and *nukA* genes.[66] In terms of amino acid composition, both nukacin core peptides are identical.[66] Another identical variant, named nukacin L217, was detected in *S. chromogenes* L217.[67] Later, a variant with three amino acid substitutions (one in the leader peptide and two in the core peptide) was discovered in *S. hominis* KQU-131 and named nukacin KQU-131.[68] The antimicrobial activity and inhibition spectrum of nukacin KQU-131 and ISK-1 are identical, indicating that the substitutions do not affect the mode of action of the lantibiotic.[68] An additional variant with six amino acid substitutions in the leader peptide and five in the core peptide, named nukacin IVK45, was discovered from *S. epidermidis* IVK45 in 2016 by Janek and coworkers.[69] Recently, a variant of nukacin IVK45 was reported by K. Nakazono et al. in *S. epidermidis* KSE650 and called nukacin KSE650.[70] Nukacin IVK45 and KSE650 differ by one amino acid in their leader peptide.[70] Compared to nukacin ISK-1, this new variant displays reduced antimicrobial activity, presumably due to the amino acid substitutions in the core peptide.[70]

Bacteriocin of *Staphylococcus aureus* Au-26 (Bsa$_{Au-26}$) and its variants

Bsa$_{Au-26}$, a class I, epidermin-like lanthipeptide produced by *S. aureus* strain 26 (a vaginal isolate), shows antimicrobial activity against a wide range of *Lactobacillus* spp. (**Table 1**).[71] The *Bsa$_{Au-26}$* BGC is located on the genome and comprises the genes *bsaA1A2BCDPFEG$_{Au-26}$*, with *bsaA1* and *A2* encoding precursor peptides that are 77% identical in their core region.[72] Compared to the epidermin and gallidermin core peptides, they exhibit between 66 and 77% sequence homology. However, unlike the epidermin and gallidermin BGC, the Bsa$_{Au-26}$ BGC lacks the genes that encode the regulator, accessory immunity protein, and ABC transporter (*epi/gdmQHT*, respectively).[72] Mass spectrometry analysis of the purified lantibiotics revealed that BsaA2$_{Au-26}$ has a molecular weight of 2089 Da, but BsaA1$_{Au-26}$ was not detected, indicating that the first precursor peptide is either not produced or is present only in very low abundance.[72]

Multiple variants of Bsa_{Au-26} have been discovered in related strains, including Bsa_{ET3-1}, agneticin 3682, Bsa, Bsa_{COL}, and BacCH91. The Bsa_{ET3-1} variant was isolated from *S. aureus* ET3-1, a bovine mastitis isolate and has a core peptide that is 86% and 81% identical to $BsaA1_{Au-26}$ and $BsaA2_{Au-26}$, respectively.[72] Bsa refers to variants that are identical to Bsa_{Au-26} and produced by several community-acquired *S aureus* strains, including methicillin-resistant, disease-causing isolates. This lantibiotic has been linked to antimicrobial activity against different skin-associated microorganisms, such as *M. luteus.*[72] Another variant, Bsa_{COL}, was discovered in *S. aureus* COL1881. Unlike the other variants, the BGC of Bsa_{COL} encodes only one precursor peptide.[73] Studies on *S. aureus* COL1881 revealed that its antimicrobial activity is due to proteolytically processed phenol-soluble modulins (PSMs), not Bsa_{COL}[73]. PSMs, produced by *S. epidermidis* and *S. aureus* strains, are small compounds with a molecular mass similar to BsaA2. The function of Bsa_{COL} remains unclear and requires further investigation, e.g. through gene deletion studies. Agneticin 3682 (formerly known as hyicin 3682) is produced by *S. agnetis* 3682.[74] The BGC of agneticin 3682 is very similar to that of BsaCOL, but unlike Bsa_{COL}, agneticin 3682 is encoded by a large plasmid, named pRJ109.[70] The lantibiotic exhibits antimicrobial activity against Gram-positive bacteria including *Listeria monocytogenes, Bacillus cereus* and *S. aureus.*[75] Intriguingly, agneticin 3682 is associated with the siblicide phenomenon, where the producer strain inhibits its own growth in broth or on solid medium due to only partial immunity to its own antimicrobial compound. It is suggested that the absence of the accessory immunity protein (encoded by *epiH* in the epidermin BGC) might be responsible for these effects.[74] Finally, BacCH19 was identified from *S. aureus* CH-19, a strain isolated from a broiler chicken with atopic dermatitis.[76] The BGC of this variant has not yet been identified, with only the structural genes reporte.[76] Like the other variants, BacCH19 shows antimicrobial activity against Gram-positive bacteria, like *M. luteus.*[76] Overall, Bsa and its variants are proposed to provide a competitive advantage to staphylococci in the vagina, on the skin and in other complex, host-associated microbial communities by eliminating competing commensal bacteria.[72]

Nisin J

Nisin J is a variant of nisin A produced by *S. capitis* APC 2923, a strain isolated from the human skin (**Table 1**).[77] The active nisin J core peptide harbors 35 amino acids, differing from nisin A by eight residues and an additional amino acid at the C-terminus.[77] The nisin J BGC (*nsjFEGBTCJP*), located on a plasmid named pJOS01, is arranged differently from that of nisin A (*nisABTCIPRKFEG*) and lacks a number of genes, including *nisRK* (regulatory genes) and *nisI* (immunity).[77] Nisin J displays activity against a wide range of Gram-positive bacteria, including MRSA. [77] A comparison of their antimicrobial activity showed that nisin J was more active than nisin A against 12 out of 13 tested strains.[77] Surprisingly, *S. capitis* APC 2923 was only partially resistant against its own lanthipeptide, which might be due to the absence of the immunity gene *nisI.* [77] It was also demonstrated that both the nisin J and nisin A producing strains are cross-resistant to each other's lantibiotics.[77]

Hominicin

Hominicin is a lanthipeptide produced by *S. hominis* MBBL 2–9 that has antimicrobial activity against both MRSA and VISA (vancomycin-intermediate *S. aureus*) (**Table 1**).[78] The active core peptide consists of 21 aa residues and differs from most lanthipeptides by the absence of thioether linkages and its acidic nature.[78] While the hominicin BGC has not yet been discovered, the amino acid sequence and structure of the peptide were determined using mass spectrometry and NMR approaches.[78] The amino acid sequence is DmIle–Dhb–Pro–Ala–Dhb–Pro–Phe–Dhb–Pro–Ala–Ile–Thr–Glu–Ile–Dhb–Ala–Ala–Val–Ile–Ala–Dmp, with DmIle and Dmp representing N_1,N_1-dimethyl-isoleucine and N_2,N_2-dimethyl-1,2-propanediamine, respectively.[78]

Staphylococcin C55

Staphylococcin C55 is a class II lanthipeptide produced by *S. aureus* C55, a strain isolated from the skin (**Table 1**).[79] The staphylococcin C55 BGC is located on a plasmid and comprises six genes, *sacαAβAM1TM2J*, with *sacαAβA* encoding the precursor peptides, *sacM1* and *M2* encoding modification enzymes, *sacT* encoding an ABC transporter with a peptidase domain and *sacJ* encoding a putative dehydrogenase.[33,79] Staphylococcin C55 exhibits strong bactericidal activity against a range of Gram-positive bacteria, including multidrug-resistant strains of *S. aureus.*[80] Interestingly, C55α and C55β were shown to work synergistically when combined in equimolar ratios.[80]

Romsacin

Recently, a novel class I two-peptide lantibiotic, named romsacin, was discovered, produced by *S. haemolyticus* (**Table 1**).[81] Romsacin exhibits activity against Gram-positive bacteria, including highly virulent and multidrug-resistant pathogens such as *S. aureus*, *S. haemolyticus,* and *E. faecium.*[81] The romsacin BGC consists of two oppositely oriented operons. The first encodes the immunity genes *romFE*, while the second encodes the precursor peptides, modification enzymes and a dual function transport and peptidase gene (*romA1A2M1T$_p$M2*).[81] A comparison of the amino acid sequences of the romsacin core peptides A1 and A2 with the plantaricin W peptides A1 and A2 revealed sequence identities of 67% and 51%, respectively.[81] Although the structures of these peptides have not yet been experimentally determined, they have been predicted based on known modifications in related lanthipeptides, such as lacticin 3147 and lichenicidin. Based on this comparison and the observed masses, RomA1 was predicted to undergo three dehydration reactions and contain four reduced cysteines, while RomA2 was predicted to undergo nine dehydration reactions with four reduced cysteine residues.[81] The mode of action of romsacin was also investigated, revealing bactericidal effects. It was shown to disrupt biofilms and compromise membrane integrity, leading to cell death. To determine whether romsacin induces pore formation, similar to other lantibiotics, a propidium iodide assay was performed.[81] The results indicated no pore formation in comparison to the positive control. However, it remains unclear whether pores were formed, as the size of the PI molecule maybe too large to pass through potential pores.[81]

Sactipeptides

Sactipeptides are a rapidly expanding class of RiPPs, many of which show potent biological activities, ranging from antimicrobial and antiviral, to spermicidal and haemolytic effects. [82,83] They are characterized by the presence of one or more sactionine bonds. These intramolecular, sulfur-to-α-carbon thioether linkages are installed between a donor cysteine residue and another amino acid acceptor through the action of a radical S-adenosyl-methionine (SAM) enzyme, called a sactisynthase. [83–86]The thioether crosslinks give sactipeptides their typical hairpin structure, with hydrophobic amino acids generally pointing outwards.[83] This hairpin structure restricts the conformation of the sactipeptide, facilitating target binding and providing a high degree of stability and resistance to proteolysis.[84,86] Some sactipeptide BGCs also encode additional tailoring enzymes that catalyse reactions such as N- to C-terminal macrocyclization.[87] To date, most sactipeptides reported derive from *Bacillus* species, with subtilosin A the most well-known and well-studied example.[87,88]

Hyicin 4244

Hyicin 4244 is the first and currently the only sactipeptide reported to be produced by a *Staphylococcus* species (**Table 1**). [88]The hyicin 4244 BGC in *S. hyicus 4244* comprises eight genes, *hyiSABCDEFG*, which code for proteins that share between 42 and 70% similarity to those encoded by the subtilosin A BGC. [88] The precursor peptide HycS consists of 43 amino acid and is highly similar

to the subtilosin A precursor SboA (**Figure 4**).[1,88] Given that the identities and positions of the Cys residues as well as the acceptor amino acids forming the sactionine linkages in subtilosin A are conserved, hyicin 4244 is also predicted to have three sactionine linkages. The exact structure of the sactipeptide, however, remains to be elucidated.[88] HycA shares high similarity with the radical SAM enzyme AlbA and is predicted to install the thioether crosslinks. HycE and HycF are putative proteases likely responsible for leader peptide cleavage and macrocyclization. The remaining genes in the cluster are believed to encode self-immunity and transport enzymes.[88] Hyicin has demonstrated activity against foodborne pathogens, such as *L. monocytogenes* and *S. aureus*, as well as biofilm-forming *S. aureus* isolates from human infections and bovine mastitis. [88,89]The sactipeptide was not only capable of preventing biofilm formation, but also decreased the viability of cells within established biofilms. These findings suggest that hyicin 4244 has the potential to be exploited for applications ranging from food preservation to the prevention or treatment of biofilm-related infections in medical settings.

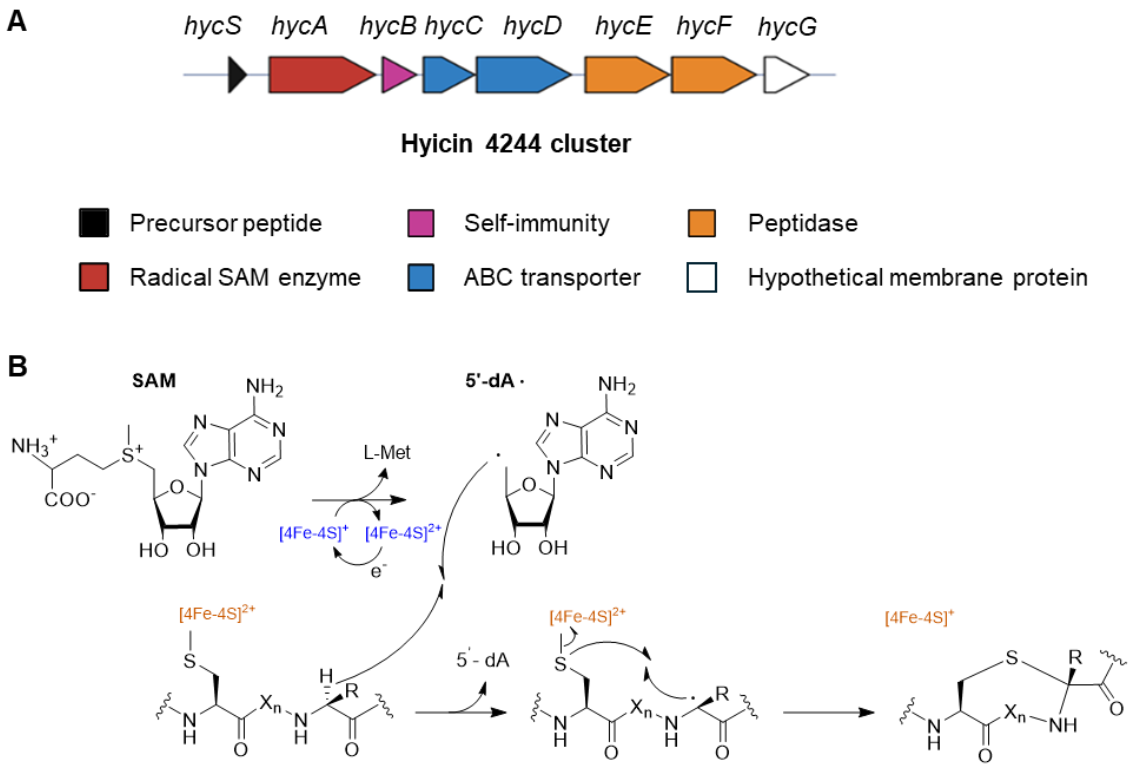


Figure 4. Biosynthesis of Hyicin 4244, a sactipeptide produced by *S. hyicus* 4244 and the only reported sactipeptide produced by the genus *Staphylococcus*. **A**. The hyicin 4244 biosynthetic gene cluster. *HycS* encodes the hyicin 4244 precursor peptide, and *hycA* the radical SAM enzyme responsible for the formation of the characteristic sactionine linkages. **B**. Possible mechanism for thioether bridge formation in hyicin 4244.

Thiopeptides

Thiopeptides are highly modified macrocyclic RiPPs with complex structures and diverse functions.[90] They are primarily characterized by three types of backbone modifications: thiazol(in)e rings, dehydrated amino acid residues (Dha and Dhb) and a class-defining six-membered nitrogen-containing piperidine, dehydropiperidine, or pyridine ring.[83,84,91] Some thiopeptides also undergo methylations, hydroxylations, decarboxylations and additional cyclizations, such as the installation of tryptophan-derived quinaldic or indolic acid moieties.[92] In the biosynthesis of thiopeptides, the thiazole moieties are formed from Cys residues through the action of a heterotrimeric complex composed of an ATP-dependent cyclodehydratase and a flavin mononucleotide (FMN)-dependent

dehydrogenase.[84,93,94] Ser and Thr residues are converted into Dha and Dhb residues, respectively, by two enzymes that are structurally and functionally similar to class I lanthipeptide LanB dehydratases.[90,95] Finally, a [4+2] cycloaddition between two Dha residues results in macrocyclization and simultaneous leader peptide elimination, giving rise to the six-membered nitrogen-containing heterocycle.[84] Thiopeptide display various activities but are most well-known for their antibiotic activity against Gram-positive bacteria. They inhibit protein synthesis either by binding to the 50S ribosomal subunit or by targeting elongation factor Tu (EF-Tu).[90]

Micrococcin P_1

Micrococcin P1 was first isolated from *Staphylococcus equorom* WS2733 in 2001 and initially thought to be a nonribosomally synthesized peptide (**Table 1**).[96] However, in 2014, Bennallack *et al*. identified the micrococcin P_1 BGC in *S. epidermidis* strain 115 on a 24kb plasmid.[97] The cluster comprises 11 genes, designated as *tcl* genes, that encode the precursor peptide (TclE), the biosynthetic machinery, as well as a self-immunity determinant (**Figure 5A**). The assembly of micrococcin P1 proceeds in a defined order. First, six Cys residues are converted into thiazoles in a two-step reaction involving TclI, TclJ and TclN. TclI acts as a scaffolding protein by recognizing the leader peptide and presenting TclE to the cyclodehydratase TclJ.[97,98] TclJ facilitates the nucleophilic attack of the Cys side chain onto the preceding amide carbon, forming a hemiorthoamide intermediate.[99] This intermediate undergoes ATP-dependent O-phosphorylation, amide nitrogen deprotonation, and phosphate elimination to yield a thiazoline heterocycle. In a second step, the FMN-dependent dehydrogenase TclN oxidizes the thiazoline rings to thiazoles (**Figure 5B**).[100,101] Subsequently, TclP decarboxylates the peptide at its C-terminus, and the LanB-like dehydratases TclK and TclL generate dehydroamino acids from Ser and Thr residues.[102] Finally, the dehydrogenase TclM catalyzes an intramolecular [4 + 2] cycloaddition reaction with concomitant leader peptide removal and pyridine ring formation (**Figure 5C**).[97,98] Micrococcin P1 producing strains from the human skin have shown potential as probiotics to reduce *S. aureus* infections and accelerate healing.[103] Aside from having potent activity against a broad range of Gram-positive bacteria, micrococcin P1 is also effective against *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*.[104] Screening studies for synergistic effects and genetic inactivation experiments have revealed that micrococcin P1 is capable of hijacking the ferrioxamine siderophore receptor to cross the outer membrane of *P. aeruginosa*.[105] Within the cell, the thiopeptide binds to the 50S ribosomal subunit, thereby blocking protein synthesis.[100,105] To protect itself against the harmful effects of its own thiopeptide antibiotic, the *S. epidermidis* producing strain encodes the self-immunity protein TclQ, a resistant homolog of the native 50S ribosomal protein L11.[98,104]

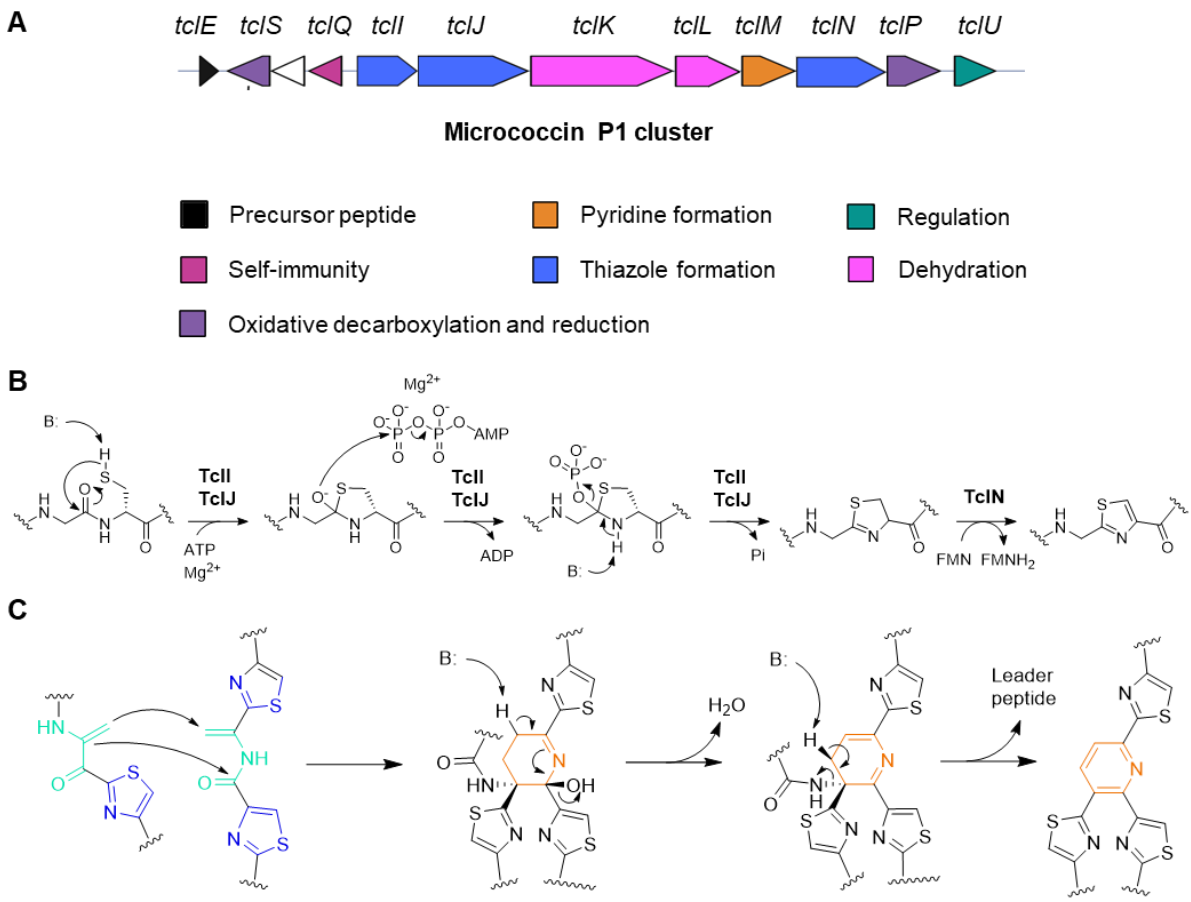


Figure 5. Micrococcin P1 biosynthesis. **A.** The biosynthesis of micrococcin P1 is directed by a gene cluster comprising 11 genes. **B.** ATP-dependent cyclodehydration and dehydrogenation by TcilJ and TclN, respectively, introduces thiazole moieties in micrococcin P1. **C.** Mechanism of pyridine ring formation via a [4+2] cycloaddition reaction catalyzed by TclM.

CLASS II AND III BACTERIOCINS

Unlike RiPPs, or class I bacteriocins, which are generally small in size (<5 kDa) and post-translationally modified, class II bacteriocins are mostly heat-stable peptides less than 10 kDa in size that are not, or only minimally modified, with modifications typically limited to disulfide bridges or circularization.[106] Class III bacteriocins, on the other hand, are defined as larger (> 30 kDa), heat-labile proteins. So far, only a handful of class II and III bacteriocins have been discovered from *Staphylococcus* species.[107]

Aureocin A53 and A70

Aureocin 53 is a class II bacteriocin produced by *S. aureus* A53. The core peptide, notably lacking the N-terminal leader peptide, is highly cationic and consists of 51 amino acid residues, including 10 lysine and 5 tryptophan residues.[108] The molecular weight of aureocin 53 was determined to be 6021.5 Da, 28 Da more than initially predicted.[33] This discrepancy was shown to be due to the formylated N-terminal methionine residue.[33] The aureocin A53 BGC is located on a plasmid named pRJ9 and comprises the genes *aucB, aucC, aucA, aucIa, aucIb*, and *aucFEG*.[109] The precursor peptide is encoded by *aucA*, while *aucFEG* encodes an ABC transporter that confers immunity and presumably mediates transport of the peptide out of the cell. *AucIa* and *aucIb are* also believed to encode proteins responsible for immunity, while AucB and AucC are proteins of unknown functions.[108,109] Aureocin A53 displays antimicrobial activity against *E. faecium, L. innocua, M. luteus, S. aureus, S. epidermidis,* and *S. simulans 22* by dissipating the membrane potential and increasing membrane permeability, resulting in cell lysis.[110] Multiple variants of aureocin A53 have been reported, including epidermicin

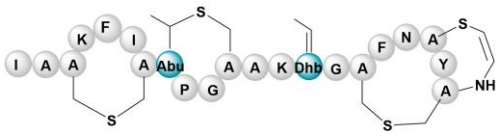
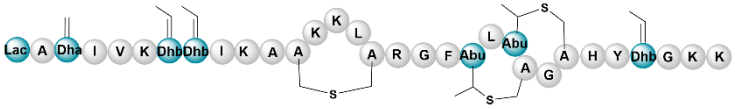
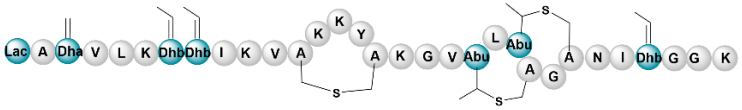
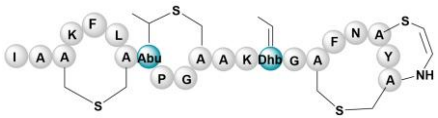
NI01, BacSp222, and capidermicin produced by *S. epidermidis*, *S. pseudintermedius,* and *S. capitis*, respectively.[1] The BGCs of these variants all show high similarity to that of aureocin 53, with only minor differences in the organization of the genes and transcriptional units.[1]

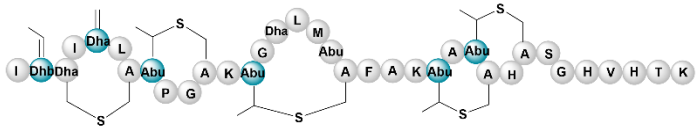
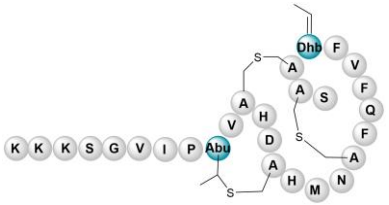
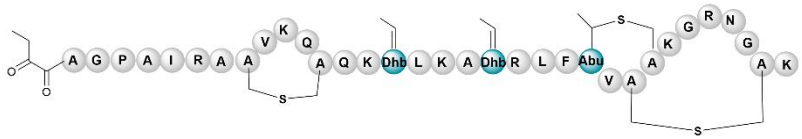
Aureocin A70 is another class II bacteriocin produced by *S. aureus* A70.[111] The BGC of aureocin A70 is located on a plasmid named pRJ6 and harbors the genes *aurRIABCDT.* A*urR* encodes a regulatory protein, *aurI* an immunity protein, *aurT* a transporter, and *aurABCD* four structural peptides.[112,113] The peptides consist of 30 to 31 amino acid residues, do not feature any post-translational modifications and have pronounced cationic and hydrophobic properties.[111] The four-peptide bacteriocin aureocin A70 has antimicrobial activity and is able to inhibit a range of Gram-positive bacteria, including *L. monocytogenes, S. aureus, S. agalactiae,* and *Corynebacterium* spp.[33] Interestingly, each of the individual peptides, except for AurD, has also been shown to be active against *M. luteus*. However, combining the peptides significantly enhanced their activity. When tested against *L. innocua* and *S. aureus*, antibiotic activity was only detected when all the peptides were mixed together.[33] Recently, a new variant of aureocin A70, named aureocin 4181, was discovered in *S. aureus* strain 4181.[114] The BGC of this variant is highly similar to that of aureocin a70, with minor differences in the encoded structural peptides, including the presence of an N-formyl group at the N-terminus of each peptide and a single amino acid substitution in the AurD peptide.[114] Compared to aureocin A70, the 4181 variant displays a broader spectrum of activity and causes more extensive cell lysis.[114]

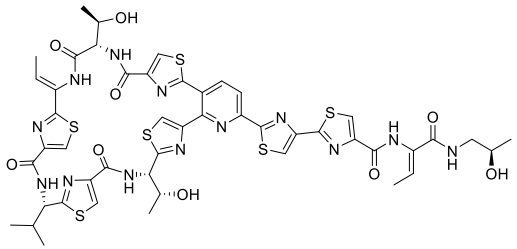
Lysostaphin

Lysostaphin is a class III bacteriocin belonging to the subclass of bacteriolysins, produced by *S. simulans* subsp. *Staphylolyticus.*[115] The BGC for lysostaphin is located on the plasmid pACK1 and encodes the precursor peptide gene *Lss* and the resistance gene *Lif*.[115] Lysostaphin is first synthesized as an inactive pre-propeptide composed of 493 amino acids, including a signal peptide, 15 tandem repeats of 13 amino acids each, and a core peptide of 246 amino acids.[115,116] Following translation, the peptide is secreted into the extracellular environment, where the signal peptide is cleaved, converting it to prolysostaphin.[115] The tandem repeat sequences are then gradually trimmed in a growth stage-dependent manner by a cysteine protease secreted into the extracellular space, resulting in the formation of mature lysostaphin.[115] The mature 27 kDa lysostaphin consists of two domains, a catalytic N-terminal peptidase domain and a C-terminal targeting domain, which binds to peptidoglycan.[115,116] Lysostaphin possesses antimicrobial activity against *Staphylococcus* spp.[116]. strains by targeting the cell wall of susceptible strains through its glycyl-glycine endopeptidase activity.[115] Interestingly, the producing strain does not possess a specific immunity mechanism against lysostaphin. Instead, it relies on a resistance mechanism involving the incorporation of serine instead of glycine at the cleavage site where lysostaphin acts.[115] This modification prevents lysostaphin from hydrolysing the cell wall of the producer strain.[115]

Table 1: Overview of ribosomally synthesized peptides produced by *Staphylococcus* spp.

Type	Producing strain	Antimicrobial activity against	BGC size (kb)	Mw (Da)	MiBIG ID	Reference
Lantibiotics						
Agneticin (hyicin) 3682	*S. agnetis* 3682	*Staphylococcus* spp., *Listeria monocytogenes*, *Bacillus cereus*	8.6	2117	BGC0001618	[74]
BsaAu-26	*S. aureus* AU-26	*Staphylococcus* spp., *Lactobacillus* spp.	10.9	2089	ND	[71]
Epicidin 280	*S. epidermidis* BN 280	*Staphylococcus* spp.	7.4	3133/3136*	BGC0000507	[49]
Epidermin 	*S. epidermidis* Tü3298	*Staphylococcus* spp., *M. luteus*, *C. pseudodiphtheriticum*, *R. mucilaginosa*	8.7	2164	BGC0000508	[29]
Epilancin 15x 	*S. epidermidis* 15X154	*Staphylococcus* spp., *Enterococcus* spp.	15.8	3017	BGC0000509/ NPA013376	[53]
Epilancin K7 	*S. epidermidis* K7	*Staphylococcus* spp., *Enterococcus* spp.	1.1	3032	BGC0000510	[53]
Gallidermin 	*S. gallinarum* (F16/P57) Tü3298	*Staphylococcus* spp., *Micrococcus* spp., *Lactococcus* spp, *Lactobacillus* spp., *Enterococcus* spp., *Listeria* spp.	12.7	2164	BGC0000514/ NPA024678	[29]
Homicorcin	*S. hominis* MBL_AB63	*Staphylococcus* spp., *M. luteus*, *Lactococcus lactis*, *Bacillus subtilis*	7.4	3043/3046*	ND	[51]

Hominicin	*S. hominis* MBBL 2–9	*Staphylococcus* spp., *M. luteus, C. pseudodiphtheriticum, R. mucilaginosa*	ND	2038.4	ND	[78]
Nisin J 	*S. capitis* APC 2923	*Staphylococcus* spp., *Lactobacillus delbrueckii*, *Lactococcus lactis*, *Enterococcus spp.*, *Listeria monocytogenes*	9.78	3458	ND	[77]
Nukacin ISK-1 	*S. warneri* ISK-1	*Staphylococcus* spp., *Streptococcus* spp., *Propionibacterium acnes*	11.7	2960	BGC0000539/ NPA007347	[61]
Pep5 	*S. epidermidis* strain 5	*Staphylococcus* spp., *M. luteus*	7.9	3488	BGC0000543	[43]
Romsacin	*S. haemolyticus*	*S. aureus, S. haemolyticus, E. faecium*	10.5	3149/3548	ND	[81]
Staphylococcin C55 α & β	*S. aureus* C55	*S. aureus, M. luteus*	11.8	3339/2993*	BGC0000555	[79,80]
SWLP1	*S. warneri* DSM 16081	*Staphylococcus* spp.	ND	2998	ND	[55]
Sactipeptides						
Hyicin 4244	*S. agnetis 4244*	*Staphylococcus* spp., *Enterococcus* spp., *Lactobacillus* spp., *Listeria* spp., *L. lactis*, *M. luteus*	8.6	3274	BGC0001618	[93]
Thiopeptides						

Micrococcin P_1 	*S. epidermidis* strain 115, *S. equorum* WS 2733, and *S. hominis* S34-1	*Staphylococcus* spp., *Enterococcus* spp., *Listeria* spp., *Lactobacillus* sp., *Bacillus cereus, Clostridium perfringens Actinobacteria: Corynebacterium* sp., *Brevibacterium* sp., *Arthrobacter* sp., *Microbacterium* sp.	10.9	1143	ND	[98]
Non-modified bacteriocins						
Aureocin A53	*S. aureus* A53	Antimicrobial activity against *E. faecium, L. innocua, M. luteus, S. aureus, S. epidermidis, S. simulans 22*	6.1	6021	ND	[108]
Aureocin A70	*S. aureus* A70 (ABCD)	Antimicrobial activity against *L. monocytogenes, S. aureus, S. agalactiae, Corynebacterium* spp.	3.4	2927/2795/ 2954/3086*	ND	[111]

*Compound that has multiple peptides or has different masses depending on the post translational modifications

ND: not determined

NONRIBOSOMAL PEPTIDES

Nonribosomal peptides (NRPs) constitute a large and diverse family of specialized metabolites with a wide range of biological activities and applications in medicine and agriculture. Well-known examples include toxins, virulence factors, insecticides, antibiotics and anticancer agents.[117] NRPs are produced by large multienzyme complexes, known as nonribosomal peptide synthetases (NRPSs).[118] These biosynthetic assembly lines have a modular architecture, each module consisting of a set of enzymatic domains responsible for the selection, activation and covalent binding of one amino acid building block, as well as its incorporation into the growing peptide chain through amide bond formation.[118–120] In addition to the standard set of 22 proteinogenic amino acids, NRPSs can utilize a variety of non-proteinogenic amino acids, along with α-hydroxy acids, carboxylic acids and even glycosylated and methylated residues, thereby generating a high level of structural diversity.[117,118] An NRPS module minimally consists of three domains: an adenylation (A) domain, which selects and loads a specific amino acid onto the phosphopantetheinyl (Ppant) prosthetic arm of a peptidyl carrier protein (PCP) domain, and a condensation (C) domain, which catalyzes chain elongation. A variety of additional domains, such as epimerisation (E) and methyltransferase (MT) domains, may be present to perform various modifications on the growing peptide.[121] Throughout the assembly process, all biosynthetic intermediates remain covalently attached to the PCP domains as thioesters. Upon completion of the final chain elongation/modification cycle, the fully assembled peptide is typically released from the assembly line through hydrolysis or macrocyclization, catalyzed by a thioesterase (TE) domain.[118,122] After the peptide is released, it can undergo additional post-assembly modifications by dedicated tailoring enzymes to obtain its biological activity. These modifications often include glycosylation, oxidative cross-linking, hydroxylation, and/or halogenation.[118,119,121,122] To date, two nonribosomal peptides have been identified as originating from *Staphylococcus* species.

Lugdunin

Lugdunin is a cyclo-hexapeptide discovered in *S. lugdunensis* IVK28, a clinical isolate from the human nasal cavity (**Table 2**).[123] It exhibits bactericidal activity against *S. aureus* and has been shown to modulate the human immune system.[123,124] Specifically, lugdunin increases the expression of the antimicrobial peptide LL-37 and the chemokine CXCL8, leading to the recruitment of neutrophils and monocytes.[124] The biosynthesis of lugdunin is directed by four genes, *lugABCD*, which encode an NRPS with one loading module and six extension modules (**Figure 6**).[123] Interestingly, although lugdunin incorporates seven amino acid residues, the NRPS only comprises five adenylation domains. Based on the atypical domain organization of LugC, which includes three extension modules and a single A domain, it has been suggested that this A domain is responsible for selecting and loading the valine residues onto the three consecutive PCP domains.[123] Such iterative A domain activity has previous been observed during yersiniabactin assembly.[125] Another unusual feature is the presence of a thiazolidine heterocycle. Thiazolidine rings are known modifications in a number of linear NRPs but had not yet been observed in macrocyclic NRPs. Zipperer *et al.* propose that the heterocycle moiety is formed by condensation of the N-terminal *L*-Cys with the C-terminal *L*-Val residue after the peptide is released as an aldehyde by the C-terminal thioester reductase (TR) domain within LugC.[123] This reaction is proposed to proceed via a macrocyclic imine intermediate (**Figure 6**). The fully assembled lugdunin is capable of eliminating several major human pathogens, including MRSA and vancomycin-resistant *Enterococcus* (VRE) strains by rapidly depleting their energy resources.[126–128] The antibiotic has been shown to dissipate the membrane potential of target bacteria by forming peptide nanotubes.[128] This process occurs in two steps. First, lugdunin partitions into the hydrophobic core of the bacterial membrane.[129] Next, the lugdunin monomers self-assemble into nanopores through intermolecular hydrogen bonds. The nanopores are formed by lugdunin trimers,

tetramers and pentamers. The dissipation of the membrane potential is driven by a water wire that fills the nanopore, facilitating the translocation of protons and monovalent cations.[129] The *S. lugdunensis* producing strain is protected from these harmful effects through the action of LugEFGH, four ABC transporters that provide self-resistance by secreting the antibiotic from the cell.[130] Notably, sensitive *S. aureus* strains did not develop resistance to lugdunin after 30 days of serial passaging at subinhibitory antibiotic concentrations.[130] The combination of broad-spectrum activity against major pathogens and a low risk of resistance development makes lugdunin a promising pharmaceutical candidate.

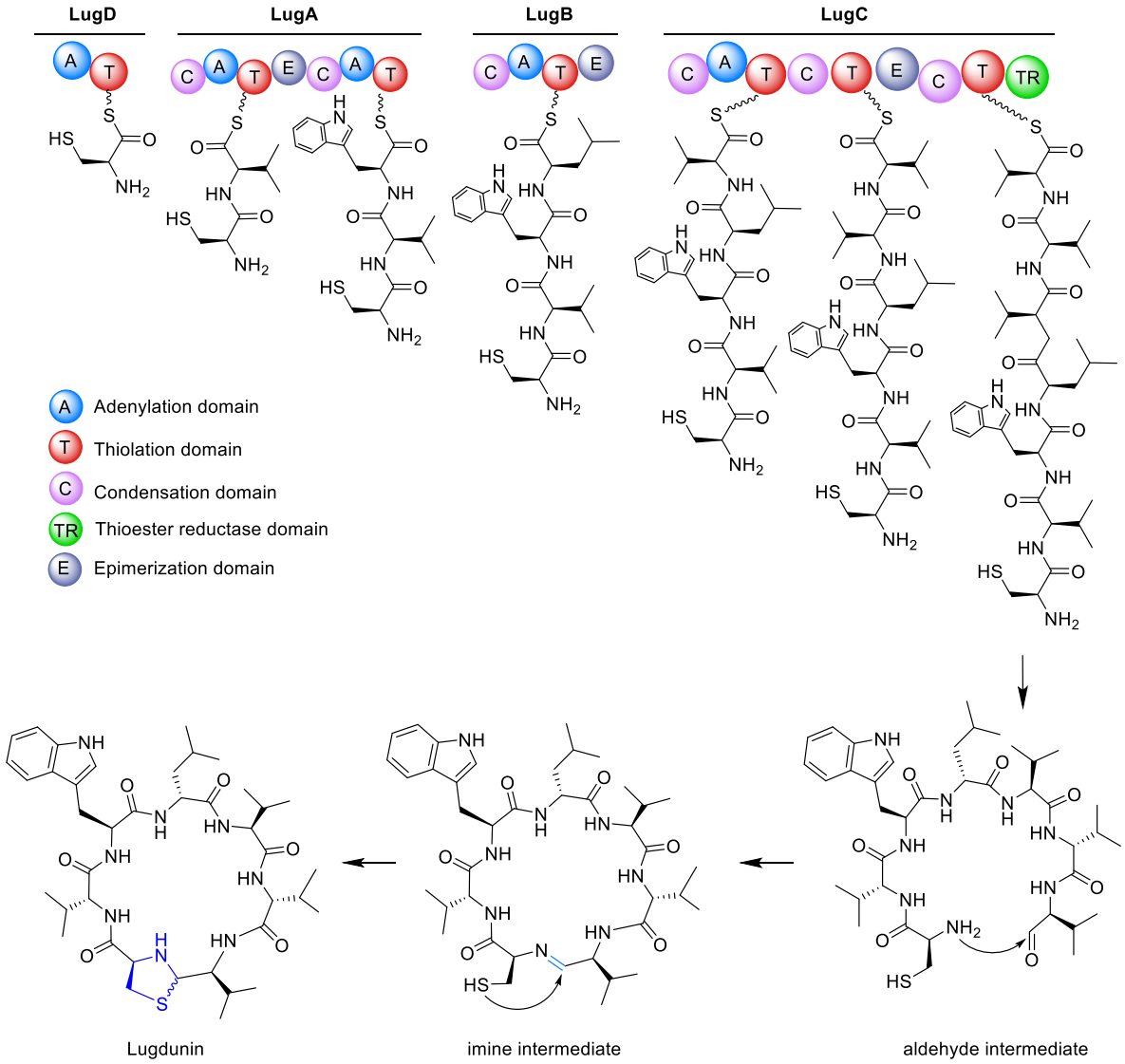


Figure 6. Biosynthetic pathway for lugdunin. The hexapeptide backbone of lugdunin is synthesized by an NRPS assembly line consisting of one loading module (LugD) and six extension modules (LugABC). The full-length peptide is released as an aldehyde by a C-terminal thioester reductase domain. Formation of the characteristic thiazolidine ring in lugdunin is proposed to occur through condensation of the N-terminal Cys with the C-terminal Val via an imine intermediate.

Aureusimine

A family of pyrazinone NRPs, known as aureusimines, was first reported to be produced by *S. aureus* in 2010.[131–133] The BGC responsible for their production consists of two genes, *ausA* and *ausB*, which direct the assembly of three distinct dipeptides: tyrvalin (aureusimine A), phevalin (aureusimine B) and leuvalin (aureusimine C).[134,135] Interestingly, phevalin was originally isolated from soil-dwelling *Streptomyces* sp. SC433, potentially serving as the source from which *S. aureus* may have acquired

the BGC.[136] The conserved BGC is not only present in all sequenced *S. aureus* isolates, including MRSA, but also in *S. epidermidis*, *S. capitis*, and *S. lugdunensis* strains. *AusB* encodes a 4-phosphopantetheinyltransferase that post-translationally primes the PCP domains within AusA by transferring a 4-phosphopantetheinyl moiety from coenzyme A onto the side chain of a conserved serine residue.[135,137] This reaction converts the PCP domains from their inactive *apo* form to their functional *holo* form. AusA is a bimodular NRPS harboring two A domains, two PCP domains and a C-terminal TR domain. The latter reductively releases the dipeptidyl thioester as an aldehyde. [135,137] The resulting aldehyde undergoes a nucleophilic attack by the N-terminal amino group to afford an imine intermediate that spontaneously oxidizes to a pyrazinone ring (**Figure 7A**).[135,137] The production of three different congeners is linked to the promiscuity of the second A domain within AusA. The first A has strict specificity for L-Val, while the second incorporates either L-Tyr (aureusimine A) or, to a lesser extent, L-Phe (aureusimine B) or L-Leu (aureusimine C) (**Figure 7B**).[134,135] It has been suggested that the dipeptide aldehyde is the active compound rather than the cyclic pyrazinone, given that the aldehyde form of aureusimine B has been found to have potent protease inhibitory activity.[135] The dipeptide aldehyde targets the cathepsins, a lysosomal cysteine protease, which plays a role in antigen presentation. It thereby modulates the innate immune system, helping the bacteria evade immune system recognition.[138] Production of phevalin helps *S. aureus* escape from phagocytes, aids with intracellular survival and inhibits neutrophil activation.[139] Indeed, clinical mutations in *ausA* have been found to reduce *S. aureus* cytotoxicity and increase persistence within epithelial cells, allowing the bacteria to evade immune responses and cause host cell death.[140] Phevalin production is elevated in *S. aureus* biofilms and has modest effects on gene expression of human keratinocytes.[134] Taken together, these findings indicate that aureusimine production provides an advantage for *S. aureus* and that these metabolites could be used, either as biomarkers or as therapeutic targets.

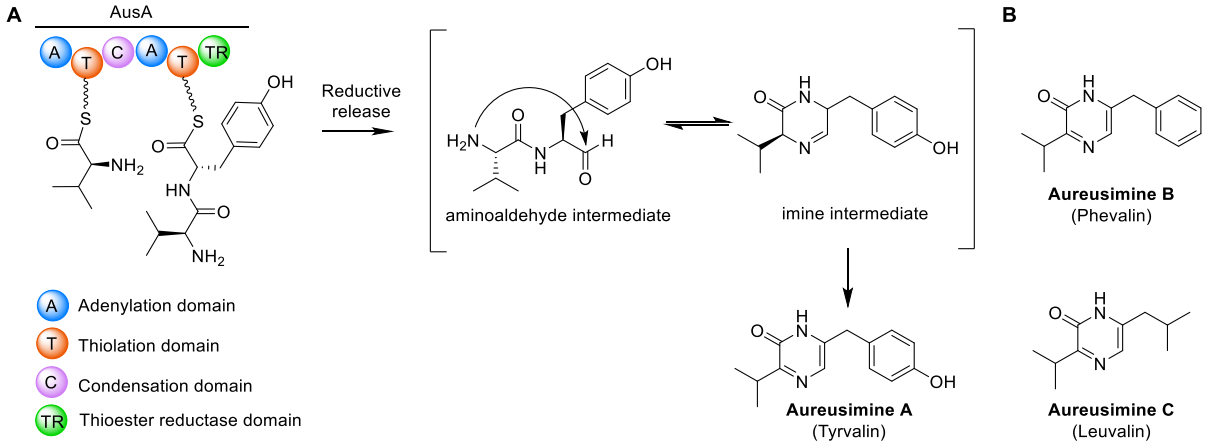


Figure 7. Biosynthesis of the aureusimines, pyrazinone NRPs from *Staphylococcus* spp. **A**. Aureusimine assembly is directed by the bimodular NRPS AusA and the 4-phosphopantetheinyltransferase AusB, which primes the T domains in AusA. AusA is highly specific for its first amino acid substrate, L-Val, while its second substrate can be L-Tyr, L-Phe or L-Leu in order of decreasing specificity, resulting in the production of three aureusimine variants. **B**. Chemical structures of aureusimine B (L-Val and L-Phe) and aureusimine C (L-Val and L-Leu).

SIDEROPHORES

Siderophores are natural products produced by organisms, such as bacteria, fungi, and plants, to sequester free iron from the environment.[141] These iron chelators are characterized by their low molecular weight and high affinity for Fe^{3+}. Siderophores are classified based on the chemical moieties that chelate the ferric iron, which include hydroxamate, catecholate, carboxylate and mixed types.[142] Siderophores are either produced by NRPSs or through NRPS-independent pathways.[142]

NRPS-independent siderophore (NIS) synthetases typically catalyze a single enzymatic reaction, often forming an amide or ester bond between an organic acid and a substrate containing an amine or hydroxyl group.[142,143] Prior to condensation, the enzymes activate the carboxyl group via adenylation using a nucleotide triphosphate (NTP).[143] NIS synthetases are classified into four types based on their substrate specificities: type A, A', B and C. Type A synthetases, exemplified by LucA, have a specificity for citric acid and mono-amine substrates.[142] These enzymes condense a prochiral carboxyl group of citric acid with the amine or hydroxyl group of the second substrate, resulting in an amide or ester bond.[143] Type A' synthetases are distinguished by their enantioselectivity and the chirality of the synthesized siderophore.[142] Type B synthetases are selective for alpha- ketoglutarate, condensing its C5 carboxyl group with the amine or hydroxyl group of the second substrate.[142,143] Type C NIS synthetases, such as LucC, are specific for monoamide and monoester substrates with citryl-or succinyl-intermediates.[142,143] The biosynthesis of siderophores is regulated by environmental iron levels; when organisms experience iron starvation, siderophores are produced and secreted to scavenge insoluble iron forms.[141] Siderophores play a crucial role by providing access to otherwise unavailable iron and can also act as virulence factors by extracting iron from host proteins.[141] *S. aureus* is known to produce four siderophores.[144]

Staphylobactin

Staphylobactin is a siderophore that increases the fitness of *S. aureus* in iron-deficient environments, such as abscesses.[145] In addition to promoting bacterial fitness, staphylobactin also functions as a virulence factor.[145,146]It was first reported to be produced in *S. aureus* RN6390 and Newman by Dale *et al.* in 2004.[145] Staphylobactin is synthesized when the bacteria are deprived of iron. Its biosynthesis is directed by an 11,5 kb BGC consisting of nine genes.[145] The cluster, *sbnABCDEFGHI*, is flanked by the *sirABC* genes, which encode proteins that show significant similarity to Fe^{3+}-siderophore transporters and are likely required for the import of iron(III)-staphylobactin into the cell.[146] Dale *et al.* also showed that *sbnE* was essential to produce staphylobactin.[145] To date, the structure of staphylobactin has not been elucidated and the *sbn* genes remain to be functionally characterized.

Staphyloferrin A and B

Staphyloferrin A is a polycarboxylate siderophore composed of two citric acid molecules linked to D-ornithine via an amide bond.[147] This siderophore is widely prevalent, having been identified in 33 different *Staphylococcus* species, and was first elucidated by Meiwes *et al*. in 1990.[147] Staphyloferrin A, classified as a nonribosomal synthetase independent siderophore, is produced by a gene cluster consisting of four genes, *sfnaABCD*. [144] The two NIS synthetases, SfnaB and SfnaD, are responsible for condensing the citric acids onto D-ornithine. The biosynthesis of staphyloferrin A proceeds in two steps: SnfaD first forms a δ-citryl-D-ornithine intermediate using the ∂-amine as a nucleophile, while SnfaB completes the synthesis by attaching the second citric acid molecule to the α-amine (**Figure 8A**).[144] Both reactions involve ATP-dependent activation of the carboxylate substrates through adenylation. The NIS cluster responsible for staphyloferrin A synthesis is regulated by the ferric uptake regulator (Fur) protein, an Fe^{2+}-dependent transcriptional repressor.[148] The gene cluster is flanked by the *htsABC* genes, which encode the ABC transporter needed for siderophore secretion.[148–150] Export of staphyloferrin A has been shown to enhance bacterial fitness within the host, particularly in subcutaneous abscesses where it aids in cellular proliferation.[151]

Staphyloferrin B was chemically characterized in 1993.[152] It is composed of 2,3-diaminopropionic acid, citrate, ethylenediamine and 2-ketoglutaric acid. [152] Initially isolated from *S. hyicus* DSM 20459 after growth under low iron conditions, it has since been identified in 16 different *Staphylococcus* species.[152] Staphyloferrin B is a α-hydroxycarboxylate siderophore of the complexone type and is

produced by a NIS gene cluster.[152,153] The *sbn* BGC responsible for synthesizing staphyloferrin B encodes three NIS synthetases: SbnC (type B), SbnE (type A), and SbnF (type A), which are necessary for the condensation of the substrates.[153] SbnE functions as an ATP-dependent citrate-desymmetrizing enzyme, adenylating one of carboxymethyl groups of citrate to facilitate a nucleophilic attack from 2,3-diaminopropionic acid (DAP), forming a citryl-DAP intermediate.[153,154] This intermediate is then decarboxylated by SbnH in a PLP-dependent manner. In the next step, SbnF adds another DAP molecule onto the free carboxyl group of the decarboxylated intermediate. Finally, SbnC attaches 2-ketoglutaric acid, yielding staphyloferrin B (**Figure 8B**).[153]

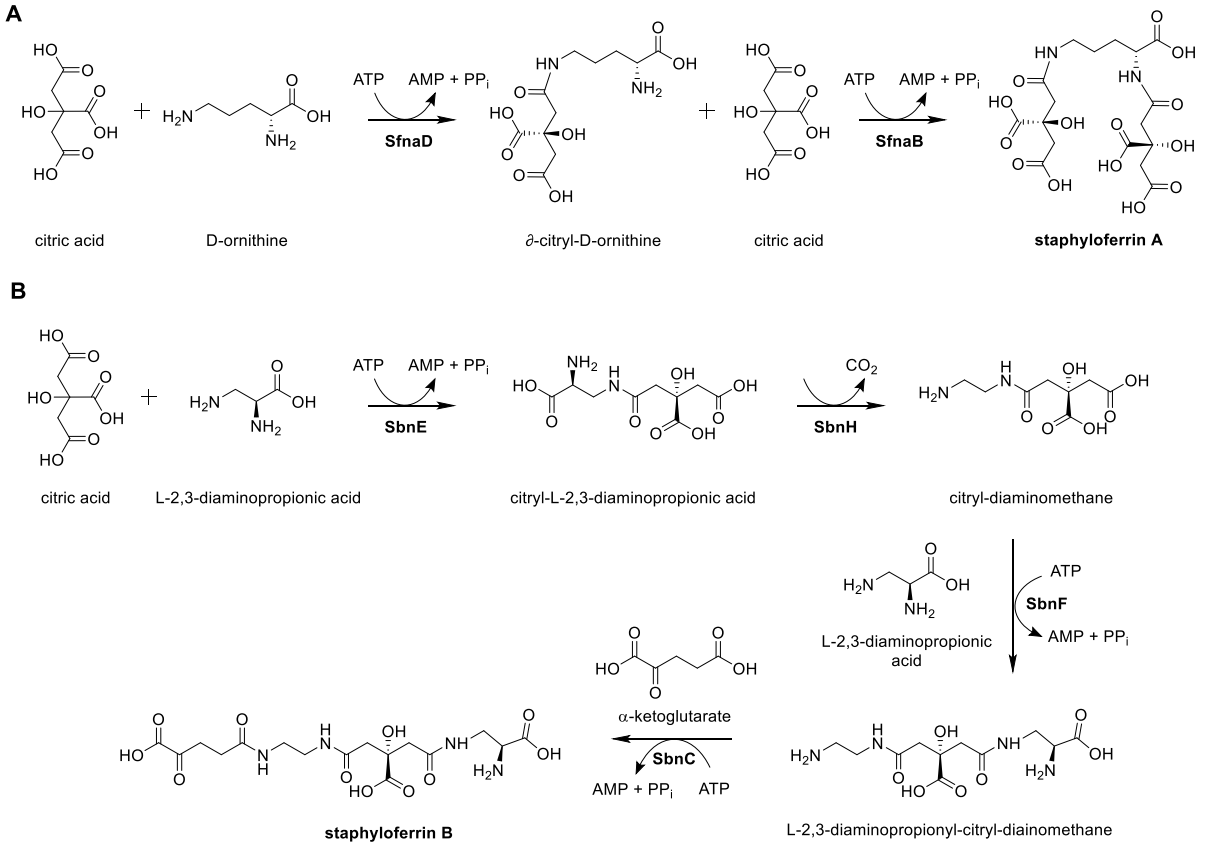


Figure 8. Biosynthesis of the NRPS-independent siderophores staphyloferrin A and B. **A**. Stepwise assembly of staphyloferrin A by SfnaD and SfnaB in an ATP-dependent matter, using D-ornithine and citric acid as substrates. **B**. Biosynthetic pathway to staphyloferrin B from citric acid, α-ketoglutarate and two molecules of L-2,3-diaminopropionic acid.

Aureochelin

Aureochelin is a siderophore produced by *S. aureus* with an as-yet-unknown biosynthetic pathway. The siderophore has a molecular mass of 577 Da, but its structure and the gene cluster responsible for its production have not yet been identified.[155]

Staphylopine

S. aureus also produces other metal-sequestering molecules, such as staphylopine, a zinc-capturing metallophore.[156] Staphylopine is the product of the *cnt* cluster, which consists of nine genes.[156,157] The *cntKLM* genes are responsible for the biosynthesis of staphylopine, while *cntABCDF* form an ATP-dependent transporter for the import of the metallophore. C*ntE* encodes an MFS transporter that allows staphylopine to exit the cell.[157,158] The biosynthetic pathway involves a histidine racemase (CntK), a nicotianamine synthase (CntL) and an opine dehydrogenase (CntM).[159,160] Staphylopine is formed in three steps, beginning with the conversion of L-His to D-His by CntK.[157,159] The D-His is then coupled with an α-butyric acid group derived from SAM in a reaction catalyzed by CntL,

producing an intermediate known as xNA. This intermediate is subsequently converted into staphylopine by CntM, which uses NADPH to reductively condense a pyruvate group.[157–159] The production of staphylopine plays a critical role in the survival of pathogenic *S. aureus* within the host, enabling the bacteria to withstand zinc starvation imposed by the host's immune system as a defense mechanism against pathogens.[156] By producing staphylopine, *S. aureus* increases its virulence, effectively evading the host's innate immune response.

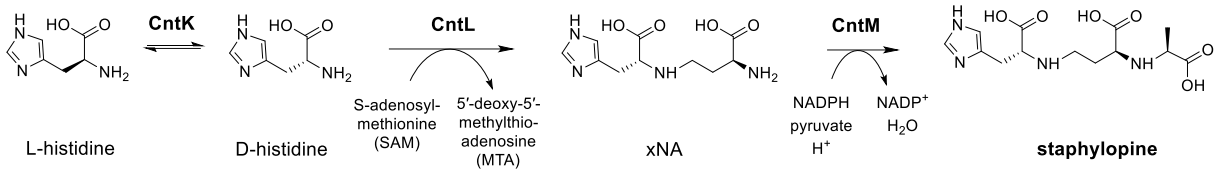


Figure 9. Biosynthesis of staphylopine, a zinc-chelating metallophore. D-histidine is formed from L-histidine by a histidine racemase and then fused to a SAM-derived alpha-butyric acid moiety to yield an intermediate product, termed xNA. This compound is then converted into staphylopine by a reductive condensation of a pyruvate group using NADPH.

TERPENES

Terpenes represent a diverse class of natural products, comprising over 70000 compounds that are categorized into more than 400 structural families.[161] Well-known terpenes include vitamins A and D, steroid hormones and the antimalarial drug artemisinin. Terpene biosynthesis starts with the assembly of five-carbon isoprene units into a linear polyene structure with branching methyl side chains.[161] This polyene serves as the carbon backbone of the terpene. Terpene cyclase enzymes play a key role by holding the linear polyene in a defined conformation, facilitating carbocation-driven cyclization and rearrangement events that shape the hydrocarbon skeleton of the terpene.[161] Subsequent modifications to this hydrocarbon skeleton give rise to the wide variety of terpenes observed in nature.

Staphyloxanthin

Staphylococcus aureus produces the terpene staphyloxanthin, an orange carotenoid responsible for the characteristic golden color of its colonies. [162] The carotenoid is embedded in the membrane of *S. aureus*, where it protects the cells against reactive oxygen species (ROS) generated by the human innate immune system.[163] It also enhances bacterial survival in neutrophils.[164] Staphyloxanthin biosynthesis is directed by an operon consisting of five genes: *crtOPQMN*.[162,165,166] The biosynthetic pathway starts with the condensation of two farnesyl diphosphate molecules to form dehydrosqualene, catalyzed by the dehydrosqualene synthase CrtM.[162,166] Dehydrosqualene then undergoes desaturation by CrtN yielding 4,4′-diaponeurosporene, a key intermediate in the pathway.[162] The next step involves oxidation of the terminal methyl group of 4,4'-diaponeurosporene by CrtP to form 4,4′-diaponeurosporenic acid. A glycosyltransferase, CrtQ catalyzes the esterification of glucose at the C1 position of 4,4'-diaponeurosporenic acid.[162] Finally, the acyltransferase CrtO attaches a 12-methyltetradecanoic acid to the C_6 position of glucose, yielding staphyloxanthin. The production of staphyloxanthin enables *S. aureus* to resist ROS produced by phagocytes, providing a competitive advantage over non-producing strains.[163,167] Inhibiting the biosynthesis of staphyloxanthin may therefore represent a promising strategy for anti-virulence therapy.[167]

OTHER PEPTIDE DERIVATIVES

Staccopins

Staccopin P1 and P2 are cysteine proteinase inhibitors produced by *S. tanabeensis*. These peptide derivatives are characterized by their low molecular weight (less than 1000 Da) and pentapeptide structure, featuring a free N-terminus and a C-terminal phenylalaninal (staccopin P1) or tyrosinal residue (staccopin P2). The amino acid sequence of staccopin P1 is H-Valyl-Valyl-Valyl-Valyl-Phenylalaninal, and that of staccopin P2 is H-Valyl-Valyl-Valyl-Valyl-Tyrosinal. Both peptides display strong and selective inhibitory activity against cysteine proteinases, such as calpain and papain. However, their precise mechanism of action, biosynthetic pathway and regulation are still not fully understood.[168]

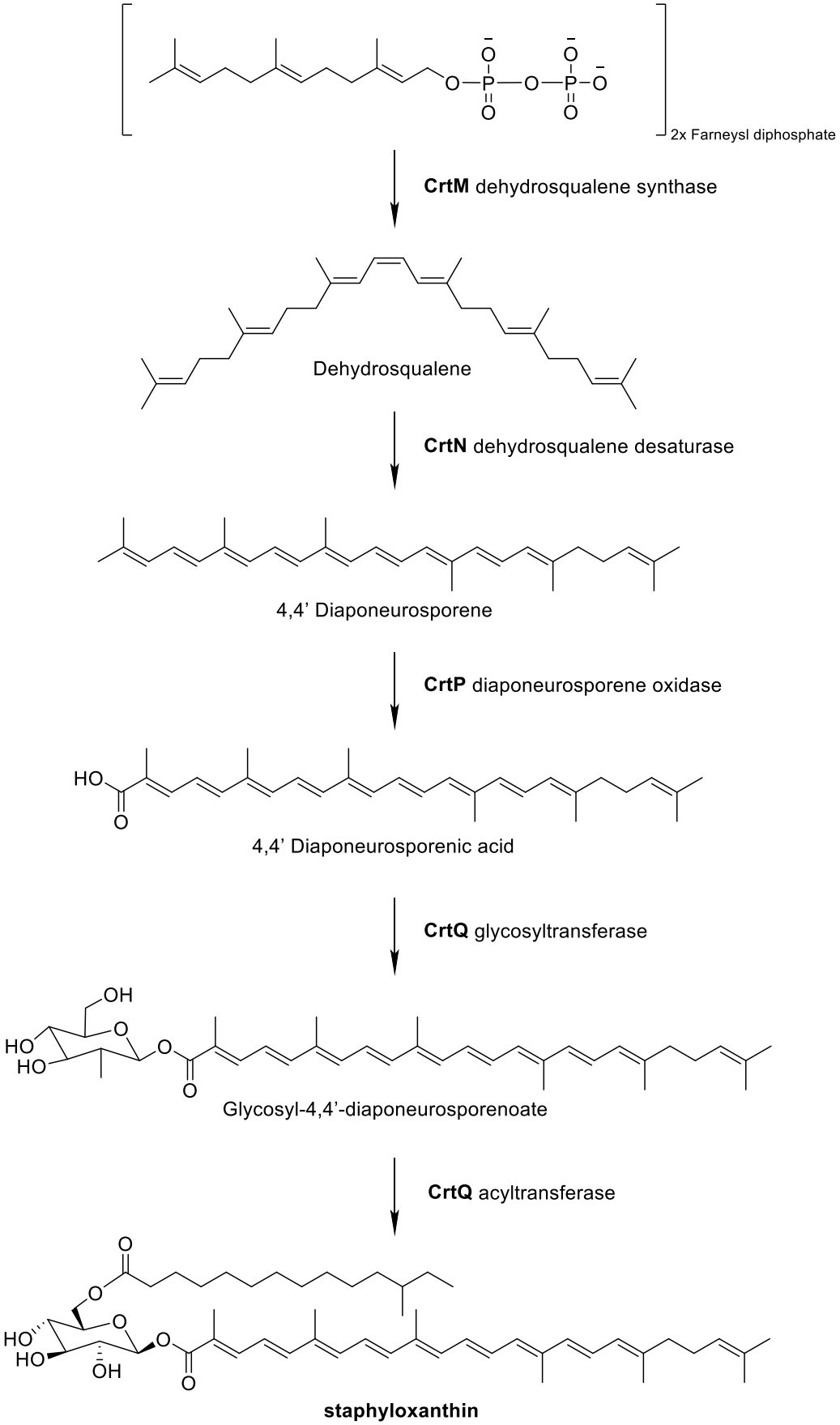
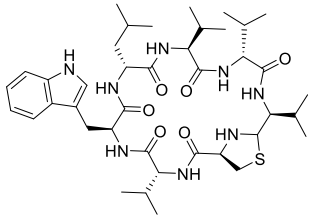
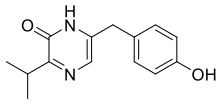
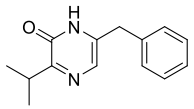
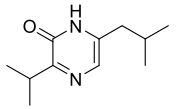
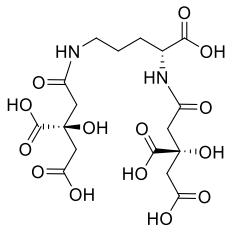
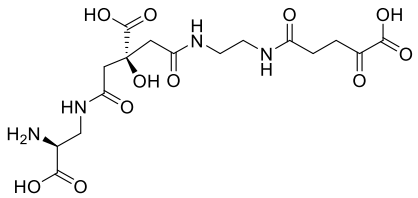
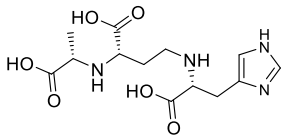
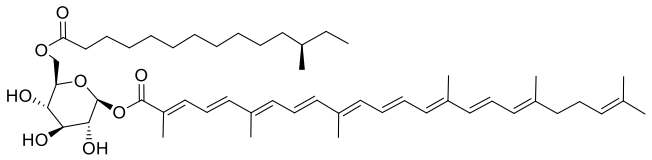


Figure 10. Biosynthesis of the terpene staphyloxanthin, an orange carotenoid that causes the characteristic yellow color of *S. aureus* colonies.

Table 2: Overview of nonribosomal peptides, siderophores and terpenes produced by *Staphylococcus* spp.

Type	Name	Producing strain	Bioactivity	BGC size (kb)	Mw (Da)	MiBIG ID	Reference
Nonribosomal peptides							
	Lugdunin 	*S. lugdunensis* N920143	Antimicrobial activity against *S. aureus, E. faecium, E. faecalis, L. monocytogenes, S. pneumoniae*	30.8	783	BGC0001393/ NPA024660	[130]
	Aureusimine A 	*S. aureus* subsp. *aureus* strain JKD6008, *S. aureus* Newman	Increased infectivity and virulence	7.9	245	BGC0000308/ NPA020444	[134]
	Aureusimine B 	*S. aureus* subsp. *aureus* strain JKD6008, *S. aureus* Newman	Increased infectivity and virulence	7.9	229	BGC0000308/ NPA020445	[134,135]
	Aureusimine C 	*S. aureus* subsp. *aureus* strain JKD6008, *S. aureus* Newman	Increased infectivity and virulence	7.9	229	BGC0000308/ NPA020445	[134,135]
Siderophores							
	Staphylobactin	*S. aureus* subsp. *aureus* NCTC 8325	Iron sequestration and virulence	16.7	822	BGC0000943	[145]

Staphyloferrin A 	*S. aureus* subsp. *aureus* NCTC 8326	Iron sequestration	6.1	479	BGC0000944/ NPA024679	[144], [147]
Staphyloferrin B 	*S. hyicus* DSM 20459	Iron sequestration	13	448	ND	[152], [153]
Aureochelin	*S. aureus*	ND	ND	577	ND	[155]
Staphylopine 	*S. aureus*	Nickel, cobalt, zinc, copper and iron chelation	9.2	328	ND	[157–159]
Terpenes						
Staphyloxanthin 	*S. aureus*	Antioxidant	5.7	819	ND	[162]
Saccharides						
Capsular polysaccharide	*S. aureus*	Increased infectivity and virulence	7.9	ND	BGC0000758	[169]

ND: not determined

THE MINING POTENTIAL OF STAPHYLOCOCCI: WHAT IS LEFT TO DISCOVER?

Thanks to advances in DNA sequencing, we now know that staphylococci harbour an immense, largely untapped potential to produce far more natural products than have been previously isolated.[170,171] Analysis of the AntiSMASH database (accessed on 9/10/2024) reveals that only 23% of the gene clusters in *Staphylococcus* genomes have been linked to their metabolic product. Over 90% of the BGCs that are predicted to direct the biosynthesis of bacteriocins, tRNA-dependent cyclodipeptide synthases, homoserine lactones, lanthidins, linear azol(in)e-containing peptides, sactipeptides, polyketides, terpenes and thiopeptides, and most hybrid BGCs show very low similarities (< 10 %) to known BGCs, with many showing no similarity at all. The Natural Products Atlas currently lists just 19 natural products produced by staphylococci. Overall, these observations highlight the large number of uncharacterized BGCs in staphylococci whose products remains to be identified and further investigated.

How to discover new BGCs or natural products in staphylococci?

While it is clear that staphylococci harbor many uncharacterized and undiscovered natural products, the challenge lies in producing, detecting, characterizing, and linking these compounds to cryptic BGCs. Depending on the starting point, there are various bioinformatic tools, laboratory protocols, and techniques available to support this process.[172] Most of the tools and protocols are developed for specific classes or subclasses of natural products. However, none have been specially developed for staphylococci. Therefore, the following tools and protocols are presented from a general perspective and may require adaptation for use with staphylococci.

Activating silent BGCs in staphylococci

Previous experiments and literature indicate that the vast majority of BGCs in staphylococci, like in many other organisms, are silent.[171,173–175] While unlocking these cryptic or silent gene clusters is an exciting prospect, determining the most effective technique to activate a specific BGC remains a significant challenge. Many comprehensive reviews have addressed this question in recent years, which are referenced here.[176] In this review, we will focus on techniques that we believe are particularly relevant or promising for use in staphylococci.

One of the most straightforward methods is the one strain-many compounds (OSMAC) approach.[177] This method involves altering easily accessible and adjustable cultivation parameters, such as media composition, aeration and temperature, to stress the bacteria and potentially activate silent BGCs. The OSMAC approach is laborious, but has been successfully applied to a wide range of BGCs across various microorganisms .[178,179] An interesting variation to this technique is the introduction of a scaffold during microbial growth. For example, introducing cotton into the growth medium has been used to activate BGCs in bacteria isolated from the surface of sponges[180]. Similarly, adding microparticles to the growth medium of *Aspergillus terreus* was shown to increase the production of lovastatin.[181] Given the notorious ability of staphylococci to grow on catheters and orthopaedic/breast implants[182] , it could interesting to investigate whether the addition of similar materials into cultures of staphylococci to see if this promotes the activation of specific BGCs.

Although the OSMAC approach allows to replicate or mimic environmental conditions and stress factors, activation of BGCs often requires specific signals from the host or from competing micro-organisms. As several staphylococcal species are human pathogens, it would be interesting to explore whether compounds associated with infection, such as hormones or cytokines, could induce the expression of silent BGCs. A recent study from Van Wezel and co-authors demonstrated that the human stress hormone epinephrine (adrenaline) elicits siderophore production in Actinobacteria.[183] The authors identified the catechol moiety as crucial for eliciting this response, making it a potential

compound that can be used to activate BGCs in other bacteria. Another example of a human protein influencing bacterial secondary metabolism comes from Zaborina et al (2007), who showed that the opioid peptide dynorphin can upregulate the virulence machinery in *P. aeruginosa*. When exposed to dynorphin, *P. aeruginosa* increased production of the secondary metabolite pyocyanin, which suppresses the growth of probiotic bacteria.[184]

Co-culture, or cultivating multiple microorganisms at the same time, has proven effective in activating BGCs on several occasions. The triggers required for activation can be molecules produced by one of member of such a microbial community, or direct physical contact between the microorganisms. Regarding using another micro-organism to activate silent BGC.[185,186] An interesting candidate for co-culture experiments aimed at activating the expression of BGCs in staphylococci is *Pseudomonas aeruginosa*. These two organisms are frequently co-isolated from burn wounds, chronic wounds and the sputum of cystic fibrosis patients.[187–189] Moreover, studies have shown that when *S. aureus* and *P. aeruginosa* are both present, they increase each other's virulence, often leading to poorer treatment outcomes. However, the interactions between these two pathogens remain poorly understood. A detailed review about this can be found here.[190]

One challenge of co-culture experiments is the inevitable difference in the growth rates of the bacterial cultures, which can influence the final outcome.[176] Therefore, high-throughput screening of potential, known elicitors could provide an alternative approach. This technique is called HiTES (high-throughput elicitor screening) and consists of two steps. First, a reporter gene is inserted into the BGC of interest. This can be achieved by inserting the silent promoter of the BGC together with the reporter at a neutral site in the genome, or by directly fusing the reporter gene downstream of the promoter in the silent BGC. In the second step, the mutant strain is screened against a library of potential BGC elicitors.[171,176,181] Alternatively, activation of BGC expression can be measured directly by visualizing the effect of each elicitor on the metabolite profile of the strain via imaging mass spectrometry.[191]

Rather than activating BGC expression via elicitors, reverse chemical-genetic methods aim to interfere with processes that are important for BGC expression. This can be done by modifying either the translational (ribosome engineering) or transcriptional machinery (RNA polymerase engineering) to increase natural product biosynthesis. Depending on the micro-organism, different antibiotics are used to select for mutated ribosomes or RNA polymerase. The readouts of these assays are similar to other techniques and are based on phenotypes, genetic reporters, bioactivity assays, or MS-based metabolomics.[176] So far, HiTES and reverse chemical-genetic methods have not been applied to staphylococci to activate silent BGCs. A drawback of this technique is that the gene clusters or at least the genome of the micro-organism of interest, must be known.

If the genome sequence of a micro-organism has been determined, genetic techniques can be used for activating silent gene clusters. One such approach is reporter-guided mutant selection, which combines two steps to activate a silent BGC. First, a genome-wide random mutant library is created using methods such as UV or transposon insertion. The second step involves analysing the mutants based on phenotypes, reporter genes or advanced metabolomics. This technique has been successfully applied to various micro-organisms and different types of BGCs. In some cases, adaptations were required to overcome natural resistance to certain selection markers.[171,176]

Genome engineering tools for staphylococci

The CRISPR-Cas genetic toolbox is increasingly used to engineer the expression of natural product BGCs. Common strategies include modification, overexpression, or deletion of regulatory genes, or replacement of the native promoter with a constitutive or inducible promoter. Specific CRISPR-Cas

systems have been developed for *S. aureus*.[192] These systems allow for rapid and efficient genome editing, such as gene deletions, insertions and single-base substitutions.[193–195] In recent years, systems have also been developed that enable multiplexing or large fragment gene editing.[196] Additionally, CRISPR interference (CRISPRi) tools have been optimized for *S. aureus*, expanding the possibilities for further unravelling the functions of cryptic BGCs.[197] However, a CRISPR activation (CRISPRa) system has yet to be developed for *S. aureus*. Several specific constitutive[198] or inducible[199–202] promoters have been identified for *S. aureus*, which can be used in conjunction with a variety of techniques to be integrated upstream of uncharacterized BGCs to activate them.

The native restriction-modification systems in *S. aureus* can hinder genetic manipulation using recombinant DNA from *E. coli*, which has historically complicated molecular analysis. However, plasmids such as the pLOW and pGL485 combination now allow for regulated gene expression in *S. aureus* using isopropyl β-d-1-thiogalactopyranose (IPTG)[201]. In addition, various other plasmids have been developed to facilitate genetic work in this organism[203]. For gene deletions via homologous recombination, suicide plasmids with a temperature-sensitive origin of replication are commonly used, along with other selectable markers, such as the TargeTron system[204], beta-galactosidase[205], secY antisense RNA,[206] phenylalanine tRNA synthetase (pheS*).[207]

For many Gram-positive genera, such as *Bacillus*[208–210] and *Clostridium*[211,212], molecular toolboxes have been developed to standardize and annotate genetic parts, facilitating easier and more efficient assembly of synthetic constructs. Similarly, for *S. aureus*, Rondthaler *et al*. have developed a standardized toolbox that includes several promoters and terminators that can easily be integrated into multipart DNA constructs using Type IIS restriction enzymes, streamlining genetic manipulation and construct assembly.[213]

CONCLUSION

Staphylococci are a remarkably versatile and diverse genus of bacteria that have developed an extensive repertoire of specialized metabolites with potent bioactivities. These metabolites, ranging from antibiotics and immunomodulators to siderophores and signalling molecules, underscore the ecological and biotechnological significance of *Staphylococcus* species. While advances in genome sequencing, bioinformatics, and molecular biology have begun to unlock their hidden biosynthetic potential, many metabolites remain uncharacterized. Harnessing this untapped potential requires an interdisciplinary approach, integrating bioinformatics, synthetic biology, analytical chemistry and microbial ecology. Such efforts not only hold promise for developing innovative strategies to combat multidrug-resistant pathogens but will also deepen our understanding of staphylococcal secondary metabolism and its critical role in microbial communities and host interactions.

References

[1] M. do C. de Freire Bastos, F. Miceli de Farias, P. Carlin Fagundes, M. L. Varella Coelho, *Appl. Microbiol. Biotechnol.* **2020**, *104*, 10339–10368.

[2] M. Kosecka-Strojek, A. Buda, J. Międzobrodzki, *Pet--Man Travel. Staphylococci World Prog.* **2018**, 11–24.

[3] Y. Lampert, D. Kelman, Y. Nitzan, Z. Dubinsky, A. Behar, R. T. Hill, *FEMS Microbiol. Ecol.* **2008**, *64*, 187–198.

[4] D. A. Stavropoulou, L. De Vuyst, F. Leroy, *J. Appl. Microbiol.* **2018**, DOI 10.1111/jam.14054.

[5] K. Becker, R. L. Skov, C. von Eiff, in *Man. Clin. Microbiol.*, John Wiley & Sons, Ltd, **2015**, pp. 354–382.

[6] O. Goldmann, E. Medina, *Int. J. Med. Microbiol. IJMM* **2018**, *308*, 625–630.

[7] S. Y. C. Tong, J. S. Davis, E. Eichenberger, T. L. Holland, V. G. Fowler, *Clin. Microbiol. Rev.* **2015**, *28*, 603–661.

[8] W. Spink, W. Hall, *Ann. Intern. Med.* **1945**, *22*, 510.

[9] Y. Fang, Y. Song, C. Li, G. Yang, R. Zheng, Y. Shi, X. Xia, *Jundishapur J. Microbiol.* **2019**, *12*, DOI 10.5812/jjm.95859.

[10] F. Micoli, F. Bagnoli, R. Rappuoli, D. Serruto, *Nat. Rev. Microbiol.* **2021**, *19*, 287–302.

[11] A. Oladipo, V. Enwemiwe, O. Ejeromedoghene, A. Adebayo, O. Ogunyemi, F. Fu, *Metabolites* **2022**, *12*, 534.

[12] S. A. Kautsar, K. Blin, S. Shaw, J. C. Navarro-Muñoz, B. R. Terlouw, J. J. J. Van Der Hooft, J. A. Van Santen, V. Tracanna, H. G. Suarez Duran, V. Pascal Andreu, N. Selem-Mojica, M. Alanjary, S. L. Robinson, G. Lund, S. C. Epstein, A. C. Sisto, L. K. Charkoudian, J. Collemare, R. G. Linington, T. Weber, M. H. Medema, *Nucleic Acids Res.* **2020**, *48*, D454–D458.

[13] K. Blin, S. Shaw, A. M. Kloosterman, Z. Charlop-Powers, G. P. Van Wezel, M. H. Medema, T. Weber, *Nucleic Acids Res.* **2021**, *49*, W29–W35.

[14] M. Montalbán-López, T. A. Scott, S. Ramesh, I. R. Rahman, A. J. van Heel, J. H. Viel, V. Bandarian, E. Dittmann, O. Genilloud, Y. Goto, M. J. Grande Burgos, C. Hill, S. Kim, J. Koehnke, J. A. Latham, A. J. Link, B. Martínez, S. K. Nair, Y. Nicolet, S. Rebuffat, H.-G. Sahl, D. Sareen, E. W. Schmidt, L. Schmitt, K. Severinov, R. D. Süssmuth, A. W. Truman, H. Wang, J.-K. Weng, G. P. van Wezel, Q. Zhang, J. Zhong, J. Piel, D. A. Mitchell, O. P. Kuipers, W. A. van der Donk, *Nat. Prod. Rep.* **2020**, DOI 10.1039/d0np00027b.

[15] M. A. Ortega, W. A. Van Der Donk, *Cell Chem. Biol.* **2016**, *23*, 31–44.

[16] E. L. Ongey, P. Neubauer, *Microb. Cell Factories 2016 151* **2016**, *15*, 1–16.

[17] Q. Zhang, Y. Yu, J. E. Vélasquez, W. A. van der Donk, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 18361.

[18] Y. Goto, B. Li, J. Claesen, Y. Shi, M. J. Bibb, W. A. van der Donk, *PLOS Biol.* **2010**, *8*, e1000339.

[19] W. M. Müller, T. Schmiederer, P. Ensle, R. D. Süssmuth, *Angew. Chem. Int. Ed.* **2010**, *49*, 2436–2440.

[20] H. Liang, I. J. Lopez, M. Sánchez-Hidalgo, O. Genilloud, W. A. van der Donk, *ACS Chem. Biol.* **2022**, *17*, 2519–2527.

[21] Z.-F. Pei, L. Zhu, R. Sarksian, W. A. van der Donk, S. K. Nair, *J. Am. Chem. Soc.* **2022**, *144*, 17549–17557.

[22] M. Xu, F. Zhang, Z. Cheng, G. Bashiri, J. Wang, J. Hong, Y. Wang, L. Xu, X. Chen, S.-X. Huang, S. Lin, Z. Deng, M. Tao, *Angew. Chem. Int. Ed Engl.* **2020**, *59*, 18029–18035.

[23] F. J. Ortiz-López, D. Carretero-Molina, M. Sánchez-Hidalgo, J. Martín, I. González, F. Román-Hurtado, M. de la Cruz, S. García-Fernández, F. Reyes, J. P. Deisinger, A. Müller, T. Schneider, O. Genilloud, *Angew. Chem. Int. Ed.* **2020**, *59*, 12654–12658.
[24] A. M. Kloosterman, P. Cimermancic, S. S. Elsayed, C. Du, M. Hadjithomas, M. S. Donia, M. A. Fischbach, G. P. van Wezel, M. H. Medema, *PLoS Biol.* **2020**, *18*, e3001026.
[25] L. M. Repka, J. R. Chekan, S. K. Nair, W. A. van der Donk, *Chem. Rev.* **2017**, *117*, 5457–5520.
[26] J. Lubelski, R. Rink, R. Khusainov, G. N. Moll, O. P. Kuipers, *Cell. Mol. Life Sci. CMLS* **2008**, *65*, 455–476.
[27] N. SCHNELL, G. ENGELKE, J. AUGUSTIN, R. ROSENSTEIN, V. UNGERMANN, F. GÖTZ, K.-D. ENTIAN, *Eur. J. Biochem.* **1992**, *204*, 57–68.
[28] G. Valsesia, G. Medaglia, M. Held, W. Minas, S. Panke, *Appl. Environ. Microbiol.* **2007**, *73*, 1635–1645.
[29] F. Götz, S. Perconti, P. Popella, R. Werner, M. Schlag, *Int. J. Med. Microbiol. IJMM* **2014**, *304*, 63–71.
[30] T. Kupke, C. Kempter, G. Jung, F. Götz, *J. Biol. Chem.* **1995**, *270*, 11282–11289.
[31] M. Blaesse, T. Kupke, R. Huber, S. Steinbacher, *EMBO J.* **2000**, *19*, 6299–6310.
[32] T. Kupke, F. Götz, *J. Biol. Chem.* **1997**, *272*, 4759–4762.
[33] M. Bastos, H. Ceotto, M. Coelho, J. Nascimento, *Curr. Pharm. Biotechnol.* **2009**, *10*, 38–61.
[34] G. Bierbaum, *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **1996**, *69*, 119–127.
[35] S. Kies, C. Vuong, M. Hille, A. Peschel, C. Meyer, F. Götz, M. Otto, *Peptides* **2003**, *24*, 329–338.
[36] S. Freund, G. Jung, O. Gutbrod, G. Folkers, W. A. Gibbons, H. Allgaier, R. Werner, *Biopolymers* **1991**, *31*, 803–811.
[37] H. Brötz, M. Josten, I. Wiedemann, U. Schneider, F. Götz, G. Bierbaum, H. G. Sahl, *Mol. Microbiol.* **1998**, *30*, 317–327.
[38] A. Müller, H. Ulm, K. Reder-Christ, H.-G. Sahl, T. Schneider, *Microb. Drug Resist.* **2012**, *18*, 261–270.
[39] R. Kellner, G. Jung, T. Hörner, H. Zähner, N. Schnell, K. D. Entian, F. Götz, *Eur. J. Biochem.* **1988**, *177*, 53–59.
[40] R. Schmucker, G. Sauermann, U. Eigener, W. Engel, *Deodorizing Lantibiotic Cosmetic Agents*, **1994**, US5318778A.
[41] J. Saising, L. Dube, A.-K. Ziebandt, S. P. Voravuthikunchai, M. Nega, F. Götz, *Antimicrob. Agents Chemother.* **2012**, *56*, 5804–5810.
[42] M. Reis, M. Eschbach-Bludau, M. I. Iglesias-Wind, T. Kupke, H. G. Sahl, *Appl. Environ. Microbiol.* **1994**, *60*, 2876–2883.
[43] H. P. Weil, A. G. Beck-Sickinger, J. Metzger, S. Stevanovic, G. Jung, M. Josten, H. G. Sahl, *Eur. J. Biochem.* **1990**, *194*, 217–223.
[44] C. Meyer, G. Bierbaum, C. Heidrich, M. Reis, J. Süling, M. I. Iglesias-Wind, C. Kempter, E. Molitor, H.-G. -G Sahl, *Eur. J. Biochem.* **1995**, *232*, 478–489.
[45] G. Bierbaum, C. Szekat, M. Josten, C. Heidrich, C. Kempter, G. Jung, H. G. Sahl, *Appl. Environ. Microbiol.* **1996**, *62*, 385–392.
[46] T. Abee, *FEMS Microbiol. Lett.* **1995**, *129*, 1–10.
[47] A. Hoffmann, T. Schneider, U. Pag, H.-G. Sahl, *Appl. Environ. Microbiol.* **2004**, *70*, 3263.

[48] U. Pag, C. Heidrich, G. Bierbaum, H.-G. Sahl, *Appl. Environ. Microbiol.* **1999**, *65*, 591.
[49] C. Heidrich, U. Pag, M. Josten, J. Metzger, R. W. Jack, G. Bierbaum, G. Jung, H. G. Sahl, *Appl. Environ. Microbiol.* **1998**, *64*, 3140–3146.
[50] M. L. Varella Coelho, J. D. Santos Nascimento, P. C. Fagundes, D. J. Madureira, S. S. D. Oliveira, M. A. Vasconcelos De Paiva Brito, M. D. C. D. Freire Bastos, *Res. Microbiol.* **2007**, *158*, 625–630.
[51] M. Aftab Uddin, S. Akter, M. Ferdous, B. Haidar, A. Amin, A. H. M. Shofiul Islam Molla, H. Khan, M. R. Islam, *Sci. Rep.* **2021**, *11*, 1–12.
[52] M. V. D. Kamp, H. W. V. D. Hooven, R. N. H. Konings, G. Bierbaum, H.-G. -G Sahl, O. P. Kuipers, R. J. Siezen, W. M. D. Vos, C. W. Hilbers, F. J. M. V. D. Ven, *Eur. J. Biochem.* **1995**, *230*, 587–600.
[53] M. B. Ekkelenkamp, M. Hanssen, S.-T. Danny Hsu, A. de Jong, D. Milatovic, J. Verhoef, N. A. J. van Nuland, *FEBS Lett.* **2005**, *579*, 1917–1922.
[54] J. E. Velásquez, X. Zhang, W. A. V. D. Donk, *Chem. Biol.* **2011**, *18*, 857–867.
[55] J. Petersen, A. Boysen, L. Fogh, K. Tabermann, T. Kofoed, A. King, P. Schrotz-King, M. C. Hansen, *Biol. Chem.* **2009**, *390*, 437–444.
[56] T. Sashihara, H. Kimura, T. Higuchi, A. Adachi, H. Matsusaki, K. Sonomoto, A. Ishizaki, *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2420–2428.
[57] Y. Aso, T. Sashihara, J. I. Nagao, Y. Kanemasa, H. Koga, T. Hashimoto, T. Higuchi, A. Adachi, H. Nomiyama, A. Ishizaki, J. Nakayama, K. Sonomoto, *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1663–1671.
[58] J. Nagao, Y. Harada, K. Shioya, Y. Aso, T. Zendo, J. Nakayama, K. Sonomoto, *Biochem. Biophys. Res. Commun.* **2005**, *336*, 507–513.
[59] J. Nagao, Y. Aso, T. Sashihara, K. Shioya, A. Adachi, J. Nakayama, K. Sonomoto, *Biosci. Biotechnol. Biochem.* **2005**, *69*, 1341–1347.
[60] K. I. Okuda, Y. Aso, J. Nakayama, K. Sonomoto, *J. Bacteriol.* **2008**, *190*, 356.
[61] Y. Aso, J.-I. Nagao, H. Koga, K.-I. Okuda, Y. Kanemasa, T. Sashihara, J. Nakayama, K. Sonomoto, *J. Biosci. Bioeng.* **2004**, *98*, 429–436.
[62] U. Roy, M. R. Islam, J. I. Nagao, H. Iida, A. A. Mahin, M. Li, T. Zendo, J. Nakayama, K. Sonomoto, *Biosci. Biotechnol. Biochem.* **2014**, *78*, 1270–1273.
[63] S. M. Asaduzzaman, J. I. Nagao, H. Iida, T. Zendo, J. Nakayama, K. Sonomoto, *Antimicrob. Agents Chemother.* **2009**, *53*, 3595.
[64] S. M. Asaduzzaman, J. I. Nagao, Y. Aso, J. Nakayama, K. Sonomoto, *Appl. Environ. Microbiol.* **2006**, *72*, 6012–6017.
[65] D. Fujinami, A.-A. -Mahin, K. M. Elsayed, M. R. Islam, J. Nagao, U. Roy, S. Momin, T. Zendo, D. Kohda, K. Sonomoto, *Commun. Biol.* **2018**, *1*, 1–9.
[66] H. Ceotto, H. Holo, K. F. S. da Costa, J. dos S. Nascimento, Z. Salehian, I. F. Nes, M. do C. de F. Bastos, *Vet. Microbiol.* **2010**, *146*, 124–131.
[67] G. Braem, B. Stijlemans, W. Van Haken, S. De Vliegher, L. De Vuyst, F. Leroy, *J. Appl. Microbiol.* **2014**, *116*, 1084–1093.
[68] P. Wilaipun, T. Zendo, K. I. Okuda, J. Nakayama, K. Sonomoto, *Biosci. Biotechnol. Biochem.* **2008**, *72*, 2232–2235.
[69] D. Janek, A. Zipperer, A. Kulik, B. Krismer, A. Peschel, *PLoS Pathog.* **2016**, *12*, e1005812.
[70] K. Nakazono, M. N.-T. Le, M. Kawada-Matsuo, N. Kimheang, J. Hisatsune, Y. Oogai, M. Nakata, N. Nakamura, M. Sugai, H. Komatsuzawa, *PloS One* **2022**, *17*, e0258283.
[71] J. C. Scott, H. G. Sahl, A. Carne, J. R. Tagg, *FEMS Microbiol. Lett.* **1992**, *72*, 97–102.

[72] K. M. Daly, M. Upton, S. K. Sandiford, L. A. Draper, P. A. Wescombe, R. W. Jack, P. M. O'Connor, A. Rossney, F. Götz, C. Hill, P. D. Cotter, R. P. Ross, J. R. Tagg, *J. Bacteriol.* **2009**, *192*, 1131.
[73] H.-S. Joo, G. Y. C. Cheung, M. Otto, *J. Biol. Chem.* **2011**, *286*, 8933–8940.
[74] P. Carlin Fagundes, I. Nascimento de Sousa Santos, M. Silva Mrancisco, R. Mattos Albano, M. do C. de Freire Bastos, *Microbiol. Res.* **2017**, *198*, 36–46.
[75] P. C. Fagundes, H. Ceotto, A. Potter, M. A. Vasconcelos de Paiva Brito, D. Brede, I. F. Nes, M. do C. de F. Bastos, *Res. Microbiol.* **2011**, *162*, 1052–1059.
[76] B. Wladyka, K. Wielebska, M. Wloka, O. Bochenska, G. Dubin, A. Dubin, P. Mak, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7229–7239.
[77] J. N. O'Sullivan, P. M. O'Connor, M. C. Rea, O. O'Sullivan, C. J. Walsh, B. Healy, H. Mathur, D. Field, C. Hill, R. Paul Ross, *J. Bacteriol.* **2020**, *202*, 1–15.
[78] P. I. Kim, J. K. Sohng, C. Sung, H. S. Joo, E. M. Kim, T. Yamaguchi, D. Park, B. G. Kim, *Biochem. Biophys. Res. Commun.* **2010**, *399*, 133–138.
[79] M. A. D. B. Navaratna, H. G. Sahl, J. R. Tagg, *Infect. Immun.* **1999**, *67*, 4268–4271.
[80] M. A. D. B. Navaratna, H.-G. Sahl, J. R. Tagg, *Appl. Environ. Microbiol.* **1998**, *64*, 4803.
[81] R. Wolden, K. V. Ovchinnikov, H. J. Venter, T. F. Oftedal, D. B. Diep, J. P. Cavanagh, *Microbiol. Spectr.* **2023**, *11*, e00869.
[82] Y. Chen, J. Wang, G. Li, Y. Yang, W. Ding, *Front. Chem.* **2021**, *9*, 595991.
[83] P. G. Arnison, M. J. Bibb, G. Bierbaum, A. A. Bowers, T. S. Bugni, G. Bulaj, J. A. Camarero, D. J. Campopiano, G. L. Challis, J. Clardy, P. D. Cotter, D. J. Craik, M. Dawson, E. Dittmann, S. Donadio, P. C. Dorrestein, K. D. Entian, M. A. Fischbach, J. S. Garavelli, U. Göransson, C. W. Gruber, D. H. Haft, T. K. Hemscheidt, C. Hertweck, C. Hill, A. R. Horswill, M. Jaspars, W. L. Kelly, J. P. Klinman, O. P. Kuipers, A. J. Link, W. Liu, M. A. Marahiel, D. A. Mitchell, G. N. Moll, B. S. Moore, R. Müller, S. K. Nair, I. F. Nes, G. E. Norris, B. M. Olivera, H. Onaka, M. L. Patchett, J. Piel, M. J. T. Reaney, S. Rebuffat, R. P. Ross, H. G. Sahl, E. W. Schmidt, M. E. Selsted, K. Severinov, B. Shen, K. Sivonen, L. Smith, T. Stein, R. D. Süssmuth, J. R. Tagg, G. L. Tang, A. W. Truman, J. C. Vederas, C. T. Walsh, J. D. Walton, S. C. Wenzel, J. M. Willey, W. A. V. D. Donk, *Nat. Prod. Rep.* **2013**, *30*, 108–160.
[84] M. Montalbán-López, T. A. Scott, S. Ramesh, I. R. Rahman, A. J. van Heel, J. H. Viel, V. Bandarian, E. Dittmann, O. Genilloud, Y. Goto, M. J. G. Burgos, C. Hill, S. Kim, J. Koehnke, J. A. Latham, A. J. Link, B. Martínez, S. K. Nair, Y. Nicolet, S. Rebuffat, H.-G. Sahl, D. Sareen, E. W. Schmidt, L. Schmitt, K. Severinov, R. D. Süssmuth, A. W. Truman, H. Wang, J.-K. Weng, G. P. van Wezel, Q. Zhang, J. Zhong, J. Piel, D. A. Mitchell, O. P. Kuipers, W. A. van der Donk, *Nat. Prod. Rep.* **2020**, DOI 10.1039/d0np00027b.
[85] Y. Li, S. Rebuffat, *J. Biol. Chem.* **2020**, *295*, 34–54.
[86] T. L. Grove, P. M. Himes, S. Hwang, H. Yumerefendi, J. B. Bonanno, B. Kuhlman, S. C. Almo, A. A. Bowers, *J. Am. Chem. Soc.* **2017**, *139*, 11734–11744.
[87] M. A. Funk, W. A. Van Der Donk, *Acc. Chem. Res.* **2017**, *50*, 1577–1586.
[88] A. F. de S. Duarte, H. Ceotto-Vigoder, E. S. Barrias, T. C. B. S. Souto-Padrón, I. F. Nes, M. do C. de F. Bastos, *Int. J. Antimicrob. Agents* **2018**, *51*, 349–356.
[89] A. F. de S. Duarte, H. Ceotto, M. L. V. Coelho, M. A. V. de P. Brito, M. do C. de F. Bastos, *Food Control* **2013**, *32*, 313–321.
[90] D. C. K. Chan, L. L. Burrows, *J. Antibiot. (Tokyo)* **2021**, *74*, 161–175.
[91] J. O. Melby, N. J. Nard, D. A. Mitchell, *Curr. Opin. Chem. Biol.* **2011**, *15*, 369–378.
[92] A. A. Vinogradov, H. Suga, *Cell Chem. Biol.* **2020**, *27*, 1032–1051.

[93] S.-H. Dong, A. Liu, N. Mahanta, D. A. Mitchell, S. K. Nair, *ACS Cent. Sci.* **2019**, *5*, 842–851.
[94] B. J. Burkhart, C. J. Schwalen, G. Mann, J. H. Naismith, D. A. Mitchell, *Chem. Rev.* **2017**, *117*, 5389–5456.
[95] M. Moutiez, P. Belin, M. Gondry, *Chem. Rev.* **2017**, *117*, 5578–5618.
[96] M. C. Carnio, T. Stachelhaus, K. P. Francis, S. Scherer, *Eur. J. Biochem.* **2001**, *268*, 6390–6401.
[97] P. R. Bennallack, S. R. Burt, M. J. Heder, R. A. Robison, J. S. Griffitts, *J. Bacteriol.* **2014**, *196*, 4344–4350.
[98] P. R. Bennallack, K. D. Bewley, M. A. Burlingame, R. A. Robison, S. M. Miller, J. S. Griffitts, *J. Bacteriol.* **2016**, *198*, 2431–2438.
[99] D. G. Calvopina-Chavez, D. M. Bursey, Y.-J. Tseng, L. M. Patil, K. D. Bewley, P. R. Bennallack, J. M. McPhie, K. B. Wagstaff, A. Daley, S. M. Miller, J. D. Moody, J. C. Price, J. S. Griffitts, *Appl. Environ. Microbiol.* **2024**, *90*, e00244-24.
[100] M. A. Ciufolini, D. Lefranc, *Nat. Prod. Rep.* **2010**, *27*, 330.
[101] D. Lefranc, M. A. Ciufolini, *Angew. Chem. Int. Ed.* **2009**, *48*, 4198–4201.
[102] Q. Zhang, W. Liu, *Nat. Prod. Rep.* **2013**, *30*, 218–226.
[103] Y. Liu, Y. Liu, Z. Du, L. Zhang, J. Chen, Z. Shen, Q. Liu, J. Qin, H. Lv, H. Wang, L. He, J. Liu, Q. Huang, Y. Sun, M. Otto, M. Li, *Microbiome* **2020**, *8*, 85.
[104] G. Degiacomi, Y. Personne, G. Mondésert, X. Ge, C. S. Mandava, R. C. Hartkoorn, F. Boldrin, P. Goel, K. Peisker, A. Benjak, M. B. Barrio, M. Ventura, A. C. Brown, V. Leblanc, A. Bauer, S. Sanyal, S. T. Cole, S. Lagrange, T. Parish, R. Manganelli, *Tuberculosis* **2016**, *100*, 95–101.
[105] Y. Liu, Q. Liu, L. Zhao, S. W. Dickey, H. Wang, R. Xu, T. Chen, Y. Jian, X. Wang, H. Lv, M. Otto, M. Li, *J. Extracell. Vesicles* **2022**, *11*, e12212.
[106] S. Soltani, R. Hammami, P. D. Cotter, S. Rebuffat, L. B. Said, H. Gaudreau, F. Bédard, E. Biron, D. Drider, I. Fliss, *FEMS Microbiol. Rev.* **2020**, *45*, fuaa039.
[107] A. Simons, K. Alhanout, R. E. Duval, *Microorganisms* **2020**, *8*, 639.
[108] D. J. A. Netz, R. Pohl, A. G. Beck-Sickinger, T. Selmer, A. J. Pierik, M. D. C. D. F. Bastos, H.-G. Sahl, *J. Mol. Biol.* **2002**, *319*, 745–756.
[109] J. dos S. Nascimento, M. L. V. Coelho, H. Ceotto, A. Potter, L. R. Fleming, Z. Salehian, I. F. Nes, M. do C. de F. Bastos, *J. Bacteriol.* **2012**, *194*, 875–883.
[110] D. J. A. Netz, M. D. C. D. F. Bastos, H.-G. Sahl, *Appl. Environ. Microbiol.* **2002**, *68*, 5274–5280.
[111] D. J. A. Netz, H. G. Sahl, R. Marcolino, J. D. S. Nascimento, S. S. D. Oliveira, M. B. Soares, M. D. C. D. F. Bastos, *J. Mol. Biol.* **2001**, *311*, 939–949.
[112] M. L. V. Coelho, L. R. Fleming, M. D. C. D. F. Bastos, *Res. Microbiol.* **2016**, *167*, 90–102.
[113] M. L. V. Coelho, B. G. Coutinho, O. Cabral da Silva Santos, I. F. Nes, M. do C. de F. Bastos, *Res. Microbiol.* **2014**, *165*, 50–59.
[114] S. L. Salustiano Marques-Bastos, M. L. Varella Coelho, H. Ceotto-Vigoder, P. Carlin Fagundes, G. Silva Almeida, D. A. Brede, I. F. Nes, M. A. Vasconcelos De Paiva Brito, M. D. C. De Freire Bastos, *Braz. J. Microbiol.* **2020**, *51*, 1527–1538.
[115] M. do C. de F. Bastos, B. G. Coutinho, M. L. V. Coelho, *Pharmaceuticals* **2010**, *3*, 1139.
[116] Y. Yue, K. Chen, C. Sun, S. Ahmed, S. C. Ojha, *BMC Microbiol.* **2023**, *23*, 311.
[117] R. D. Süssmuth, A. Mainz, *Angew. Chem. - Int. Ed.* **2017**, *56*, 3770–3821.
[118] K. J. Weissman, *Nat. Chem. Biol.* **2015**, *11*, 660–670.
[119] S. L. Wenski, S. Thiengmag, E. J. N. Helfrich, *Synth. Syst. Biotechnol.* **2022**, *7*, 631–647.

[120] S. A. Sieber, M. A. Marahiel, *Chem. Rev.* **2005**, *105*, 715–738.
[121] M. Strieker, A. Tanović, M. A. Marahiel, *Curr. Opin. Struct. Biol.* **2010**, *20*, 234–240.
[122] L. Du, L. Lou, *Nat. Prod. Rep.* **2010**, *27*, 255–278.
[123] A. Zipperer, M. C. Konnerth, C. Laux, A. Berscheid, D. Janek, C. Weidenmaier, M. Burian, N. A. Schilling, C. Slavetinsky, M. Marschal, M. Willmann, H. Kalbacher, B. Schittek, H. Brötz-Oesterhelt, S. Grond, A. Peschel, B. Krismer, *Nature* **2016**, *535*, 511–516.
[124] K. Bitschar, B. Sauer, J. Focken, H. Dehmer, S. Moos, M. Konnerth, N. A. Schilling, S. Grond, H. Kalbacher, F. C. Kurschus, F. Götz, B. Krismer, A. Peschel, B. Schittek, *Nat. Commun.* **2019**, *10*, DOI 10.1038/s41467-019-10646-7.
[125] T. Chaaban, Y. Mohsen, Z. Ezzeddine, G. Ghssein, *Biology* **2023**, *12*, 598.
[126] G. Wei, Y. He, *Int. J. Mol. Sci.* **2022**, *23*, 8029.
[127] S.-C. Chang, C.-Y. Kao, L.-C. Lin, J. H. Hidrosollo, J.-J. Lu, *Microbiol. Spectr.* **2023**, *11*, e01298-23.
[128] N. A. Schilling, A. Berscheid, J. Schumacher, J. S. Saur, M. C. Konnerth, S. N. Wirtz, J. M. Beltrán-Beleña, A. Zipperer, B. Krismer, A. Peschel, H. Kalbacher, H. Brötz-Oesterhelt, C. Steinem, S. Grond, *Angew. Chem. Int. Ed.* **2019**, *58*, 9234–9238.
[129] D. Ruppelt, M. F. W. Trollmann, T. Dema, S. N. Wirtz, H. Flegel, S. Mönnikes, S. Grond, R. A. Böckmann, C. Steinem, *Nat. Commun.* **2024**, *15*, 3521.
[130] S. Krauss, A. Zipperer, S. Wirtz, J. Saur, M. C. Konnerth, S. Heilbronner, B. O. Torres Salazar, S. Grond, B. Krismer, A. Peschel, *Antimicrob. Agents Chemother.* **2021**, *65*, 1–10.
[131] M. Zimmermann, M. A. Fischbach, *Chem. Biol.* **2010**, *17*, 925–930.
[132] M. A. Wyatt, W. Wang, C. M. Roux, F. C. Beasley, D. E. Heinrichs, P. M. Dunman, N. A. Magarvey, *Science* **2010**, *329*, 294–296.
[133] F. Sun, H. Cho, D. W. Jeong, C. Li, C. He, T. Bae, *PLoS ONE* **2010**, *5*, DOI 10.1371/journal.pone.0015703.
[134] P. R. Secor, L. K. Jennings, G. A. James, K. R. Kirker, E. de L. Pulcini, K. McInnerney, R. Gerlach, T. Livinghouse, J. K. Hilmer, B. Bothner, P. Fleckman, J. E. Olerud, P. S. Stewart, *PLoS ONE* **2012**, *7*, 1–10.
[135] M. A. Wyatt, M. C. Y. Mok, M. Junop, N. A. Magarvey, *ChemBioChem* **2012**, *13*, 2408–2415.
[136] N. Böhringer, M. Gütschow, G. M. König, T. F. Schäberle, *Nat. Prod. Commun.* **2017**, *12*, 107–109.
[137] D. J. Wilson, C. Shi, A. M. Teitelbaum, A. M. Gulick, C. C. Aldrich, *Biochemistry* **2013**, *52*, 926–937.
[138] B. O. Torres Salazar, S. Heilbronner, A. Peschel, B. Krismer, *Microb. Physiol.* **2021**, *31*, 198–216.
[139] S. Blättner, S. Das, K. Paprotka, U. Eilers, M. Krischke, D. Kretschmer, C. W. Remmele, M. Dittrich, T. Müller, C. Schuelein-Voelk, T. Hertlein, M. J. Mueller, B. Huettel, R. Reinhardt, K. Ohlsen, T. Rudel, M. J. Fraunholz, *PLOS Pathog.* **2016**, *12*, e1005857.
[140] A. Hachani, S. G. Giulieri, R. Guérillot, C. J. Walsh, M. Herisse, Y. M. Soe, S. L. Baines, D. R. Thomas, S. D. Cheung, A. S. Hayes, E. Cho, H. J. Newton, S. Pidot, R. C. Massey, B. P. Howden, T. P. Stinear, *eLife* **2023**, *12*, e84778.
[141] M. Albelda-Berenguer, M. Monachon, E. Joseph, *Siderophores: From Natural Roles to Potential Applications*, Elsevier Inc., **2019**.
[142] C. S. Carroll, M. M. Moore, *Crit. Rev. Biochem. Mol. Biol.* **2018**, *53*, 356–381.

[143] F. C. Beasley, D. E. Heinrichs, *J. Inorg. Biochem.* **2010**, *104*, 282–288.
[144] J. L. Cotton, J. Tao, C. J. Balibar, *Biochemistry* **2009**, *48*, 1025–1035.
[145] S. E. Dale, A. Doherty-Kirby, G. Lajoie, D. E. Heinrichs, *Infect. Immun.* **2004**, *72*, 29–37.
[146] S. E. Dale, M. T. Sebulsky, D. E. Heinrichs, *J. Bacteriol.* **2004**, *186*, 8356–8362.
[147] J. Meiwes, H.-P. Fiedler, H. Haag, H. Zähner, S. Konetschny-Rapp, G. Jung, *FEMS Microbiol. Lett.* **1990**, *67*, 201–206.
[148] F. C. Beasley, E. D. Vinés, J. C. Grigg, Q. Zheng, S. Liu, G. A. Lajoie, M. E. P. Murphy, D. E. Heinrichs, *Mol. Microbiol.* **2009**, *72*, 947–963.
[149] F. C. Beasley, C. L. Marolda, J. Cheung, S. Buac, D. E. Heinrichs, *Infect. Immun.* **2011**, *79*, 2345–2355.
[150] J. C. Grigg, J. D. Cooper, J. Cheung, D. E. Heinrichs, M. E. P. Murphy, *J. Biol. Chem.* **2010**, *285*, 11162–11171.
[151] H. Nakaminami, C. Chen, Q. C. Truong-Bolduc, E. S. Kim, Y. Wang, D. C. Hooper, *Infect. Immun.* **2017**, *85*, e00358-17.
[152] H. Haag, H.-P. Fiedler, J. Meiwes, H. Drechsel, G. Jung, H. ZÃ¤hner, *FEMS Microbiol. Lett.* **1994**, *115*, 125–130.
[153] J. Cheung, F. C. Beasley, S. Liu, G. A. Lajoie, D. E. Heinrichs, *Mol. Microbiol.* **2009**, *74*, 594–608.
[154] F. C. Beasley, J. Cheung, D. E. Heinrichs, *BMC Microbiol.* **2011**, *11*, 199.
[155] R. J. Courcol, D. Trivier, M. C. Bissinger, G. R. Martin, M. R. Brown, *Infect. Immun.* **1997**, *65*, 1944–1948.
[156] K. P. Grim, B. San Francisco, J. N. Radin, E. B. Brazel, J. L. Kelliher, P. K. Párraga Solórzano, P. C. Kim, C. A. McDevitt, T. E. Kehl-Fie, *mBio* **2017**, *8*, e01281-17.
[157] G. Ghssein, C. Brutesco, L. Ouerdane, C. Fojcik, A. Izaute, S. Wang, C. Hajjar, R. Lobinski, D. Lemaire, P. Richaud, R. Voulhoux, A. Espaillat, F. Cava, D. Pignol, E. Borezée-Durant, P. Arnoux, *Science* **2016**, *352*, 1105–1109.
[158] J. R. Morey, T. E. Kehl-Fie, *mSystems* **2020**, *5*, e00554-20.
[159] J. S. McFarlane, C. L. Davis, A. L. Lamb, *J. Biol. Chem.* **2018**, *293*, 8009–8019.
[160] J. S. McFarlane, J. Zhang, S. Wang, X. Lei, G. R. Moran, A. L. Lamb, *J. Biol. Chem.* **2019**, *294*, 17988–18001.
[161] E. J. N. Helfrich, G. M. Lin, C. A. Voigt, J. Clardy, *Beilstein J. Org. Chem.* **2019**, *15*, 2889–2906.
[162] A. Pelz, K. P. Wieland, K. Putzbach, P. Hentschel, K. Albert, F. Götz, *J. Biol. Chem.* **2005**, *280*, 32493–32498.
[163] A. Clauditz, A. Resch, K. P. Wieland, A. Peschel, F. Götz, *Infect. Immun.* **2006**, *74*, 4950–4953.
[164] A. E. Campbell, A. R. McCready-Vangi, A. Uberoi, S. M. Murga-Garrido, V. M. Lovins, E. K. White, J. T.-C. Pan, S. A. B. Knight, A. R. Morgenstern, C. Bianco, P. J. Planet, S. E. Gardner, E. A. Grice, *Cell Rep.* **2023**, *42*, 113281.
[165] K. Siems, K. Runzheimer, K. Rebrosova, L. Etzbach, A. Auerhammer, A. Rehm, O. Schwengers, M. Šiler, O. Samek, F. Růžička, R. Moeller, *Front. Microbiol.* **2023**, *14*, 1272734.
[166] S. H. Kim, P. C. Lee, *J. Biol. Chem.* **2012**, *287*, 21575–21583.
[167] L. Xue, Y. Y. Chen, Z. Yan, W. Lu, D. Wan, H. Zhu, *Infect. Drug Resist.* **2019**, *12*, 2151–2160.
[168] M. Saito, N. Kawaguchi, M. Hashimoto, T. Kodama, N. Higuchi, T. Tanaka, K. Nomoto, T. Murachi, *Agric. Biol. Chem.* **1987**, *51*, 861–868.

[169] W. S. Lin, T. Cunneen, C. Y. Lee, *J. Bacteriol.* **1994**, *176*, 7005–7016.
[170] K. Scherlach, C. Hertweck, *Org. Biomol. Chem.* **2009**, *7*, 1753–1760.
[171] D. Mao, B. K. Okada, Y. Wu, F. Xu, M. R. Seyedsayamdost, *Curr. Opin. Microbiol.* **2018**, *45*, 156.
[172] K. Scherlach, C. Hertweck, *Nat. Commun.* **2021**, *12*, 1–12.
[173] P. J. Rutledge, G. L. Challis, *Nat. Rev. Microbiol. 2015 138* **2015**, *13*, 509–523.
[174] R. H. Baltz, *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 573–588.
[175] M. Nett, H. Ikeda, B. S. Moore, *Nat. Prod. Rep.* **2009**, *26*, 1362–1384.
[176] B. C. Covington, F. Xu, M. R. Seyedsayamdost, **2021**, DOI 10.1146/annurev-biochem-081420.
[177] H. B. Bode, B. Bethe, R. Höfs, A. Zeeck, *ChemBioChem* **2002**, *3*, 619–627.
[178] S. Romano, S. A. Jackson, S. Patry, A. D. W. Dobson, *Mar. Drugs 2018 Vol 16 Page 244* **2018**, *16*, 244.
[179] B. C. Covington, J. A. McLean, B. O. Bachmann, *Nat. Prod. Rep.* **2017**, *34*, 6–24.
[180] M. L. Timmermans, K. J. Picott, L. Ucciferri, A. C. Ross, *MicrobiologyOpen* **2019**, *8*, e00724.
[181] H. A. Tomm, L. Ucciferri, A. C. Ross, *J. Ind. Microbiol. Biotechnol.* **2019**, *46*, 1381–1400.
[182] W. F. Oliveira, P. M. S. Silva, R. C. S. Silva, G. M. M. Silva, G. Machado, L. C. B. B. Coelho, M. T. S. Correia, *J. Hosp. Infect.* **2018**, *98*, 111–117.
[183] D. A. van Bergeijk, S. S. Elsayed, C. Du, I. N. Santiago, A. M. Roseboom, L. Zhang, V. J. Carrión, H. P. Spaink, G. P. van Wezel, *Commun. Chem. 2022 51* **2022**, *5*, 1–12.
[184] O. Zaborina, F. Lepine, G. Xiao, V. Valuckaite, Y. Chen, T. Li, M. Ciancio, A. Zaborin, E. Petroff, J. R. Turner, L. G. Rahme, E. Chang, J. C. Alverdy, *PLOS Pathog.* **2007**, *3*, e35.
[185] B. K. Okada, M. R. Seyedsayamdost, *FEMS Microbiol. Rev.* **2017**, *41*, 19.
[186] H. Onaka, Y. Mori, Y. Igarashi, T. Furumai, *Appl. Environ. Microbiol.* **2011**, *77*, 400–406.
[187] L. M. Filkins, J. A. Graber, D. G. Olson, E. L. Dolben, L. R. Lynd, S. Bhuju, G. A. O'Toole, *J. Bacteriol.* **2015**, *197*, 2252–2264.
[188] S. DeLeon, A. Clinton, H. Fowler, J. Everett, A. R. Horswill, K. P. Rumbaugh, *Infect. Immun.* **2014**, *82*, 4718–4728.
[189] I. Pastar, A. G. Nusbaum, J. Gil, S. B. Patel, J. Chen, J. Valdes, O. Stojadinovic, L. R. Plano, M. Tomic-Canic, S. C. Davis, *PLOS ONE* **2013**, *8*, e56846.
[190] D. B. Y. Yung, K. J. Sircombe, D. Pletzer, *Mol. Microbiol.* **2021**, *116*, 1–15.
[191] F. Xu, Y. Wu, C. Zhang, K. M. Davis, K. Moon, L. B. Bushin, M. R. Seyedsayamdost, *Nat. Chem. Biol.* **2019**, *15*, 161.
[192] K. Penewit, S. J. Salipante, *Methods Mol. Biol. Clifton NJ* **2020**, *2050*, 127–143.
[193] "Rapid and Efficient Genome Editing in Staphylococcus aureus by Using an Engineered CRISPR/Cas9 System | Journal of the American Chemical Society," can be found under https://pubs.acs.org/doi/10.1021/jacs.6b13317, **n.d.**
[194] P. Yang, J. Yang, T. Lin, Q. Liu, Y. Yin, D. Chen, S. Yang, *ACS Synth. Biol.* **2023**, *12*, 3340–3351.
[195] T. Gu, S. Zhao, Y. Pi, W. Chen, C. Chen, Q. Liu, M. Li, D. Han, Q. Ji, *Chem. Sci.* **2018**, *9*, 3248–3253.
[196] Z. Wang, Y. Wang, Y. Wang, W. Chen, Q. Ji, *ACS Synth. Biol.* **2022**, *11*, 3049–3057.
[197] P. Reed, M. Sorg, D. Alwardt, L. Serra, H. Veiga, S. Schäper, M. G. Pinho, *mBio* **2023**, *15*, e02773-23.
[198] Q. Liu, D. Li, N. Wang, G. Guo, Y. Shi, Q. Zou, X. Zhang, *Front. Microbiol.* **2022**, *13*, 818307.

[199] B. T. Bateman, N. P. Donegan, T. M. Jarry, M. Palma, A. L. Cheung, *Infect. Immun.* **2001**, *69*, 7851.
[200] W. Shang, Y. Rao, Y. Zheng, Y. Yang, Q. Hu, Z. Hu, J. Yuan, H. Peng, K. Xiong, L. Tan, S. Li, J. Zhu, M. Li, X. Hu, X. Mao, X. Rao, *mBio* **2019**, *10*, 10.1128/mbio.00880-19.
[201] A. T. F. Liew, T. Theis, S. O. Jensen, J. Garcia-Lara, S. J. Foster, N. Firth, P. J. Lewis, E. J. Harry, *Microbiology* **2011**, *157*, 666–676.
[202] R. M. Corrigan, T. J. Foster, *Plasmid* **2009**, *61*, 126–129.
[203] A. J. Brzoska, N. Firth, *Appl. Environ. Microbiol.* **2013**, *79*, 3133–3136.
[204] J. Yao, J. Zhong, Y. Fang, E. Geisinger, R. P. Novick, A. M. Lambowitz, *RNA* **2006**, *12*, 1271–1281.
[205] M. Arnaud, A. Chastanet, M. Débarbouillé, *Appl. Environ. Microbiol.* **2004**, *70*, 6887–6891.
[206] T. Geiger, P. Francois, M. Liebeke, M. Fraunholz, C. Goerke, B. Krismer, J. Schrenzel, M. Lalk, C. Wolz, *PLOS Pathog.* **2012**, *8*, e1003016.
[207] C. F. Schuster, S. A. Howard, A. Gründling, *Microbiology* **2019**, *165*, 572–584.
[208] P. F. Popp, M. Dotzler, J. Radeck, J. Bartels, T. Mascher, *Sci. Rep.* **2017**, *7*, 15058.
[209] Y. Liu, L. Liu, J. Li, G. Du, J. Chen, *Trends Biotechnol.* **2019**, *37*, 548–562.
[210] H. Put, H. Gerstmans, H. V. Capelle, M. Fauvart, J. Michiels, J. Masschelein, *Nat. Prod. Rep.* **2024**, *41*, 1113–1151.
[211] P. Yang, J. Tian, L. Zhang, H. Zhang, G. Yang, Y. Ren, J. Fang, Y. Gu, W. Jiang, *Synth. Syst. Biotechnol.* **2024**, *9*, 43–54.
[212] I. C. Cañadas, D. Groothuis, M. Zygouropoulou, R. Rodrigues, N. P. Minton, *ACS Synth. Biol.* **2019**, *8*, 1379–1390.
[213] S. N. Rondthaler, B. Sarker, N. Howitz, I. Shah, L. B. Andrews, *ACS Synth. Biol.* **2024**, *13*, 103–118.