Nisin J, a Novel Natural Nisin Variant, Is Produced by Staphylococcus capitis Sourced from the Human Skin Microbiota

Julie N. O’Sullivan,a,b,c Paula M. O’Connor,a,c Mary C. Rea,a,c Orla O’Sullivan,a,c Calum J. Walsh,a,c Brian Healy,c Harsh Mathur,a,c Des Field,b,c Colin Hill,b,c R. Paul Ross,a,b,c

a Teagasc Food Research Centre, Fermoy, County Cork, Ireland
b School of Microbiology, University College Cork, Cork, Ireland
c APC Microbiome Ireland, University College Cork, Cork, Ireland

ABSTRACT The skin microbiota is thought to play a key role in host protection from infection. Nisin J is a novel nisin variant produced by Staphylococcus capitis APC 2923, a strain isolated from the toe web space area in a screening study performed on the human skin microbiota. Whole-genome sequencing and mass spectrometry of the purified peptide confirmed that S. capitis APC 2923 produces a 3,458-Da bacteriocin, designated nisin J, which exhibited antimicrobial activity against a range of Gram-positive pathogens, including methicillin-resistant Staphylococcus aureus and Cutibacterium acnes. The gene order in the nisin J gene cluster (nsjFEGBTCJP) differs from that of other nisin variants in that it is lacking the nisin regulatory genes, nisRK, as well as the nisin immunity gene nisI. Nisin J has 9 amino acid changes compared to prototypical nisin A, with 8 amino acid substitutions, 6 of which are not present in other nisin variants (Ile4Lys, Met17Gln, Gly18Thr, Asn20Phe, Met21Ala, Ile30Gly, Val33His, and Lys34Thr), and an extra amino acid close to the C terminus, rendering nisin J the only nisin variant to contain 35 amino acids. This is the first report of a nisin variant produced by a Staphylococcus species and the first nisin producer isolated from human skin.

IMPORTANCE This study describes the characterization of nisin J, the first example of a natural nisin variant, produced by a human skin isolate of staphylococcal origin. Nisin J displays inhibitory activity against a wide range of bacterial targets, including MRSA. This work demonstrates the potential of human commensals as a source for novel antimicrobials that could form part of the solution to antibiotic resistance across a broad range of bacterial pathogens.

KEYWORDS antimicrobial agents, antimicrobial peptides, antimicrobial structure, bacteriocins, microbiota, natural antimicrobial products, skin microbiota, nisin

The human skin microbiome is home to ~10^{12} bacteria (1), and interest in the potential of skin bacteria to produce antimicrobials is growing, given the spread of antibiotic resistance (AR). Staphylococcus capitis is a member of the resident skin microbiota. First isolated from human skin in 1975, it has since been regarded as an opportunistic pathogen and has been associated with sepsis in neonates, meningitis, and endocarditis (2). Little is known about the inhibitory nature or antimicrobial activity of S. capitis, with only one report of S. capitis EPK-1 producing the glycolglycine endopeptidase ALE-1, an enzyme that targets the cell wall of Staphylococcus aureus (3). More recently, genomic analysis of an S. capitis strain isolated from the skin of a human toe revealed the presence of gene clusters capable of encoding gallidermin, epidermin, and phenol soluble modulins, highlighting its potential to produce antimicrobial
peptides (AMPs) (4). In a recent study, our group detected antimicrobial activity by a number of S. capitis strains isolated from different areas of the human skin (5) and highlighted the potential for S. capitis species to produce bacteriocins (small ribosomally synthesized peptides produced by a range of bacteria which kill other bacteria). Interestingly, bacteriocin production is considered to be a probiotic trait in that bacteriocins function in helping the producer strain to become established in a niche, by killing off competitors and interacting with the immune system. Although the impact of nisin on immune systems has not yet been completely elucidated, this peptide stimulates a wide array of effects, and it influences various populations of cells involved in immunity (6–12).

One of the oldest known and most intensively studied bacteriocins is nisin, which was first described in this journal by Rogers and Whittier in 1928 (13). Nisin has been used in food preservation since 1953 (14) and was granted generally regarded as safe (GRAS) status in 1988 by the Food and Drug Administration (FDA). It is also approved by the World Health Organization (WHO) as a food additive and has been assigned the E number E234. Since the discovery of nisin, interest in bacteriocins has grown rapidly. Nisin A, composed of 34 amino acids, is produced by several strains of Lactococcus lactis (15). Nisin is a lantibiotic and thus a member of the class I bacteriocins (16). Lantibiotics are small peptides (<5 kDa) and are produced by many Gram-positive bacteria to inhibit or kill other Gram-positive bacteria (17). Production of other lantibiotics is common among commensal coagulase-negative staphylococci. For example, Staphylococcus gallinarum, Staphylococcus epidermidis, and Staphylococcus hominis produce the lantibiotics gallidermin, epidermin, and hominicin, respectively (18–20). Class I bacteriocins consist of posttranslationally modified bacteriocins which are subdivided into 4 classes, as follows: class Ia, lanthipeptides (of which nisin is the most prominent member); class Ib, head-to-tail cyclized peptides; class Ic, sactibiotics; and class Id, linear azol(in)e-containing peptides (8, 21). Lantibiotics are characterized by the presence of lanthionine/β-methyl-lanthionine residues and are produced through the dehydration of serine and threonine residues to form dehydroalanines and dehydrobutyryl residues, respectively. These dehydrated residues in turn react with cysteine thioles, forming lanthionine bridges (22, 23). The lantibiotics are subdivided based on the enzymes catalyzing the formation of lanthionines. Subclass I requires two distinct enzymes, LanB and LanC, whereas subclass II is modified by a single enzyme, LanM. Subclass III has no associated antimicrobial activity and is modified by a single enzyme, LanKC, while subclass IV is modified by LanL (24). Studies have revealed that nisin and other structurally related lantibiotics use the membrane-bound peptidoglycan precursor lipid II as a docking molecule, which consequently promotes two bactericidal activities, pore formation and inhibition of peptidoglycan biosynthesis (25). Significantly, lantibiotics have been shown to possess activity against antibiotic-resistant targets such as vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) and may have the potential to mitigate the looming global AR crisis (26).

A number of nisin variants have been discovered since the original nisin A was characterized (Fig. 1A). Nisin variants of lactococcal origin are more similar to each other than to variants from other genera such as Streptococcus (Fig. 1B). Nisin Z is the most closely related nisin variant to nisin A, with only a single amino acid substitution, His27Asn. Nisin U, U2, and P each contain 31 amino acids, nisins O1–3 contain 33 amino acids, and nisin O4 contains 32 amino acids, making them shorter than other previously described nisin variants. Here, we describe nisin J, produced by the S. capitis strain APC 2923, isolated in a screening study of the human skin microbiota. At 35 amino acids, nisin J is the longest nisin variant identified to date and has antimicrobial activity against significant human pathogens, including staphylococci, streptococci, and Cutibacterium acnes.

RESULTS

A nisin-like gene cluster exists within the S. capitis APC 2923 genome. S. capitis APC 2923 was previously isolated from the toe web space area in a screening study of
the human skin microbiota that sought to identify novel antimicrobial-producing strains (5). This strain was of particular interest due to its potent activity against the indicator strain *Lactobacillus delbrueckii* subsp. *bulgaricus* LMG 6901 and its broad inhibitory spectrum against a panel of *Staphylococcus*, *Streptococcus*, and *Corynebacterium* species and against *Cutibacterium acnes*. Whole-genome sequencing of this strain revealed a nisin gene cluster of ~9.78 kb compared to ~13.3 kb for nisin A. The structural gene nisJ encodes a peptide with the following eight amino acid variations compared to nisin A: Ile4Lys, Met17Gln, Gly18Thr, Asn20Phe, Met21Ala, Ile30Gly, Val33His, and Lys34Thr. Nisin J also contains an extra amino acid at the C terminus, making nisJ the longest nisin variant identified to date (Fig. 1A). A dendrogram of the natural nisin variants (Fig. 1B) demonstrates that peptides which have a closer common ancestor are more similar than are peptides than have more distant branching points. Lactococcal nisin variants are structurally distinct from all other nisin variants. Staphylococcal nisin J groups in the middle of the tree and appears to be more similar to streptococcal nisin than to lactococcal nisins. Nisins of *Blautia* origin appear to be more phylogenetically distinct due to longer branching. Streptococcal nisins H and J are more closely related to lactococcal nisins than to other streptococcal nisins, U, U2, and P. The gene order of the nisin J cluster (*FEGBTCJP*) also differs from that of the nisin A
in that it contains eight as opposed to the 11 genes within the cluster (Fig. 2). The BAGEL4 bacteriocin genome mining tool predicted that the nisin J prepeptide is composed of 61 amino acids with a leader sequence consisting of 26 amino acids. Overall, the nisin J mature peptide has 62.5% identity to the nisin H structural peptide produced by *Streptococcus hyointestinalis* (27). The identity and function of features of the nisin J operon are listed in Table 1.

**Other genes contained in the *S. capitis* APC 2923 draft genome.** In addition to the nisin J cluster, BAGEL4 and antiSMASH3.0 also highlighted a small gene cluster containing the *lanB* and *lanC* genes and a gene encoding a peptide with 93% identity to the gallidermin family in *S. capitis* APC 2923. These were located on a different contig from that of the nisin J gene cluster, and this mass was not detected from either the colony or purified cell free supernatants.

**Purification and predicted structure of nisin J.** Nisin J was purified in four steps using Amberlite XAD-16N solid-phase extraction (SPE), SP Sepharose cation exchange, C₁₈ SPE, and reversed-phase high-performance liquid chromatography (HPLC). Antimicrobial activity correlated with the most dominant peak eluting at 24.5 min in the HPLC chromatogram, and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) revealed that the corresponding fractions had a mass of 3,458 Da (Fig. 3). This correlates with the predicted mass of the putative nisin J bacteriocin (following subsequent dehydration and ring formation reactions) as calculated from the draft genome sequence. Fractions deemed pure by MALDI-TOF MS were combined and lyophilized to give a yield of 3.00 mg/liter. Given that nisin J is a natural nisin variant with demonstrable conservation between key structural amino acids common to all natural nisin variants, it is predicted that the structure will be in line with those of other lactococcal nisins, as shown in Fig. 4.

**Comparing the activities of purified nisins A, Z, and J.** The spectrum of activity of pure nisin A, nisin Z, and nisin J, by means of a well diffusion assay (WDA), was performed on several target indicator strains. Nisin J was more active than nisin A against 12 of the 13 strains tested, while nisin J was more active than nisin Z for 7 of the target strains tested, including *Corynebacterium xerosis*, MRSA, *Streptococcus uberis*, and *S. aureus* (Table 2). However, in an MIC assay using *L. lactis* HP as the indicator, no difference was observed between nisins A, Z, and J, with all exhibiting MICs of 32 nM.

**The nisin J-producing strain is cross-immune to nisin A and H but not to nisin U producers.** Cross-immunity assays were performed to investigate whether the nisin
| Feature name | Start codon | Stop codon | Size (aa) | E value | Putative function (conserved domain) | % identity to best match |
|--------------|-------------|------------|----------|---------|-------------------------------------|--------------------------|
| J1           | 3           | 518        | 172      | 3E−124  | DDE_Tnp, IS240 superfamily; Rve transposase | 100 to IS6 family transposase of *S. epidermidis* |
| J2           | 562         | 1221       | 220      | 6E−149  | ABC2 membrane superfamily; NosY ABC-type transport system involved in multicopper enzyme maturation, permease component | 100 to ABC transporter permease subunit of *S. capitis* |
| J3           | 1749        | 1874       | 125      | 3E−17   | DUF2648 superfamily; unknown function | 100 to multiple species; DUF2648 domain-containing protein |
| J4           | 1886        | 3385       | 500      | 0       | NADB_Rossmann superfamily; MqO malate: quinone oxidoreductase | 100 to multiple species; malate dehydrogenase (quinone) (*Staphylococcus*) |
| J5           | 3446        | 5050       | 535      | 0       | L-Lactate permease superfamily (energy production and conversion) | 100 to L. lactate permease (*Staphylococcus*) |
| J6           | 5085        | 5789       | 235      | 6E−171  | Alpha-acetolactate decarboxylase superfamily (secondary metabolite biosynthesis, transport, and catabolism) | 100 to alpha-acetolactate decarboxylase |
| J7           | 5823        | 7487       | 555      | 0       | Acetolactate synthase superfamily (PRK08617) (amino acid transport and metabolism, coenzyme transport, and metabolism) | 100 to acetolactate synthase |
| J8           | 8213        | 8413       | 67       | 8E−39   | CspA family (transcription) DNA binding domain | 100 to cold shock protein (*Staphylococcus*) |
| CdR          | 8807        | 9424       | 206      | 1E−139  | Cadmium resistance transporter superfamily; CadD protein, predicted permease (inorganic ion transport and metabolism) | 100 to cadmium resistance transporter (*Mycobacteroides abscessus* subsp. massiliense) |
| J10          | 9442        | 9789       | 116      | 4E−74   | Arsenical resistance operon repressor family; DNA-binding transcriptional regulator (transcription) | 100 to HTH transcriptional regulator (*Staphylococcus*) |
| J11          | 10002       | 10610      | 203      | 2E−144  | Serine recombinase family | 100 to recombinase family protein (*Staphylococcus*) |
| J12          | 10716       | 11276      | 187      | 1E−124  | None detected | 100 to hypothetical protein (*Staphylococcus*) |
| J13          | 11884       | 12369      | 162      | 2E−112  | None detected | 100 to hypothetical protein (*Staphylococcus*) |
| J14          | 12632       | 13309      | 226      | 5E−166  | NlpC/P60 family; the function of this domain is unknown; it is found in several lipoproteins | 100 to hypothetical protein (*Staphylococcus*) |
| PSM          | 13578       | 13712      | 45       | 1E−22   | *Staphylococcus* hemolysin protein | 100 to beta class phenol-soluble modulin |
| J16          | 13944       | 14054      | 37       | 4E−17   | DUF2648 superfamily; protein of unknown function | 100 to multiple species; DUF2648 domain-containing protein (*Staphylococcus*) |
| J17          | 14064       | 15560      | 499      | 0       | NADB_Rossmann superfamily; MqO malate: quinone oxidoreductase | 100 to malate dehydrogenasequinone (*S. capitis*) |
| J18          | 15780       | 16118      | 113      | 6E−75   | DNA binding transcription regulator | 100 to transcriptional regulator HXIR family (*Staphylococcus caprae*) |
| RepA         | 17825       | 18760      | 312      | 0       | Replication initiator protein A (RepA) N terminus family; DNA replication initiator in plasmids | 100 to replication initiator protein A (*Staphylococcus*) |
| J20          | 19190       | 19957      | 256      | 1E−178  | Polar chromosomal segregation protein | 100 to DUF536 binding domain (*Staphylococcus*) |
| J21          | 20132       | 20734      | 201      | 2E−140  | NADB Rossmann superfamily; PRK07578 short-chain dehydrogenase | 100 to short-chain dehydrogenase (bacteria) |
| J22          | 21220       | 21894      | 225      | 7E−165  | DDE_Tnp, IS240 superfamily; Rve transposase | 100 to IS6-like element IS257 family transposase |
| nsjF         | 22148       | 22855      | 236      | 6.00E−119 | ABC-type multidrug transport system, ATPase component (defense mechanisms) | 75 to Lan protection ABC transporter ATP binding subunit in *Staphylococcus succinus* |
| nsjE         | 22857       | 23603      | 249      | 4E−85   | Lantibiotic protection ABC transporter permease subunit, MutE/EpiE family; ABC-2 membrane superfamily | 61.29 to hypothetical protein BU069_09230 in *S. succinus* |
| nsjG         | 23600       | 24337      | 246      | 1E−73   | Lantibiotic protection ABC transporter permease subunit, MutG family; ABC-2 membrane superfamily | 52.92 to hypothetical protein in *S. succinus* |
| nsjB         | 24362       | 27277      | 972      | 8E−90   | Lantibiotic dehydratase C-terminal, thiopeptide-type bacteriocin biosynthesis domain | 30.11 to lantibiotic dehydratase *Lactobacillus bombicola* |

(Continued on next page)
J+, A+, H-, and U-producing strains were cross-immune to one another (Table 3). No zones were observed between nisins A, H, and J, indicating that these producing strains are all cross-immune. However, a zone was observed from the nisin J-producing strain against the nisin U producer (S. uberis strain 42), demonstrating that the strain is sensitive to nisin J.

**Not all S. capitis strains contain a nisin-like gene cluster.** The nisJ structural gene was amplified from nine antimicrobial-producing S. capitis strains isolated from human skin in a previous study by our group (5). Two of the nine S. capitis strains (APC 2918 and APC 2934) did not contain the nisJ structural gene. The other seven S. capitis strains tested positive for the nisJ structural gene, correlating with findings from our earlier...
study which found these strains to be cross-immune and to possess the same pulso-
type, indicating that they were the same strain or very closely related strains and were
therefore most likely producing the same bacteriocin (5). These 7 strains were isolated
from 4 different subjects, indicating that the same pulsotype is shared across a number
of individuals, implying that the ability to produce nisin J may be a dominant feature
and thus an ecological advantage for this \textit{S. capitis} strain.

The \textbf{nisin J gene cluster resides on a plasmid}. Analysis of the \textit{S. capitis} APC 2923
tcontig harboring the nisin J gene cluster identified the presence of a plasmid replication
protein A (RepA) and other plasmid replication-associated proteins, suggesting
that it was of plasmid origin. Plasmid DNA was readily obtained from \textit{S. capitis} APC 2923
using a commercially available plasmid maxi kit (data not shown). Short-read sequenc-
ing was performed on the plasmid DNA using the Illumina MiSeq platform to approx-
imately 200-fold coverage. \textit{De novo} assembly resulted in four contigs (Fig. 5), with a
combined size of 49,951 bp. A plasmid map of pJOS01 (GenBank accession number
MN602039) shows all of the genes encoding immunity and the biosynthetic machinery
for nisin J (\textit{nsjFEG, njsb, njsT, njsC, nisJ, and njsP}) reside on one of the contigs, supporting
The plasmid association of the nisin J gene cluster (Fig. 5). Furthermore, three genes encoding plasmid replication functions (RepA and RepB) as well as genes encoding other nonessential plasmid-associated roles were present on the other contigs (Fig. 5 and Table 1). Restriction digestion with EcoRI yielded a profile comparable to the virtual digestion of the generated plasmid sequence, supporting the predicted size of 50 kb (data not shown). Subsequent analysis revealed a GC content of 28%, which is considerably lower than that of S. capitis chromosomal DNA (32 to 33%), a characteristic that has been observed for plasmids of many Gram-positive species (28).

**Nisin J exhibits resistance to NSR.** Deferred antagonism assays using *L. lactis* subsp. *diacetylactis* DRC3 (nisin resistance protein positive [NSR⁺]) as a target indicator strain revealed that nisin J is partially resistant to NSR (result not shown). To establish if nisin J had increased inhibitory activity against NSR compared to that of nisin A, further WDAs were conducted using the NSR⁺ and NSR⁻ strains *L. lactis* MG1614/pNP40 and *L. lactis* MG1614, respectively. While the inhibition zone of the nisin J producer is slightly decreased against the NSR-positive strain compared to the NSR-negative strain, it appears that nisin J is more active than nisin A and may be less susceptible to the proteolytic effects of NSR (Fig. 6A), which was also demonstrated in agarose assays (Fig. 6B). The analysis revealed a significant difference in the zones of inhibition between nisin A and nisin J against an NSR⁻ strain (MG1614/pNP40), with a *P* value of 0.0001 compared to zone sizes against an NSR⁻ strain (MG1614), where no statistical difference (*P* = 0.1701) was observed (these data support Fig. 6).

**DISCUSSION**

As the burden of antibiotic resistance increases globally, there is an urgent need for novel therapeutic options. In addition to the well-established use of nisin as a food

### TABLE 2 Inhibition spectra of purified peptides of nisins A, Z, and J against indicator strains using well diffusion assays and expressed as the area of the zone of inhibition

| Target microorganism                  | Strain | Area of zone of inhibition (mm²) for nisin: |
|---------------------------------------|--------|------------------------------------------|
|                                       |        | A  | Z  | J  |
| Corynebacterium xerosis               | DPC 5629 | 51.5 | 66.2 | 133.8 |
| Cutibacterium acnes                   | LMG 16711 | 537 | 587.5 | 469 |
| Lactobacillus delbrueckii subsp. bulgaricus | LMG 6901 | 555.7 | 672.7 | 651.44 |
| Lactococcus lactis subsp. cremoris    | HP     | 241.9 | 325.8 | 363 |
| Listeria monocytogenes                | WSLC 112 | 60  | 73  | 37  |
| Enterococcus faecium                  | APC 852 | 93.2 | 120.6 | 170.9 |
| Enterococcus faecalis                 | ATCC 19433 | 101.2 | 120.8 | 102.97 |
| Methicillin-resistant *Staphylococcus aureus* | DPC 5645 | 77  | 115.9 | 135.8 |
| *Staphylococcus aureus*               | DPC 7016 | 109.4 | 143.4 | 153.1 |
| *Staphylococcus epidermidis*          | DPC 5990 | 136.8 | 180.3 | 159.5 |
| *Staphylococcus simulans*             | APC 3482 | 148.7 | 197.1 | 395.5 |
| *Streptococcus agalactiae*            | ATCC 13813 | 174.4 | 221.7 | 136.8 |
| *Streptococcus uberis*                | DPC 5344 | 98.5  | 153.9 | 248.8 |

*Calculated as the area of zone of inhibition (πr²) — area of well (πr²) in millimeters. Assays were carried out in duplicate; mean zone areas shown.

### TABLE 3 Cross-immunity of nisin A-, U-, H-, and J-producing strains using well diffusion assays and expressed as the area of the zone of inhibition

| Target organism                  | Strain | Nisin produced | Area of zone of inhibition (mm²) against nisin: |
|----------------------------------|--------|----------------|-----------------------------------------------|
|                                  |        |                | A  | U  | H  | J  |
| *Lactococcus lactis*             | NZ9700 | A              | 0  | 0  | 0  | 0  |
| *Streptococcus uberis*           | 42     | U              | 0  | 0  | 85 |    |
| *Streptococcus hyointestinalis*  | DPC 6484 | H             | 0  | 0  | 0  | 0  |
| *Staphylococcus capitis*         | APC 2923 | J             | 0  | 0  | 0  |    |

*Calculated as the area of zone of inhibition (πr²) — area of well (πr²) in millimeters. Values are the means from triplicate assays. 0, no zone observed.
preservative, many studies have focused on using nisin against drug-resistant pathogens in clinical or veterinary settings due to its high potency and multiple mechanisms of action (10–12). Nisin J is a novel nisin variant and the first such variant reported from a Staphylococcus species. A combination of whole-genome sequencing of S. capitis APC 2923 and peptide purification resulted in the identification of this broad-spectrum lantibiotic. The nisin J-producing S. capitis strain was isolated from the toe web space, an area associated with high microbial load. This suggests that the production of a broad-spectrum bacteriocin confers an advantage on this strain over competing commensal skin flora, as was also observed by O’Sullivan and colleagues (5) when four of the twenty subjects screened in the study exhibit the same pulsotype. The residence of the nisin J gene cluster on a plasmid is significant in that it may facilitate its dissemination to other skin microbes.

As mentioned previously, nisin J has eight amino acid changes and one extra amino acid near the C-terminal end compared to nisin A. Interestingly, six of the eight changes are unique compared to natural nisin variants. Natural nisin variants are tolerant to some amino acid changes at the N terminus, with Ile4 being the most commonly

![Figure 5](image_url)
substituted amino acid. Nisin J contains an Ile4Lys substitution which is also seen in nisins P, U, U₂ and O₁–₃, but remains unchanged in lactococcal nisins (A, Z, F, and Q) and nisin H. Nisin J differs most from other natural nisin variants in the center and at the C terminus of the peptide, which could be key to nisin J’s enhanced activity. At the center of nisin J, amino acid positions 17 to 21, there are 4 amino acids that differ compared to nisin A. It contains a Met17Gln substitution which is unique, as all other natural nisin variants that demonstrate antimicrobial activity have Met at this position. The Gly18Thr change is also interesting, as it is observed in nisins H, U, U₂, P, and O₁–₃ and is proposed to be modified to dehydrobutyrine (Dhb), in light of the dehydration observed in a M17Q-G18T derivative of nisin Z (29). At position 20, nisin J has a highly hydrophobic residue, phenylalanine, compared to the polar asparagine in nisin A. Li et al. (30) found that extending the C terminus of nisin improves both its ability to permeate membranes and its inhibitory potential against Gram-negative bacteria. Therefore, nisin J’s longer C terminus (compared to other nisin variants) could be more attracted to negatively charged cell membranes resulting in enhanced membrane insertion, which may be responsible for its broader host range. The skin origin of this nisin J producer suggests that its exposure to many competitors from the external environment may be responsible for the greater variation in the structure of nisin J.

Analysis of the nisin J gene cluster identified several key features associated with bacteriocin operons. These include a structural gene (nisJ), 2 genes associated with enzymatic modification (nsjB and nsjC), a gene involved in transport (nsjT), and immunity genes (nsjFEG) (Table 1 lists the identity and functions of features of the nisin J gene cluster). The arrangement of genes in the nisin J gene cluster differs from that of other nisin operons. Interestingly, the only conservation of gene order throughout all operons of natural nisin variants is lanBTC. Similarities in the structural peptides of different nisin variants from different origins indicate the possibility that an evolutionary link exists between lactococcal, streptococcal, Blautia, and now, staphylococcal species, a link previously mentioned by O’Connor et al. (27) with reference to streptococcal and lactococcal species. A dendrogram based on the primary structures of all known natural nisin variants highlights the genetic relatedness between the nisin-producing species and further suggests the likelihood of this evolutionary link. The FEG locus is present in lantibiotic systems other than nisin, including subtilin (31) and epidermin (32), and has been linked to transport, immunity, and defense (33). Inactivation of these
genes in the nisin A gene cluster decreased nisin production and immunity, confirming their role in immunity (34). Although the nsjFEG genes are present in the nisin J gene cluster, the absence of a specific immunity gene, nsjI, as well as the absence of an expression regulatory system, nsjRK, could explain why nisin J immunity mechanisms appear to be less able to protect the cell. It also further supports the finding that the producing strain was more sensitive to its own purified nisin J peptide than was a nisin A producer with a specific nisin immunity determinant.

The production of lantibiotics such as gallidermin and epidermin is associated with increased release of lipids and ATP and protein excretion, which are indicators of cell membrane damage (35). Thus, the production of these lantibiotics has been deemed a “burden” to staphylococci that produce them; therefore, the incomplete lantibiotic gene cluster, having only the lanB and lanC genes present, may be either an evolutionary feature of S. capitis genomes or may be an incomplete cluster of lantibiotic biosynthetic genes previously shown to occur in many microbes (35).

As previously discussed, the nisin J gene cluster resides on a plasmid, inviting the speculation that S. capitis acquired its antimicrobial ability through horizontal gene transfer. Indeed, residence on mobile genetic elements is a feature of natural nisin variants, as observed with nisins A and H, and may explain their presence in many different species.

Purification of nisin J resulted in a peptide with a mass of 3,458 Da. The mass of nisin J was predicted to be 3,622 Da, where the difference between predicted and observed masses can be accounted for by 9 dehydration reactions (−18 Da per loss of water residue) involved in the formation of lanthionine and β-methyllanthionine bridges (36). The predicted peptide structure was based on the nisin A template, with a lanthionine bridge likely to occur between Ser3 and Cys7 and four β-methyllanthionine bridges between Thr8 and Cys11, Thr13 and Cys19, Thr23 and Cys26, and Thr25 and Cys28.

True to all nisin variants, nisin J is a broad-spectrum lantibiotic with inhibitory activity similar to that of nisins A and Z, as can be seen in Table 2, inhibiting a wide range of bacterial genera with greater inhibition of staphylococcal targets than with nisins A and Z. This suggests that the nisin J-producing S. capitis strain may have naturally evolved to produce a nisin peptide with enhanced activity against other staphylococci in the skin microbiota (Table 2). Nisin J-, A-, and H-producing strains are immune to nisin peptides J, A, H, and U; however, the nisin U-producing strain is not immune to nisin J (Table 3). This may be due to the lack of the nsjI immunity gene in the nisin J cluster.

The nisin resistance protein (NSR) is a protease which cleaves nisin A at Ser29, significantly reducing the activity of the peptide. Employing a bioengineering strategy, Field et al. (37) demonstrated that the substitution of residues 29 and 30 with proline and valine, respectively (derivative designated S29PV), rendered the peptide resistant to proteolytic digestion by NSR. In this study, we found that the nisin J producer displays a higher resistance to NSR proteolytic enzymes than does nisin A, which is possibly due to a glycine residue at position 30 instead of the isoleucine as found in nisin A. Interestingly, a study carried out by Simões et al. (38) involving a multidrug-resistant S. capitis clone, NRCS-A, a major pathogen involved in sepsis in preterm neonates, demonstrated the presence of an NSR-encoding gene. PCR analysis failed to detect the presence of any nsr gene in any nisin J-producing S. capitis strain from our previous study (5).

Nisin J may have evolved to be more potent against specific competing organisms in a particular niche environment such as the skin. Employing a bioengineering strategy, Rink et al. (39) demonstrated that the replacement of residues I, S, and L at positions 4, 5, and 6 in nisin A with the residues K, S, and I, respectively, resulted in enhanced bioactivity. Notably, the residues K-S-L are naturally present in nisin J at the same positions. In a separate bioengineering study, Kuipers et al. (29) generated a novel nisin variant (M17Q/G18T) exhibiting enhanced bioactivity. It is interesting that both of these mutations are naturally present in nisin J. Furthermore, Field et al. (40) reported
that a nisin A derivative, M21A, demonstrated enhanced bioactivity. Remarkably, alanine is naturally present at position 21 in nisin J.

In conclusion, we have identified a new natural nisin variant, nisin J, produced by *S. capitis* APC 2923, which was isolated from the human skin microbiota. Nisin J represents the first nisin variant isolated from *Staphylococcus* species and the first to demonstrate partial recalcitrance to NSR. Indeed, the enhanced activity of nisin J compared to that of nisin A and Z as observed against all staphylococcal strains utilized in this study is notable. The production of bacteriocins such as nisin J from skin bacteria highlights the potential of bacterial strains of skin origin to be used as live biotherapeutics.

**MATERIALS AND METHODS**

The antimicrobial-producing strain *S. capitis* APC 2923 was isolated in a previous screening study of the human skin microbiota by our group (5).

**Bacterial strains and culture conditions.** The growth conditions of the bacterial strains used in this study are listed in Table 4. Anaerobic conditions, where appropriate, were attained using anaerobic jars and Anaerocult A gas packs (Merck, Darmstadt, Germany).

**Draft genome sequence of *S. capitis* APC 2923 and in silico analysis of the nisin J gene cluster.** Bacterial DNA was extracted using the GenElute kit, as described by the manufacturer (Sigma-Aldrich Ireland Limited, Arklow, County Wicklow, Ireland), and was prepared for sequencing following the Nextera XT DNA library prep reference guide (Illumina, Inc.). Sequencing was performed at the Teagasc/APC Microbiome Ireland Sequencing facility, Teagasc Food Research Centre, Moorepark, Fermoy, County Cork, Ireland. In total, 94 contigs, including 16 large contigs, were revealed by *de novo* assembly using SPAdes (version 3.10.0). A total of 2,453 open reading frames (ORFs) and 60 tRNAs were detected and subsequently annotated using Prokka (version 1.11). The online tools Bacteriocin GEnome mining tooL (BAGEL4) and antiSMASH 3.0 were employed to identify bacteriocin operons/gene clusters in the genomes of interest, and by combining these software programs with the ARTEMIS genome viewer, the presence of the nisin J gene cluster was confirmed.

**Evolutionary links between natural nisin variants.** The European Bioinformatics Institute toolkit (https://www.ebi.ac.uk/services) was used to investigate the evolutionary relationships between the nisin structural variants. A multiple-sequence alignment was generated using MUSCLE (version 3.8) and visualized on a neighbor-joining tree without distance corrections. This tree was visualized using the ggtree package (version 1.10.5) in R (version 3.4.4).

**Purification of the antimicrobial produced by *S. capitis* APC 2923.** To purify the antimicrobial produced by *S. capitis* APC 2923, the culture was grown in a shaking 37°C incubator overnight in 1,800 ml of

### Table 4 Growth conditions of the bacterial strains used in this study

| Species                  | Strain | Temp (°C) | Atmosphere | Growth medium(a)                  |
|--------------------------|--------|-----------|------------|-----------------------------------|
| *Corynebacterium xerosis*| DPC 5629 | 37        | Aerobic    | BHI                               |
| *Cutibacterium acnes*    | LMG 16711 | 37        | Anaerobic  | mRCM and RCA                      |
| *Enterococcus faecalis*  | ATCC 19433 | 37        | Anaerobic  | MRS                               |
| *Enterococcus faecium*   | APC 852 | 37        | Anaerobic  | MRS                               |
| *Lactobacillus delbrueckii subsp. bulgaricus* | LMG 6901 | 37        | Anaerobic  | MRS                               |
| *Lactococcus lactis*     | NZ9700 | 30        | Aerobic    | GM17                              |
| *Lactococcus lactis subsp. cremoris* | HP | 30        | Aerobic    | GM17                              |
| *Lactococcus lactis*     | MG1614 | 30        | Aerobic    | GM17                              |
| *Lactococcus lactis*     | MG1614/pNP40 | 30 | Aerobic    | GM17                              |
| *Lactococcus lactis subsp. diacetylactis* | DRC3 | 37        | Aerobic    | BHI                               |
| *Listeria monocytogenes* | WSLC 1211 | 37        | Aerobic    | BHI                               |
| *Staphylococcus aureus*  | DPC 5645 | 37        | Aerobic    | BHI                               |
| *Staphylococcus capitis* | DPC 7016 | 37        | Aerobic    | BHI                               |
| *Staphylococcus epidermidis* | DPC 5990 | 37        | Aerobic    | BHI                               |
| *Staphylococcus simulans*| APC 3482 | 37        | Aerobic    | BHI                               |
| *Streptococcus agalactiae* | ATCC 13813 | 37 | Aerobic    | BHI                               |
| *Streptococcus hyointestinalis* | DPC 6484 | 37 | Anaerobic | GM17                              |
| *Streptococcus uberis*   | DPC 5344 | 37        | Aerobic    | BHI                               |
| *Streptococcus uberis*   | Strain 42 | 37       | Anaerobic  | GM17                              |

(a) ATCC, American Type Culture Collection; APC, APC Microbiome Ireland Culture Collection; DPC, Teagasc Culture Collection; WSLC, Welhenstephan Listeria Collection; LMG, Laboratorium voor Microbiologie.

(b) Anaerobic conditions, where appropriate, were achieved through the use of anaerobic jars and Anaerocult A gas packs (Merck, Darmstadt, Germany).

(c) MRS, de Man-Rogosa-Sharpe; mRCM, modified reinforced Clostridium medium (made following the ATCC medium: 2107 modified reinforced clostridial agar/broth [prereduced] protocol); RCA, reinforced Clostridium agar; BHI, brain heart infusion; GM17, 0.5% glucose added to M17 agar.

(d) MRSA, methicillin-resistant *S. aureus*.
of brain heart infusion (BHI) which had been passed through an XAD column to remove hydrophobic peptides before autoclaving (XAD-BHI). The culture supernatant was applied to an EconoColumn containing 60 g Amberlite XAD-16N beads (Sigma Aldrich, Arklow, Co. Wicklow, Ireland). The column was then washed with 350 ml of 30% ethanol, and the antimicrobial activity was eluted with 70% propan-2-ol (IPA) containing 0.1% trifluoroacetic acid (TFA) (Sigma Aldrich). The IPA was removed from the active column eluent and the pH adjusted to 4.4 with 7.5 N NaOH. The sample was then applied to an EconoColumn containing 90 ml SP Sepharose beads preequilibrated with 20 mM sodium acetate buffer (pH 4.4) (buffer A). The column was washed with 50 ml of buffer A and the antimicrobial activity eluted in 250 ml buffer A containing 1 M NaCl. The salt-containing eluent was applied to 60 ml of a 10-g C18 solid-phase extraction (SPE) column (Phenomenex, Cheshire, United Kingdom) preequilibrated with methanol and water. The column was washed with 60 ml of 25% ethanol, and nisin was eluted in 60 ml IPA (0.1% TFA), which was subjected to reversed-phase high-performance liquid chromatography (RP-HPLC). The sample was applied to a semipreparative Proteo Jupiter (250 mm [length] by 10 mm [inside diameter], 90 Å [pore size], 4 μm [particle size]) RP-HPLC column (Phenomenex) running a gradient of 25 to 40% acetonitrile and 0.1% TFA, where buffer B was 90% acetonitrile and 0.1% TFA. The resulting eluent was monitored at 214 nm, and fractions were collected at 1-min intervals. Column eluents and HPLC fractions were assayed for antimicrobial activity by well diffusion assays (WDAs), according to the method of Parente and Hill (41), using L. delbrueckii subsp. bulgaricus LMG 6901 as the target organism. Column eluents and HPLC fractions displaying antimicrobial activity were assayed for the nisin J molecular mass by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on an Axima TOF/TOF MALDI-TOF MS in positive-ion reflectron mode (Shimadzu Biotech, Manchester, United Kingdom). Fractions containing pure nisin J were pooled and lyophilized in a Genevac lyophilizer (Suffolk, United Kingdom). Pure nisin A peptide was prepared from L. lactis NZ9700 as described for nisin J but excluding the SP Sepharose step. Nisin Z pure peptide was sourced from Handay (Fleureus, Belgium).

**Comparison of the inhibitory spectra of nisins A, Z, and J.** Pure nisins A, Z, and J were resuspended in RNase-free water to a final concentration of 1 mg/ml and subsequently assayed by WDA against a range of target indicator strains (Table 2). Zone diameters were measured in millimeters using Vernier calipers (DML-Digital Micrometers Ltd., Sheffield, United Kingdom) and recorded in Table 2 as the area of the zone (πr²) minus the area of the well (πω²) in millimeters.

**MIC determinations.** MICs were determined in triplicate from pure nisins A, Z, and J against approximately 1 × 10⁸ CFU/ml of the target indicator strain Lactococcus lactis subsp. cremoris HP using 96-well microtiter plates (Sarstedt, Co. Wexford, Ireland) and using a Libra S2 colorimeter (Biochrom Ltd., Kingdom). Sequencing data were analyzed employing the Lasergene 8 software (DNAStar, Inc., Madison, Wisconsin). Oligonucleotide primers designed to specifically amplify the nisin J structural gene (Table 1) were used in a PCR under the following conditions: initial denaturation, 94°C for 5 min; 35 cycles of 94°C for 40 s, 52°C for 30 s, and 72°C for 1 min; and a final extension 72°C for 10 min. Primers were designed according to the manufacturer's instructions following an adapted user-developed protocol specific to staphylococcal species (https://www.qiagen.com/ie/resources/resourcedetail?id=82dd6d61-fab4d35-81c9-defd6269fc644e9-en), using lysostaphin (Sigma Aldrich Ireland Limited, Arklow, County Wicklow, Ireland). The resulting DNA extract was sequenced by Illumina MiSeq technology (250-bp paired-end reads; GenProbio, Parma, Italy). De novo sequence assemblies and automated gene calling were performed using the MEGAnnotator pipeline (44) and assessed for predicted tRNA genes via transcend-SE version 1.2.1 (45). Predicted open reading frames (ORFs) were determined via Prodigal version 2.6 and Genemark.hmm (46). A BLASTP (47) analysis was performed to assign functional annotations to the predicted ORFs (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 1). PlasmidFinder (version 2.0) was employed to confirm that the generated assembled contigs were plasmid sequences based on the identification of Rep proteins. SnapGene version 2.3.2 was employed to generate a map of the plasmid harboring the nisJ gene cluster (designated pJO501 here). In additional to the sequence data analysis to confirm the plasmid association of the nisin J cluster, PCR-based analysis was undertaken using the plasmid DNA extract as the template. Oligonucleotide primers designed to specifically amplify the nisin J structural gene (nisJ F, 5'-ACTTATAACTAAGATTAG

---

### Table 1

| Nisin | Gene Cluster | Description |
|------|--------------|-------------|
| A    | pJO501       | Nisin A     |
| Z    | pJO501       | Nisin Z     |
| J    | pJO501       | Nisin J     |

### Table 2

| Strain | MIC (mm) | Comparison |
|-------|----------|------------|
| S. capitis APC 2923 | >10       | Positive   |
| L. delbrueckii subsp. cremoris | >10       | Positive   |
| BHI | >10       | Positive   |

---

**Cross-immunity of nisin J-producing S. capitis APC 2923 to other nisin-producing strains.** To investigate if the nisin J-producing S. capitis APC 2923 strain was immune to other nisin-producing cultures (L. lactis NZ9700 producing nisin A, Streptococcus hyointestinalis DPC 6484 producing nisin H, and S. uberis 42 producing nisin U), cross-immunity assays were performed based on the WDA method, whereby each strain was tested as an indicator and a producer (43).

**Determining if the nisin J structural gene is unique to S. capitis APC 2923.** To determine if the nisin J structural gene was present in other S. capitis strains isolated from the study by O’Sullivan et al. (5), oligonucleotide primers designed to specifically amplify the nisin J structural gene (nisJ F, 5'-ACTTATAACTAAGATTAGC3') and nisJ R, 5'-TCCGTCTTTATTATGATGACG-3') were used in a PCR under the following conditions: initial denaturation, 94°C for 5 min; 35 cycles of 94°C for 40 s, 52°C for 30 s, and 72°C for 1 min; and a final extension 72°C for 10 min. Sequencing data were analyzed employing the Lasergene 8 software (DNASTar, Inc., Madison, WI) and subsequently input into the Expasy online translate tool (https://www.expasy.org/translate/) to translate the nucleotides into amino acid sequences.

**Sequence analysis of the nisin J plasmid pJO501.** To confirm that the nisin J gene cluster was plasmid associated, the plasmid DNA of S. capitis APC 2923 was extracted using the Plasmid maxi kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions following an adapted user-developed protocol specific to staphylococcal species (https://www.qiagen.com/ie/resources/resourcedetail?id=82dd6d61-fab4d35-81c9-defd6269fc644e9-en), using lysostaphin (Sigma Aldrich Ireland Limited, Arklow, County Wicklow, Ireland). The resulting DNA extract was sequenced by Illumina MiSeq technology (2 × 250-bp paired-end reads; GenProbio, Parma, Italy). De novo sequence assemblies and automated gene calling were performed using the MEGAnnotator pipeline (44) and assessed for predicted tRNA genes via transcend-SE version 1.2.1 (45). Predicted open reading frames (ORFs) were determined via Prodigal version 2.6 and Genemark.hmm (46). A BLASTP (47) analysis was performed to assign functional annotations to the predicted ORFs (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 1). PlasmidFinder (version 2.0) was employed to confirm that the generated assembled contigs were plasmid sequences based on the identification of Rep proteins. SnapGene version 2.3.2 was employed to generate a map of the plasmid harboring the nisJ gene cluster (designated pJO501 here). In additional to the sequence data analysis to confirm the plasmid association of the nisin J cluster, PCR-based analysis was undertaken using the plasmid DNA extract as the template. Oligonucleotide primers designed to specifically amplify the nisin J structural gene (nisJ F, 5'-ACTTATAACTAAGATTAG
C^-3\textsuperscript{-} and nis^R, 5'-TGGTATTATTATGATGCAGC-3') were used in a PCR using Phusion Green Hot Start II high-fidelity PCR master mix with the following conditions: initial denaturation, 98°C for 5 min; 30 cycles of 98°C for 10 s, 52°C for 30 s, and 72°C for 15 s; and a final extension of 72°C for 10 min. Validation of the amplicon was performed by Sanger sequencing of the generated product (Source BioScience, Waterford, Ireland). Furthermore, restriction digestion of the plasmid DNA was carried out using EcoRI in 10\textsuperscript{\textdegree}C CutSmart buffer (New England BioLabs, Herts, United Kingdom).

**Investigation for the presence of nisin-resistant determinants in S. capitis APC 2923.** To determine if the gene encoding the nisin resistance protein (NSR) was present in *S. capitis* APC 2923 and the 7 other *S. capitis* isolates previously identified from the O’Sullivan et al. study (3), PCR was employed using the primers and reaction conditions described by Simões et al. (38). To determine if the nisin J-producing *S. capitis* strain APC 2923 was cross-immune to or sensitive to NSR-producing strains, bioassays were carried out by spotting 10 \mu{l} of the nisin J overnight culture onto 1.5% BHI agar (Merck, Darmstadt, Germany). Following overnight incubation at 37\textdegreeC, the plates were then overlaid with soft (0.75%) GM17 agar (BD Difco Trafalgar Scientific Ltd, Leicester, United Kingdom) seeded with 0.25% of an overnight culture of the NSR-positive strain *L. lactis* subsp. *diacetylactis* DRC3. To directly compare the resistance levels of nisin A and nisin J to NSR, WDAs were carried out as previously described (43), employing *L. lactis* MG1614/pNP40 (NSR-positive strain) and *L. lactis* MG1614 (NSR-negative strain) as target indicators. All lactococcal NSR indicator strains were grown aerobically overnight at 30\textdegreeC. Agarose assays were subsequently performed as outlined in reference 42. Data obtained from the agarose assays were subjected to normality tests prior to statistical analysis using the GraphPad Prism software (version 8.2.1). P values were calculated using an unpaired t test.

**Data availability.** The plasmid map of pOSO1 has been deposited in GenBank under accession number MN602039. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number WHYU00000000. The version described in this paper is version WHYU00000001.

**ACKNOWLEDGMENTS**

J.N.O. was supported by a studentship from Science Foundation Ireland (SFI; https://www.sfi.ie/) under grant SFI/12/RC/2273.

We declare no competing interests.

**REFERENCES**

1. Sender R, Fuchs S, Milo R. 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 14:e1002533. https://doi.org/10.1371/journal.pbiol.1002533.
2. Cameron DR, Jiang JH, Hassan KA, Elbourne LDH, Tuck KL, Paulsen IT. 2013. The effects of continuous in vivo hyperglycaemia on gut microbiota and host function. Proc Natl Acad Sci USA 110:4048–4053. https://doi.org/10.1073/pnas.1210823110.
3. Sugai M, Fujiwara T, Akiyama T, Ohara M, Komatsuzawa H, Inoue S, Suginaka H. 1997. Purification and molecular characterization of glycyl-glycine endopeptidase produced by Staphylococcus capitis EPK1. J Bacteriol 179:1193–1202. https://doi.org/10.1128/JB.179.4.1193-1202.1997.
4. Kumar R, Jangir PK, Das J, Taneja B, Sharma R. 2017. Genome analysis of *Staphylococcus capitis* TE8 reveals repertoire of antimicrobial peptides and adaptation strategies for growth on human skin. Sci Rep 7:10447. https://doi.org/10.1038/s41598-017-11020-7.
5. O’Sullivan JN, Rea MC, O’Connor PM, Hill C, Ross RP. 2019. Human skin microbiota is a rich source of bacteriocin-producing staphylococci that kill human pathogens. FEMS Microbiol Ecol 95:fy241. https://doi.org/10.1093/femsec/fy241.
6. Dobson A, Cotter PD, Paul Ross R, Hill C. 2012. Bacteriocin production: a probiotic trait? Appl Environ Microbiol 78:1–6. https://doi.org/10.1128/AEM.05576-11.
7. Corr SC, Li Y, Riedel CU, O’Toole PW, Hill C, Gahan CGM. 2007. Bacteriocin production as a mechanism for the antinfective activity of Lactobacillus salivarius UCC118. Proc Natl Acad Sci U S A 104:7617–7621. https://doi.org/10.1073/pnas.0700440104.
8. Cotter PD, Hill C, Ross RP. 2005. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3:777–788. https://doi.org/10.1038/nrmicro1273.
9. de Pablo MA, Gaforio JJ, Gallego AM, Ortega E, Gálvez AM, Alvarez de Cienfuegos López G. 1999. Evaluation of immunomodulatory effects of nisin-containing diets on mice. FEMS Immunol Med Microbiol 24:35–42. https://doi.org/10.1111/j.1574-695X.1999.tb01262.x.
10. Brand AM, Smith C, Dicks L. 2013. The effects of continuous in vivo administration of nisin on *Staphylococcus aureus* aureus infection and immune response in mice. Probiotics Antimicrob Proteins 5:279–286. https://doi.org/10.1007/s12602-013-9141-3.
11. Małaczewska J, Kaszorek-Łukowska E, Wójcik R, Rękaewek W, Siwicki AK. 2019. In vitro immunomodulatory effect of nisin on porcine leucocytes. J Anim Physiol Anim Nutr (Berl) 103:882–893. https://doi.org/10.1111/janp.13085.
12. Kindrachuk J, Jenssen H, Elliott M, Nijnik A, Magrandeas-Janot L, Pasupuleti M, Thorsen L, Ma S, Easton DM, Bains M, Finlay B, Breukink EJ, Georg-Sahl H, Hancock R. 2013. Manipulation of innate immunity by a bacterial secreted peptide: lantibiotic nisin Z is selectively immunomodulatory. Innate Immun 19:315–327. https://doi.org/10.1017/S1753425912461456.
13. Rogers LA, Whittier ED. 1928. Limiting factors in the lactic fermentation. J Bacteriol 16:211–229.
14. Delves-Brown and J. 2001. Nisin as a food preservative. Food Aust 57:525–527.
15. Cheigh CI, Pyun YR. 2005. Nisin biosynthesis and its properties. Biotechnol Lett 27:1641–1648. https://doi.org/10.1023/B:MBIO.000005297-x.
16. Cotter PD, Ross RP, Hill C. 2013. Bacteriocins—a viable alternative to antibiotics? Nat Rev Microbiol 11:95–105. https://doi.org/10.1038/nrmicro2937.
17. McAuliffe O, Ross RP, Hill C. 2001. Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol Rev 25:285–308. https://doi.org/10.1111/j.1574-6976.2001.tb00579.x.
18. Kellner R, Jung G, Horner T, Zahnert H, Schnell N, Entian K-D, Gotz F. 1988. Gallidermin: a new lanthionine-containing polypeptide antibiotic. Eur J Biochem 177:53–59. https://doi.org/10.1111/j.1432-1033.1988.tb14344.x.
19. Schnell N, Entian K-D, Schneider U, Götz F, Zahnert H, Kellner R, Jung G. 1988. Preptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings: Nature 333:276–278. https://doi.org/10.1038/333276a0.
20. Kim PI, Sohng JK, Sung C, Joo H-S, Kim E-M, Yamaguchi T, Park D, Kim B-G. 2010. Characterization and structure identification of an antimicrobial peptide, hominicin, produced by *Staphylococcus hominis* MBBL 2-9. Biochem Biophys Res Commun 399:133–138. https://doi.org/10.1016/j.bbrc.2010.07.024.
21. Alvarez-Sieiro P, Montalbán-López M, Mu D, Kuipers OP. 2016. Bacteriocins of lactic acid bacteria: extending the family. Appl Microbiol Biotechnol 100:2939–2951. https://doi.org/10.1007/s00253-016-7343-9.

February 2020 Volume 202 Issue 3 e00639-19

jb.asm.org 14
22. Ingram LC. 1969. Synthesis of the antibiotic nisin: formation of lantio-
 nine and beta-methyl-lantionine. Biochim Biophys Acta 184:216–219. https://doi.org/10.1016/0304-4165(69)90121-4.

23. Ingram L. 1970. A ribosomal mechanism for synthesis of peptides related
 to nisin. Biochim Biophys Acta 224:263–265. https://doi.org/10.1016/0006-2787(70)90642-8.

24. van der Donk WA, Nair SK. 2014. Structure and mechanism of lantih-
 peptide biosynthetic enzymes. Curr Opin Struct Biol 25:58–66. https://doi.org/10.1016/j.sbi.2014.09.006.

25. Bauer R, Dicks LM. 2005. Mode of action of lipid II-targeting lantibiotics.
 Int J Food Microbiol 101:201–216. https://doi.org/10.1016/j.ijfoodmicro.2004.11.007.

26. Egan K, Ross RP, Hill C. 2017. Bacteriocins: antibiotics in the age of
 the microbiome. Emerg Top Life Sci 1:55–63. https://doi.org/10.1042/ETLS20160015.

27. O’Connor PM, O’Shea EF, Guinan CM, O’Sullivan O, Cotter PD, Ross RP, Hill C. 2015. Nisin H is a new nisin variant produced by the gut-derived
 strain Streptococcus hyointestinalis DPC6484. Appl Environ Microbiol 81:3953–3960. https://doi.org/10.1128/AEM.00212-15.

28. Nishida H. 2012. Comparative analyses of base compositions, DNA sizes, and dinucleotide frequency profiles in archaeal and bacterial chromo-
 somes and plasmids. J Evol Biol 2012:342482. https://doi.org/10.1155/2012/342482.

29. Kuipers OP, Rollema HS, Yap WM, Boot HJ, Siezen RJ, de Vos WM. 1992.
 Engineering dehydrated amino acid residues in the antimicrobial peptide
 nisin. J Biol Chem 267:24340–24346.

30. Li Q, Montalban-Lopez M, Kuipers OP. 2018. Increasing the antimicrobial
 activity of nisin-based lantibiotics against Gram-negative pathogens.
 Appl Environ Microbiol 84:e00052-18. https://doi.org/10.1128/AEM .00052-18.

31. Stein T, Heinzmann S, Düsterhus S, Borchert S, Entian K-D. 2005. Expres-
 sion and functional analysis of the subtilin immunity genes spaFEG in the
 subtilin-sensitive host Bacillus subtilis MO1099. J Bacteriol 187:
 822–828. https://doi.org/10.1128/JB.187.3.822-828.2005.

32. Otto M, Peschel A, Götz F. 1998. Producer self-protection against the
 lantibiotic epidermin by the ABC transporter EpiFEG of Staphylococcus
epidermidis TSU298. FEMS Microbiol Lett 166:203–211. https://doi.org/10.1111/j.1574-6968.1998.tb13891.x.

33. Stein T, Heinzmann S, Solovieva I, Entian KD. 2003. Function of Lacto-
coccus lactis nisin immunity genes nisF and nisFEG after coordinated
 expression in the surrogate host Bacillus subtilis. J Biol Chem 278:89–94. https://doi.org/10.1074/jbc.M207237200.

34. Siegers K, Entian KD. 1995. Genes involved in immunity to the lantibiotic
 nisin produced by Lactococcus lactis 6F3. Appl Environ Microbiol 61:
 1082–1089.

35. Ebner P, Reichert S, Luqman A, Krismer B, Popella P, Götz F. 2018.
 Lantibiotic production is a burden for the producing staphylococci. Sci Rep 8:7471. https://doi.org/10.1038/s41598-018-25935-2.

36. Wysocki VH, Raising KA, Zhang Q, Cheng G. 2005. Mass spectrometry of
 peptides and proteins. Methods 35:211–222. https://doi.org/10.1016/j.
ymeth.2004.08.013.

37. Field D, Blake T, Mathur H, O’Connor PM, Cotter PD, Ross RP, Hill C.
 Bioengineering nisin to overcome the nisin resistance protein. Mol Microbiol 111:717–731. https://doi.org/10.1111/mmi.14183.

38. Simões PM, Lemirri H, Dumont Y, Lemirri S, Rasigade J-P, Assant-
 Trouillet S, Ibrahimii A, El Kabbaj S, Butin M, Laurent F. 2016. Single-
molecule sequencing (PaBio) of the Staphylococcus capitis NRCS—a clone reveals the basis of multidrug resistance and adaptation to the
 prolonged intensive care unit environment. Front Microbiol 7:1991. https://doi.org/10.3389/fmicb.2016.01991.

39. Rink R, Wierenga J, Kuipers A, Kluksens LD, Driessen AJM, Kuipers OP, Moll GN. 2007. Dissection and modulation of the four distinct activities
 of nisin by mutagenesis of rings A and B and by C-terminal truncation.
 Appl Environ Microbiol 73:5809–5816. https://doi.org/10.1128/AEM.01104-07.

40. Field D, O’Connor PM, Cotter PD, Hill C, Ross GP. 2008. The generation of
 nisin variants with enhanced activity against specific Gram-positive
 pathogens. Mol Microbiol 69:218–230. https://doi.org/10.1111/j.1365-
 2958.2008.06279.x.

41. Parente E, Hill C. 1992. A comparison of factors affecting the production
 of two bacteriocins from lactic acid bacteria. J Appl Bacteriol 73:
 290–298. https://doi.org/10.1111/j.1365-2672.1992.tb04990.x.

42. Field D, Begley M, O’Connor PM, Daly KM, Hugenholtz F, Cotter PD, Hill
 C, Ross RP. 2012. Bioengineered nisin A derivatives with enhanced
 activity against both gram positive and gram negative pathogens. PLoS One 7:e46884. https://doi.org/10.1371/journal.pone.0046884.

43. Ryan MP, Rea MC, Hill C, Ross RP. 1996. An application in cheddar cheese
 manufacture for a strain of Lactococcus lactis producing a novel broad-
spectrum bacteriocin, lacticiin 3147. Appl Environ Microbiol 62:612–619.

44. Lugli GA, Milani C, Mancabelli L, van Sinderen D, Ventura M. 2016. MEGAnnotator: a user-friendly pipeline for microbial genomes assembly
 and annotation. FEMS Microbiol Lett 363:fnw049. https://doi.org/10.1093/femsle/fnw049.

45. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detec-
tion of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964. https://doi.org/10.1093/nar/25.5.955.

46. Besemer J, Borodovsky M. 1999. Heuristic approach to deriving models
 for gene finding. Nucleic Acids Res 27:3911–3920. https://doi.org/10.1038/3
 .nar.27.19.3911.

47. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman
 DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein
database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar.25.17.3389.

48. Zhang Q, Yu Y, Velasquez JE, van der Donk WA. 2012. Characterization of
 the structural gene encoding nisin F, a new lantibiotic produced by a
 Lactococcus lactis subsp. lactis isolate from freshwater catfish (Clarias
 gariepinus). Appl Environ Microbiol 74:547–549. https://doi.org/10.1128/AEM.01862-07.

49. De Kwastteniet M, Ten Doeschate K, Dicks LMT. 2008. Characterization of
 the structural gene encoding nisin Z, a natural nisin variant. Eur J Biochem 201:581–584.

50. Zhang Q, Yu Y, Velasquez JE, van der Donk WA. 2012. Evolution of
 lantipeptide synthetases. Proc Natl Acad Sci 109:18361–18366. https://doi.org/10.1073/pnas.1210393109.

51. Hatzioannou D, Gherghisan-Filip C, Saalbach G, Horn N, Wegmann U, Dun-
 chan SH, Flint HJ, Mayer MJ, Narbad A. 2017. Discovery of a novel
 lantibiotic nisin O from Blautia obeum A2-162, isolated from the human
 gastrointestinal tract. Microbiology 163:1292–1305. https://doi.org/10.1099/mic.0.000515.