Supplementary material

Therapeutic application of lantibiotics and other lanthipeptides: old and new findings

Anton Du Preez van Staden[a,b]*, Winschau van Zyl[a], Marla Trindade[c], Leon M.T Dicks[a], Carine Smith[b]

[a] Department of Microbiology, Stellenbosch University, South Africa

[b] Division of Clinical Pharmacology, Department Medicine, Stellenbosch University, South Africa

[c] Institute for Microbial Biotechnology and Metagenomics, University of the Western Cape, South Africa

* Corresponding Author: AD van Staden (advstaden@outlook.com)

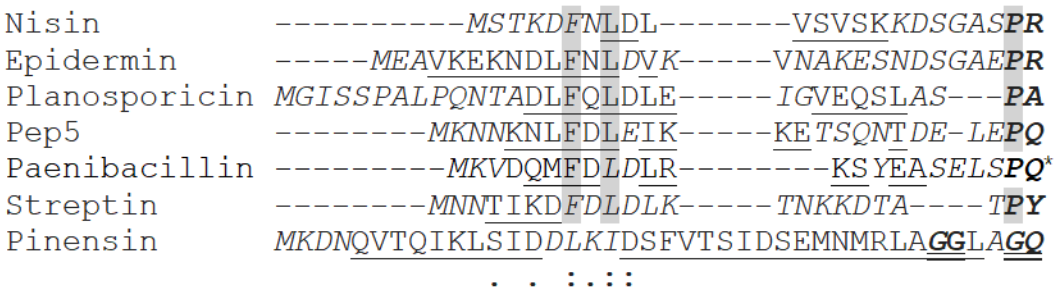
Classification of Lanthipeptides

For the purpose of this review, a combined classification system, based on amino acid sequence similarity of unmodified precursor peptides (without leader sequence) and biosynthetic machinery, was followed [1,2]. According to this classification scheme, lanthipeptides are separated into four classes and 16 subgroups (Fig. S1-S4; Fig. 1 main text). It should be mentioned that the classification of lanthipeptides will evolve with the emergence of new methods for classifying lanthipeptides and the discovery of putative lanthipeptides from newly reported genome sequences [3–5]. The reader is referred to the review by Repka et al [6] for an extensive overview on the mechanistic action of the modification enzymes involved in PTMs.

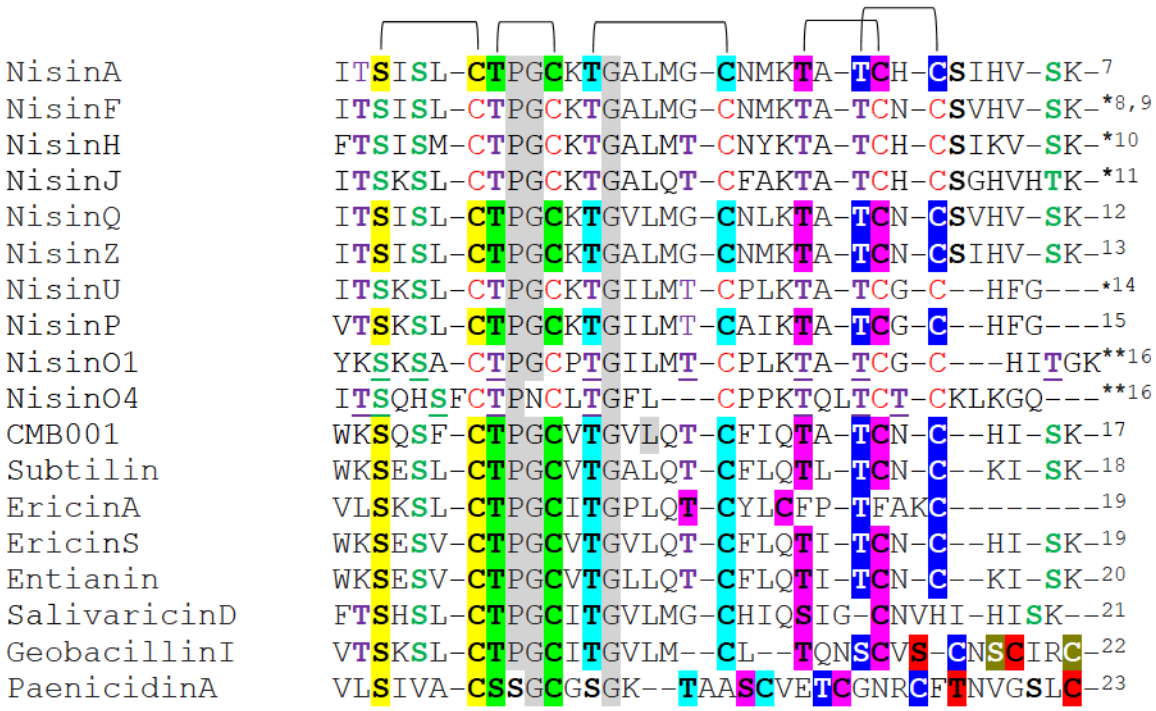
Sequence similarity network of lanthipeptide core peptides (Fig. 1 main text) were generated with the Enzyme Function Initiative-Enzyme Similarity Tool (https://efi.igb.illinois.edu/efi-est/) using an E-value cutoff of 10^{-3} and visualized using Cytoscape (v3.8.0). Core peptides used for analysis were selected based availability of experimental data and hypothetical peptides were excluded. Prediction of helical motifs in leader peptides were done using PEP2D (http://crdd.osdd.net/raghava/pep2d/index.html).

Figure S1

A

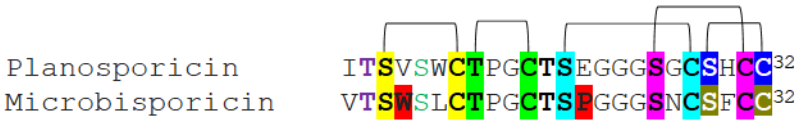


B

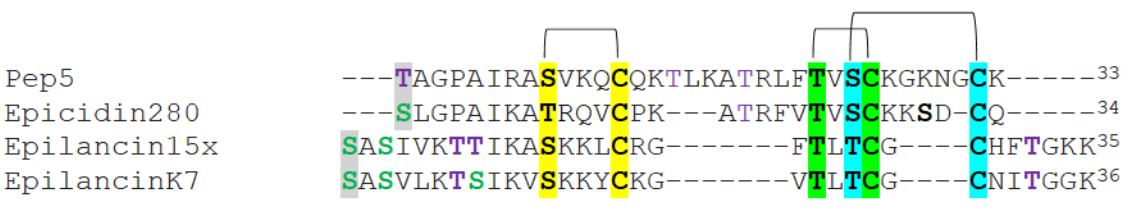


C

D



E



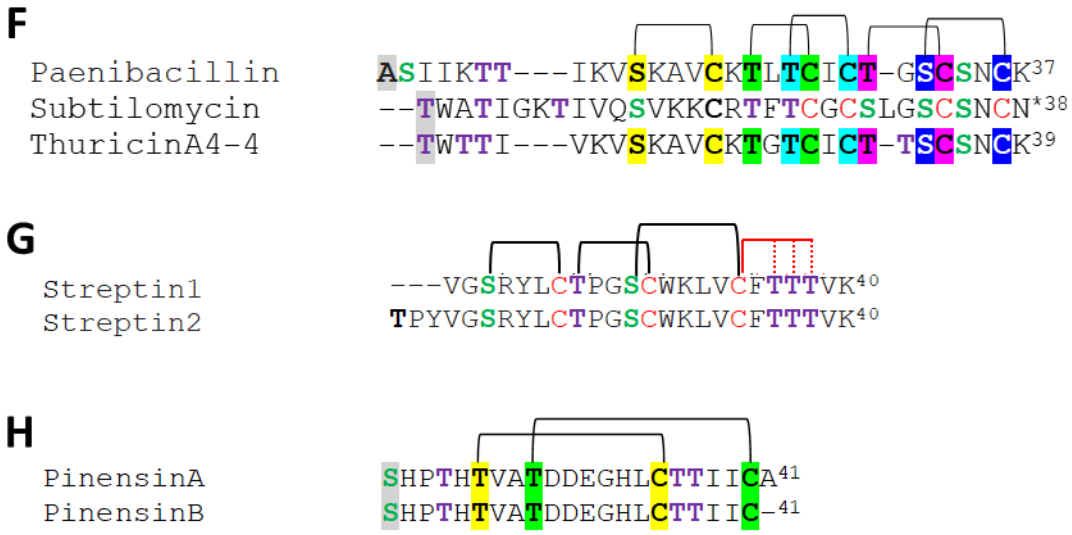
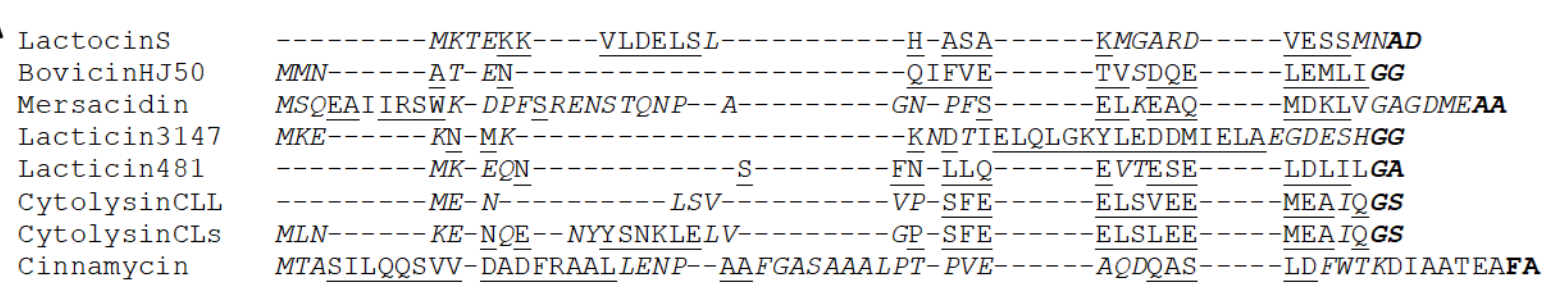


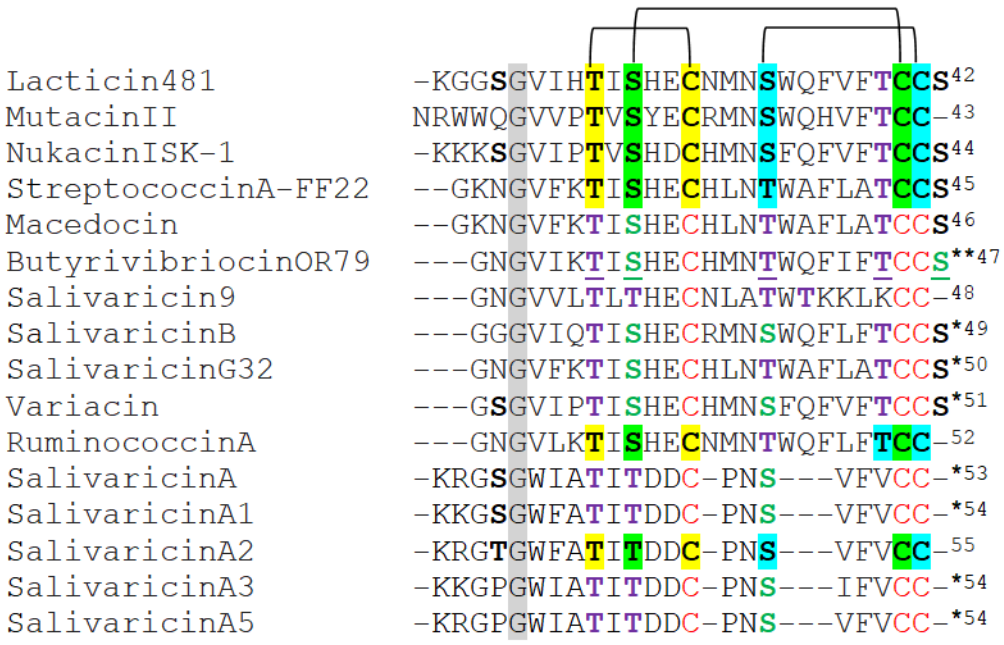
Figure S1: Class I lanthipeptides. Where possible structural information is given and lines above letters indicate ring topology of prototypical lanthipeptide. Unless otherwise stated; Letters highlighted in corresponding colours indicates residues that participate in Lan/Melan formation. Bold letters in purple and green designate Thr/Ser that are dehydrated, respectively. Bold black letters indicate Thr/Ser that escapes dehydration. **A) Leader peptides of prototypical class I lanthipeptides.** Strongly- and weakly-conserved residues are indicated by (:) and (.), respectively. Bold letters indicate LanP (lanthipeptide protease) cleavage sites and bold double underlined letters indicates putative cleavage sites for pinensin. Highlighted grey letters indicate conserved residues. * Predicted from WP_080561132.1. Residues predicted to be involved in helical or coiled structures are underlined and italicized, respectively. Structure prediction performed using PEP2D (http://crdd.osdd.net/raghava/pep2d/index.html). **B) Nisin-like lanthipeptides.** * Dehydrated Ser/Thr residues predicted based on mass of modified peptide and sequence similarity. ** No accurate mass data reported, dehydratable residues coloured and underlined. **C) Epidermin-like lanthipeptides.** Blue line above letters represents C-terminal AviCys. * Dehydrated Ser/Thr residues predicted based on mass of modified peptide and sequence similarity. **D) Planosporicin-like lanthipeptides.** Red highlighted Trp and Pro are chlorinated to chlorotryptophan and hydroxylated to dihydroxyproline, respectively. Gold highlighted letters in microbisporicin indicate amino acids involved in AviCys formation. **E) Pep5-like lanthipeptides.** Grey highlighted Ser/Thr indicate residues that undergo hydration-deamination. **F) Paenibacillin-like lanthipeptides.** Grey highlighted letters represent Ala/Thr that undergo acetylation and hydration-deamination, respectively. **G) Streptin-like lanthipeptides.** Red lines indicate alternative ring C for streptin with dashed lines indicating possible bridging patterns. **H) Pinensin-like lanthipeptides.** Grey highlighted letters represent Ser that undergoes spontaneous hydration-deamination.

Figure S2

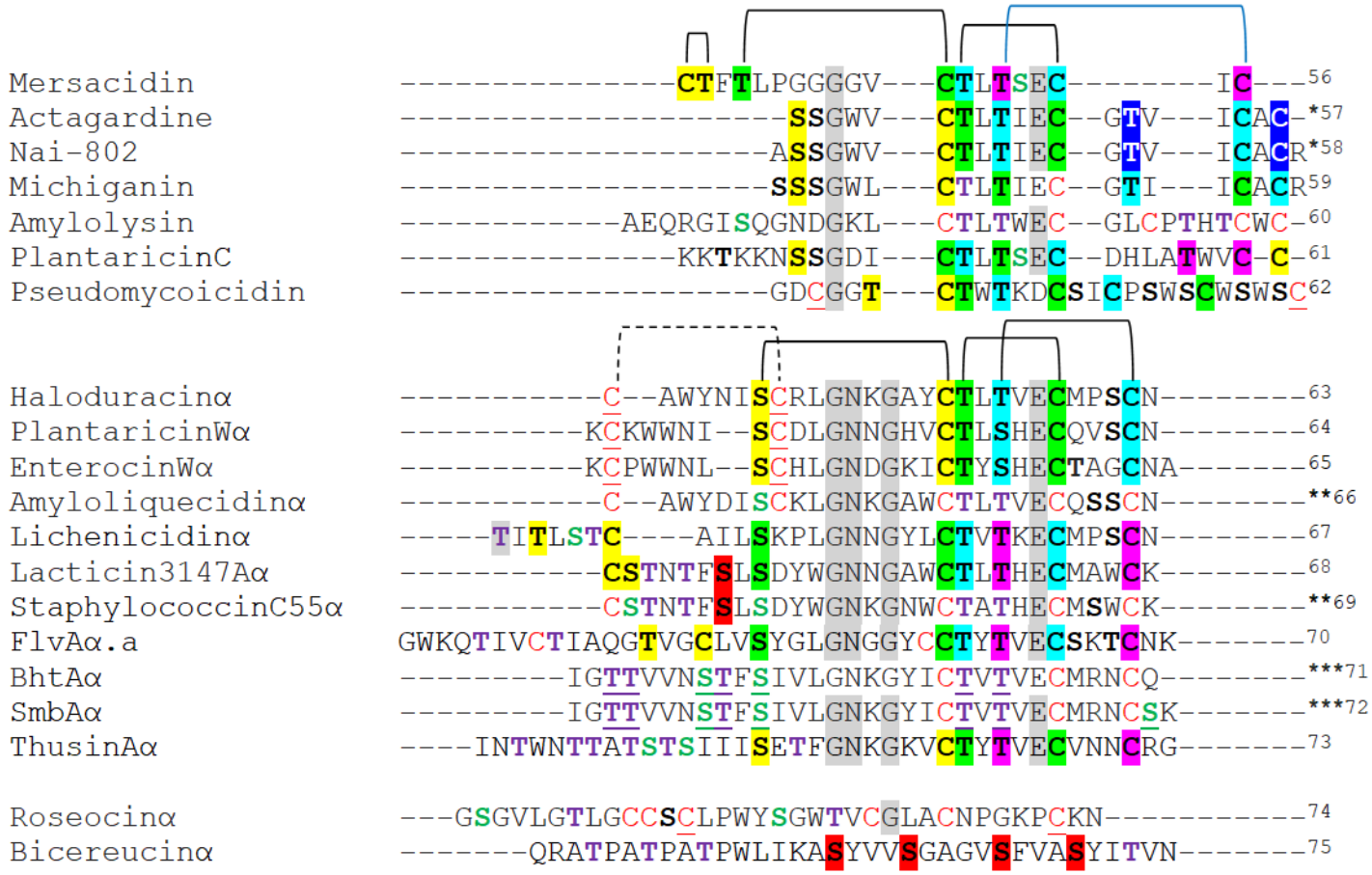
A



B



C



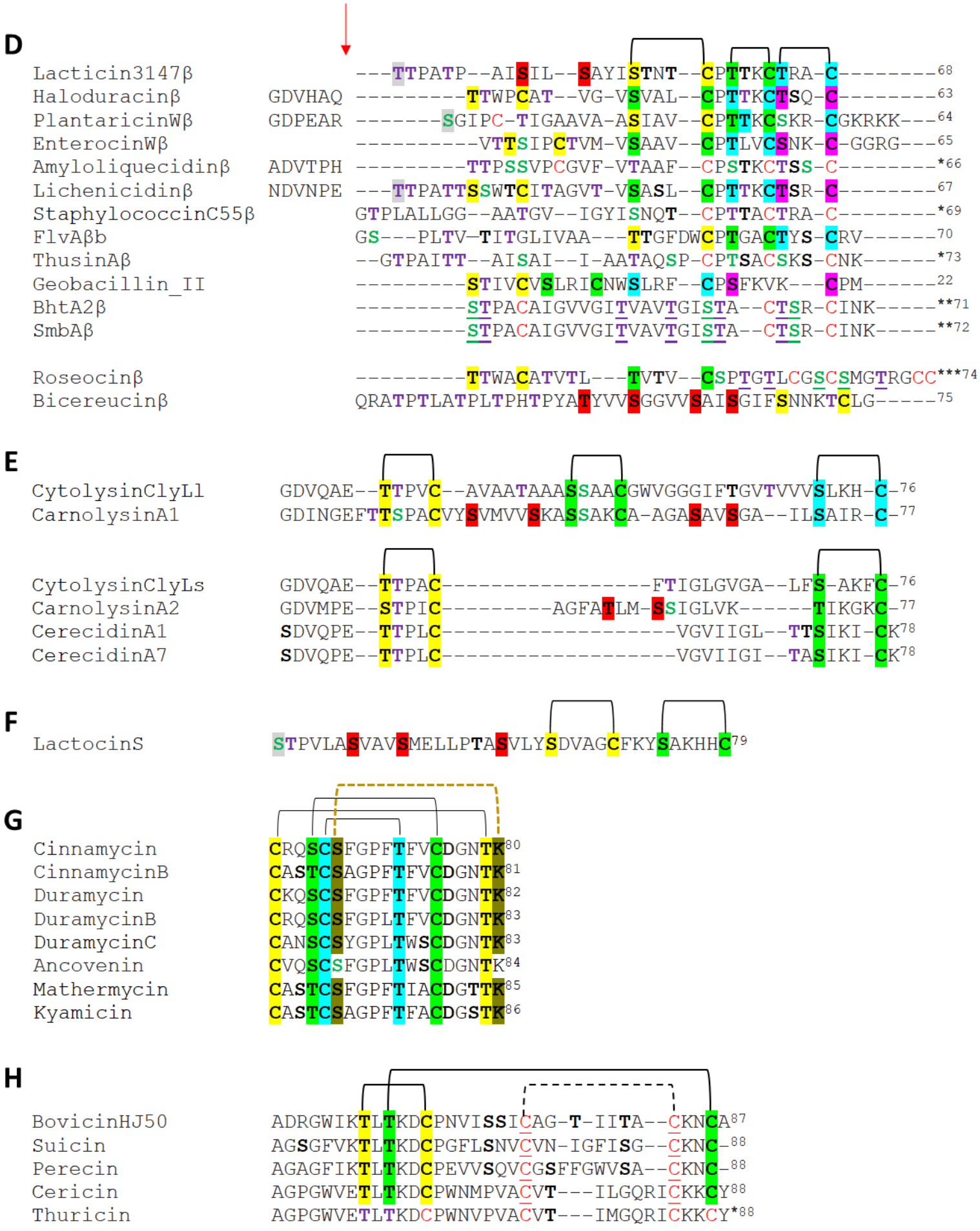

D
Lacticin3147β
HaloduracinβGDVHAQ
PlantaricinWβGDPEAR
EnterocinWβ
AmyloliquecidinβADVTPH
LichenicidinβNDVNPE
StaphylococcinC55β
FlvAβb
ThusinAβ
Geobacillin_II
BhtA2β
SmbAβ
Roseocinβ
Bicereucinβ
E
CytolysinClyLl
CarnolysinA1
CytolysinClyLs
CarnolysinA2
CerecidinA1
CerecidinA7
F
LactocinS
G
Cinnamycin
CinnamycinB
Duramycin
DuramycinB
DuramycinC
Ancovenin
Mathermycin
Kyamicin
H
BovicinHJ50
Suicin
Perecin
Cericin
Thuricin

Figure S2: Class II lanthipeptides. Where possible structural information is given and lines above letters indicate ring topology of prototypical lanthipeptide. Unless otherwise stated; Letters highlighted in corresponding colours indicates residues that participate in Lan/Melan formation. Bold letters in purple and green designate Thr/Ser that are dehydrated, respectively. Bold black letters indicate Thr/Ser that escapes dehydration. **A) Leader peptides of prototypical class II lanthipeptides.** Bold letters indicate site of leader cleavage. Residues predicted to be involved in helical or coiled structures are underlined and italicized, respectively. Structure prediction performed using PEP2D (http://crdd.osdd.net/raghava/pep2d/index.html). **B) Lacticin 481-like lanthipeptides.** Grey highlighted Gly is conserved throughout group. * Position of dehydrated Ser/Thr residues predicted based on mass of modified peptide and sequence similarity. ** No accurate mass data reported, dehydratable residues coloured and underlined. **C) Mersacidin-like lanthipeptides.** Lines above letters indicates the ring topology of mersacidin (blue line indicates AviMeCys) and haloduracin-α, respectively. Dashed line indicates a disulphide bridge in haloduracin-α, with underlined Cys residues taking part in disulphide bridge formation. Grey highlighted residues are conserved throughout group. Red highlighted Ser are converted to $_{D}$-Ala. * Blue highlighted letters form methyllanthionine sulfoxide. ** Position of dehydrated Ser/Thr residues predicted based on mass of modified peptide and sequence similarity. *** Exact position of dehydrated residues unknown, dehydratable residues coloured and underlined. **D) LtnA2-like lanthipeptides.** Grey highlighted Thr are converted to 2-oxobutyryl. Red highlighted Ser/Thr are converted to $_{D}$-Ala/ $_{D}$-Abu, respectively. Red arrow indicates site of second proteolytic cleavage. * Position of dehydrated Ser/Thr residues predicted based on mass of modified peptide and sequence similarity. ** Exact position of dehydrated residues unknown, dehydratable residues coloured and underlined. *** Dehydration status of underlined letters unknown (six out of nine dehydrated Ser/Thr known). **E) Cytolysin-like lanthipeptides.** Lines above letters indicate the ring topology of cytolysinC_L and cytolysinC_S, respectively. Red highlighted Ser and Thr are converted to $_{D}$-Ala and $_{D}$-Abu, respectively. **F) Lactocin-like lanthipeptides.** Red highlighted Ser are converted to $_{D}$-Ala. Grey highlighted letter represents Ser that undergoes hydration-deamination. **G) Cinnamycin-like lanthipeptides.** Bold black letters indicate Ser/Thr that escapes dehydration and Asp that undergoes hydroxylation, respectively. Dashed gold line and highlighted letters indicates lysinoalanine bridge and residues involved. **H) Bovicin HJ50-like lanthipeptides.** Dashed line indicates a disulphide bridge with underlined Cys residues taking part in disulphide bridge formation. * Position of dehydrated Ser/Thr residues predicted based on mass of modified peptide and sequence similarity.

Figure S3

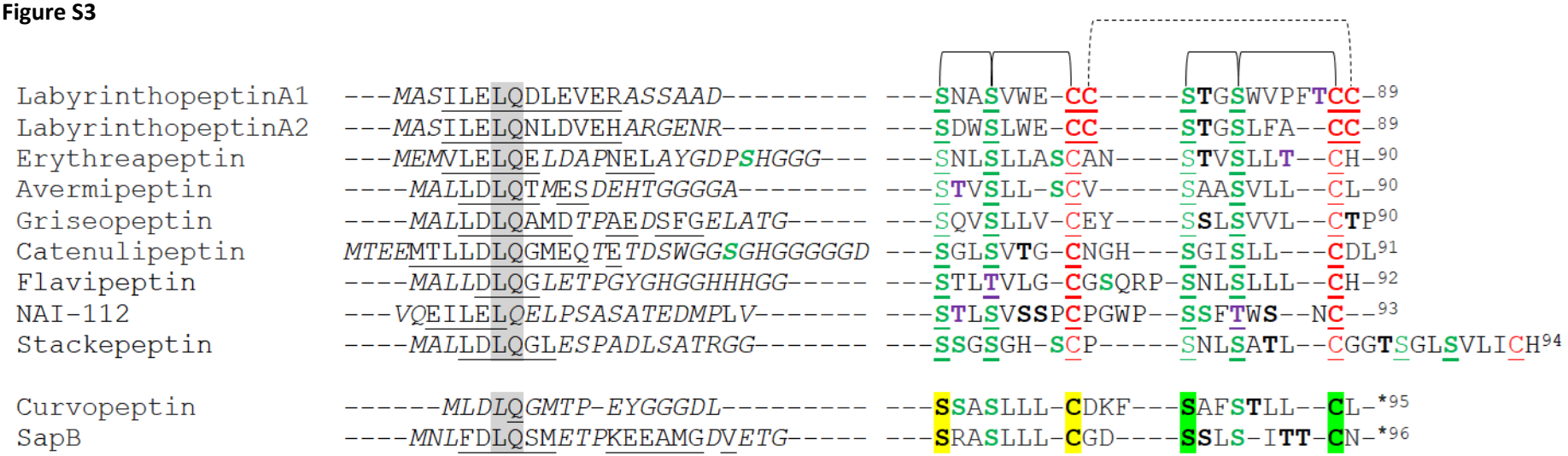


Figure S3: Labyrinthopeptin-like lanthipeptides (Class III). Precursor sequences are illustrated due to conflicting results regarding leader peptide processing. Residues predicted to be involved in helical or coiled structures are underlined and italicized, respectively. Structure prediction performed using PEP2D (http://crdd.osdd.net/raghava/pep2d/index.html). Lines above letters indicate the ring topology of labyrinthopeptinA1 (labionin rings) and dashed line indicates a disulphide bridge. Amino acids involved in lanthionine/labionin formation are indicated Cys residues are indicated in red. Purple and green letters indicate dehydrated Thr and Ser, respectively. Bold and underlined letters indicate residues that take part in labionin formation (Ser/Thr must be dehydrated). Letters underlined and not in bold indicate residues that can be involved in lanthionine or labionin formation. Bold black letters indicate Thr that escape dehydration. * Labionin residues only present in trace amounts, residues involved in Lan formation are highlighted in corresponding colours.

Figure S4

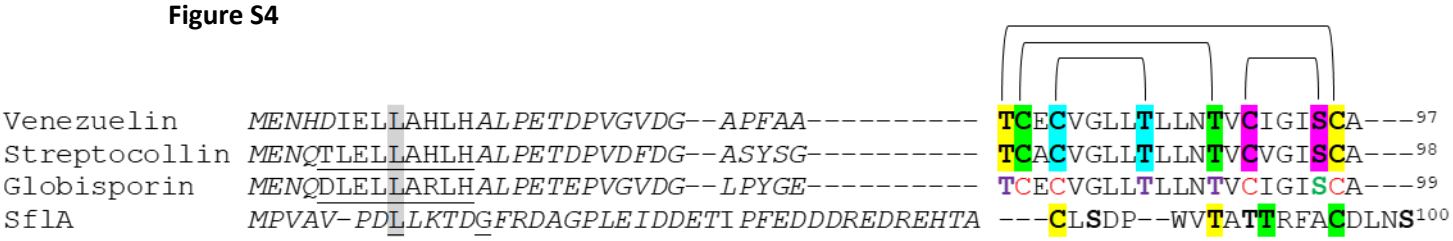


Figure S4: Venezuelin-like lanthipeptides (Class IV). Precursor sequences are illustrated due to conflicting results regarding leader peptide processing. Residues predicted to be involved in helical or coiled structures are underlined and italicized, respectively. Structure prediction performed using PEP2D (http://crdd.osdd.net/raghava/pep2d/index.html). Lines above letters indicates ring topology for venezuelin. Highlight letters indicate residue that participate in Lan/MeLan formation. Bold purple and green Thr/Ser are dehydrated and bold black Thr/Ser escapes dehydration.

References

(1) Cotter, P.; Hill, C.; Ross, R. Bacterial Lantibiotics: Strategies to Improve Therapeutic Potential. *Curr. Protein Pept. Sci.* **2005**, *6* (1), 61–75. https://doi.org/10.2174/1389203053027584.

(2) Arnison, P. G.; Bibb, M. J.; Bierbaum, G.; Bowers, A. A.; Bugni, T. S.; Bulaj, G.; Camarero, J. A.; Campopiano, D. J.; Challis, G. L.; Clardy, J.; Cotter, P. D.; Craik, D. J.; Dawson, M.; Dittmann, E.; Donadio, S.; Dorrestein, P. C.; Entian, K.-D.; Fischbach, M. A.; Garavelli, J. S.; Göransson, U.; Gruber, C. W.; Haft, D. H.; Hemscheidt, T. K.; Hertweck, C.; Hill, C.; Horswill, A. R.; Jaspars, M.; Kelly, W. L.; Klinman, J. P.; Kuipers, O. P.; Link, A. J.; Liu, W.; Marahiel, M. A.; Mitchell, D. A.; Moll, G. N.; Moore, B. S.; Müller, R.; Nair, S. K.; Nes, I. F.; Norris, G. E.; Olivera, B. M.; Onaka, H.; Patchett, M. L.; Piel, J.; Reaney, M. J. T.; Rebuffat, S.; Ross, R. P.; Sahl, H.-G.; Schmidt, E. W.; Selsted, M. E.; Severinov, K.; Shen, B.; Sivonen, K.; Smith, L.; Stein, T.; Süssmuth, R. D.; Tagg, J. R.; Tang, G.-L.; Truman, A. W.; Vederas, J. C.; Walsh, C. T.; Walton, J. D.; Wenzel, S. C.; Willey, J. M.; van der Donk, W. A. Ribosomally Synthesized and Post-Translationally Modified Peptide Natural Products: Overview and Recommendations for a Universal Nomenclature. *Nat. Prod. Rep.* **2013**, *30* (1), 108–160. https://doi.org/10.1039/C2NP20085F.

(3) Hetrick, K. J.; van der Donk, W. A. Ribosomally Synthesized and Post-Translationally Modified Peptide Natural Product Discovery in the Genomic Era. *Curr. Opin. Chem. Biol.* **2017**, *38*, 36–44. https://doi.org/10.1016/j.cbpa.2017.02.005.

(4) Velásquez, J. E.; van der Donk, W. A. Genome Mining for Ribosomally Synthesized Natural Products. *Curr. Opin. Chem. Biol.* **2011**, *15* (1), 11–21. https://doi.org/10.1016/j.cbpa.2010.10.027.

(5) Zhang, Q.; Yu, Y.; Vélasquez, J. E.; Van Der Donk, W. A. Evolution of Lanthipeptide Synthetases. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (45), 18361–18366. https://doi.org/10.1073/pnas.1210393109.

(6) Repka, L. M.; Chekan, J. R.; Nair, S. K.; van der Donk, W. A. Mechanistic Understanding of Lanthipeptide Biosynthetic Enzymes. *Chem. Rev.* **2017**, *117* (8), 5457–5520. https://doi.org/10.1021/acs.chemrev.6b00591.

(7) Gross, E.; Morell, J. L. Structure of Nisin. *J. Am. Chem. Soc.* **1971**, *93* (18), 4634–

4635. https://doi.org/10.1021/ja00747a073.

(8) De Kwaadsteniet, M.; Ten Doeschate, K.; Dicks, L. M. T. 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.* **2008**, *74* (2), 547–549. https://doi.org/10.1128/AEM.01862-07.

(9) Piper, C.; Hill, C.; Cotter, P. D.; Ross, R. P. Bioengineering of a Nisin A-Producing Lactococcus Lactis to Create Isogenic Strains Producing the Natural Variants Nisin F, Q and Z. *Microb. Biotechnol.* **2011**, *4* (3), 375–382. https://doi.org/10.1111/j.1751-7915.2010.00207.x.

(10) O'Connor, P. M.; O'Shea, E. F.; Guinane, C. M.; O'Sullivan, O.; Cotter, P. D.; Ross, R. P.; Hill, C. Nisin H Is a New Nisin Variant Produced by the Gut-Derived Strain Streptococcus Hyointestinalis DPC6484. *Appl. Environ. Microbiol.* **2015**, *81* (12), 3953–3960. https://doi.org/10.1128/AEM.00212-15.

(11) O'Sullivan, J. N.; O'Connor, P. M.; Rea, M. C.; O'Sullivan, O.; Walsh, C. J.; Healy, B.; Mathur, H.; Field, D.; Hill, C.; Paul Ross, R. Nisin J, a Novel Natural Nisin Variant, Is Produced by Staphylococcus Capitis Sourced from the Human Skin Microbiota. *J. Bacteriol.* **2020**, *202* (3), e00639-19. https://doi.org/10.1128/JB.00639-19.

(12) Fukao, M.; Obita, T.; Yoneyama, F.; Kohda, D.; Zendo, T.; Nakayama, J.; Sonomoto, K. Complete Covalent Structure of Nisin Q, New Natural Nisin Variant, Containing Post-Translationally Modified Amino Acids. *Biosci. Biotechnol. Biochem.* **2008**, *72* (7), 1750–1755. https://doi.org/10.1271/bbb.80066.

(13) Mulders, J. W.; Boerrigter, I. J.; Rollema, H. S.; Siezen, R. J.; de Vos, W. M. Identification and Characterization of the Lantibiotic Nisin Z, a Natural Nisin Variant. *Eur. J. Biochem.* **1991**, *201* (3), 581–584. https://doi.org/10.1111/j.1432-1033.1991.tb16317.x.

(14) Wirawan, R. E.; Klesse, N. A.; Jack, R. W.; Tagg, J. R. Molecular and Genetic Characterization of a Novel Nisin Variant Produced by Streptococcus Uberis Molecular and Genetic Characterization of a Novel Nisin Variant Produced by Streptococcus Uberis. **2006**, *72* (2), 1148-1156.

https://doi.org/10.1128/AEM.72.2.1148.

(15) Garcia-Gutierrez, E.; O'Connor, P. M.; Saalbach, G.; Walsh, C. J.; Hegarty, J. W.; Guinane, C. M.; Mayer, M. J.; Narbad, A.; Cotter, P. D. First Evidence of Production of the Lantibiotic Nisin P. *Sci. Rep.* **2020**, *10* (1), 1–15. https://doi.org/10.1038/s41598-020-60623-0.

(16) Hatziioanou, D.; Gherghisan-Filip, C.; Saalbach, G.; Horn, N.; Wegmann, U.; Duncan, S. H.; Flint, H. J.; Mayer, M. J.; Narbad, A. Discovery of a Novel Lantibiotic Nisin O from Blautia Obeum A2-162, Isolated from the Human Gastrointestinal Tract. *Microbiology* **2017**, *163* (9), 1292–1305. https://doi.org/10.1099/mic.0.000515.

(17) Karczewski, J.; Krasucki, S. P.; Asare-Okai, P. N.; Diehl, C.; Friedman, A.; Brown, C. M.; Maezato, Y.; Streatfield, S. J. Isolation, Characterization and Structure Elucidation of a Novel Lantibiotic From Paenibacillus Sp. *Front. Microbiol.* **2020**, *11*, 2905. https://doi.org/10.3389/fmicb.2020.598789.

(18) Chan, W. C.; Bycroft, B. W.; Leyland, M. L.; Lian, L.-Y.; Yang, J. C.; Roberts, G. C. K. Sequence-Specific Resonance Assignment and Conformational Analysis of Subtilin by 2D NMR. *FEBS Lett.* **1992**, *300* (1), 56–62. https://doi.org/10.1016/0014-5793(92)80163-B.

(19) Stein, T.; Borchert, S.; Conrad, B.; Feesche, J.; Hofemeister, B.; Hofemeister, J.; Entian, K. Two Different Lantibiotic-like Peptides Originate from the Ericin Gene Cluster of Bacillus Subtilis A1/3. *J. Bacteriol.* **2002**, *184* (6), 1703–1711. https://doi.org/10.1128/jb.184.6.1703-1711.2002.

(20) Fuchs, S. W.; Jaskolla, T. W.; Bochmann, S.; Kötter, P.; Wichelhaus, T.; Karas, M.; Stein, T.; Entian, K.-D. Entianin, a Novel Subtilin-like Lantibiotic from Bacillus Subtilis Subsp. Spizizenii DSM 15029T with High Antimicrobial Activity. *Appl. Environ. Microbiol.* **2011**, *77* (5), 1698–1707. https://doi.org/10.1128/AEM.01962-10.

(21) Birri, D. J.; Brede, D. A.; Nes, I. F. Salivaricin D, a Novel Intrinsically Trypsin-Resistant Lantibiotic from Streptococcus Salivarius 5M6c Isolated from a Healthy Infant. *Appl. Environ. Microbiol.* **2012**, *78* (2), 402–410. https://doi.org/10.1128/AEM.06588-11.

(22) Garg, N.; Tang, W.; Goto, Y.; Nair, S. K.; van der Donk, W. A. Lantibiotics from

Geobacillus Thermodenitrificans. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (14), 5241–5246. https://doi.org/10.1073/pnas.1116815109.

(23) Lohans, C. T.; Huang, Z.; van Belkum, M. J.; Giroud, M.; Sit, C. S.; Steels, E. M.; Zheng, J.; Whittal, R. M.; McMullen, L. M.; Vederas, J. C. Structural Characterization of the Highly Cyclized Lantibiotic Paenicidin A via a Partial Desulfurization/Reduction Strategy. *J. Am. Chem. Soc.* **2012**, *134* (48), 19540–19543. https://doi.org/10.1021/ja3089229.

(24) Allgaier, H.; Jung, G.; Werner, R. G.; Schneider, U.; Zähner, H. Epidermin: Sequencing of a Heterodetic Tetracyclic 21-Peptide Amide Antibiotic. *Eur. J. Biochem.* **1986**, *160* (1), 9–22. https://doi.org/10.1111/j.1432-1033.1986.tb09933.x.

(25) Kellner, R.; Jung, G.; Hörner, T.; Zähner, H.; Schnell, N.; Entian, K. D.; Götz, F. Gallidermin: A New Lanthionine-Containing Polypeptide Antibiotic. *Eur. J. Biochem.* **1988**, *177* (1), 53–59. https://doi.org/10.1111/j.1432-1033.1988.tb14344.x.

(26) Furmanek, B.; Kaczorowski, T.; Bugalski, R.; Bielawski, K.; Bohdanowicz, J.; Podhajska, A. J.; Bogdanowicz, J. Identification, Characterization and Purification of the Lantibiotic Staphylococcin T, a Natural Gallidermin Variant. *J. Appl. Microbiol.* **1999**, *87* (6), 856–866. https://doi.org/10.1046/j.1365-2672.1999.00937.x.

(27) Mota-Meira, M.; Lacroix, C.; Lapointe, G.; Lavoie, M. C. Purification and Structure of Mutacin B-Ny266: A New Lantibiotic Produced by Streptococcus Mutans. *FEBS Lett.* **1997**, *410* (2–3), 275–279. https://doi.org/10.1016/S0014-5793(97)00425-0.

(28) Smith, L.; Zachariah, C.; Thirumoorthy, R.; Rocca, J.; Novák, J.; Hillman, J. D.; Edison, A. S. Structure and Dynamics of the Lantibiotic Mutacin 1140. *Biochemistry* **2003**, *42* (35), 10372–10384. https://doi.org/10.1021/bi034490u.

(29) Bouhss, A.; Al-Dabbagh, B.; Vincent, M.; Odaert, B.; Aumont-Nicaise, M.; Bressolier, P.; Desmadril, M.; Mengin-Lecreulx, D.; Urdaci, M. C.; Gallay, J. Specific Interactions of Clausin, a New Lantibiotic, with Lipid Precursors of the Bacterial Cell Wall. *Biophys. J.* **2009**, *97* (5), 1390–1397. https://doi.org/10.1016/j.bpj.2009.06.029.

(30) Qi, F.; Chen, P.; Caufield, P. W. Purification and Biochemical Characterization of Mutacin I from the Group I Strain of Streptococcus Mutans, CH43, and Genetic Analysis of Mutacin I Biosynthesis Genes. *Appl. Environ. Microbiol.* **2000**, *66* (8),

3221–3229. https://doi.org/10.1128/aem.66.8.3221-3229.2000.

(31) Daly, K. M.; Upton, M.; Sandiford, S. K.; Draper, L. A.; Wescombe, P. A.; Jack, R. W.; O'Connor, P. M.; Rossney, A.; Götz, F.; Hill, C.; Cotter, P. D.; Ross, R. P.; Tagg, J. R. Production of the Bsa Lantibiotic by Community-Acquired Staphylococcus Aureus Strains. *J. Bacteriol.* **2010**, *192* (4), 1131–1142. https://doi.org/10.1128/JB.01375-09.

(32) Vasile, F.; Potenza, D.; Marsiglia, B.; Maffioli, S.; Donadio, S. Solution Structure by Nuclear Magnetic Resonance of the Two Lantibiotics 97518 and NAI-107. *J. Pept. Sci.* **2012**, *18* (2), 129–134. https://doi.org/10.1002/psc.1425.

(33) Kellner, R.; Jung, G.; Josten, M.; Kaletta, C.; Entian, K.-D.; Sahl, H.-G. Pep5: Structure Elucidation of a Large Lantibiotic. *Angew. Chemie Int. Ed. English* **1989**, *28* (5), 616–619. https://doi.org/10.1002/anie.198906161.

(34) Heidrich, C.; Pag, U.; Josten, M.; Metzger, J.; Jack, R. W.; Bierbaum, G.; Jung, G.; Sahl, H. G. Isolation, Characterization, and Heterologous Expression of the Novel Lantibiotic Epicidin 280 and Analysis of Its Biosynthetic Gene Cluster. *Appl. Environ. Microbiol.* **1998**, *64* (9), 3140–3146. https://doi.org/10.1128/AEM.64.9.3140-3146.1998.

(35) Ekkelenkamp, M. B.; Hanssen, M.; Danny Hsu, S.-T.; de Jong, A.; Milatovic, D.; Verhoef, J.; van Nuland, N. A. J. Isolation and Structural Characterization of Epilancin 15X, a Novel Lantibiotic from a Clinical Strain of Staphylococcus Epidermidis. *FEBS Lett.* **2005**, *579* (9), 1917–1922. https://doi.org/10.1016/j.febslet.2005.01.083.

(36) Kamp, M.; Horstink, L. M.; Hooven, H. W.; Konings, R. N. H.; Hilbers, C. W.; Frey, A.; Sahl, H.-G.; Metzger, J. W.; Ven, F. J. M. Sequence Analysis by NMR Spectroscopy of the Peptide Lantibiotic Epilancin K7 from Staphylococcus Epidermidis K7. *Eur. J. Biochem.* **1995**, *227* (3), 757–771. https://doi.org/10.1111/j.1432-1033.1995.tb20199.x.

(37) He, Z.; Yuan, C.; Zhang, L.; Yousef, A. E. N-Terminal Acetylation in Paenibacillin, a Novel Lantibiotic. *FEBS Lett.* **2008**, *582* (18), 2787–2792. https://doi.org/10.1016/j.febslet.2008.07.008.

(38) Phelan, R. W.; Barret, M.; Cotter, P. D.; O'Connor, P. M.; Chen, R.; Morrissey, J. P.;

Dobson, A. D. W.; O'Gara, F.; Barbosa, T. M. Subtilomycin: A New Lantibiotic from Bacillus Subtilis Strain MMA7 Isolated from the Marine Sponge Haliclona Simulans. *Mar. Drugs* **2013**, *11* (6), 1878–1898. https://doi.org/10.3390/md11061878.

(39) Xin, B.; Zheng, J.; Xu, Z.; Song, X.; Ruan, L.; Peng, D.; Sun, M. The Bacillus Cereus Group Is an Excellent Reservoir of Novel Lanthipeptides. *Appl. Environ. Microbiol.* **2015**, *81* (5), 1765–1774. https://doi.org/10.1128/AEM.03758-14.

(40) Wescombe, P. A.; Tagg, J. R. Purification and Characterization of Streptin, a Type A1 Lantibiotic Produced by Streptococcus Pyogenes. *Appl. Environ. Microbiol.* **2003**, *69* (5), 2737–2747. https://doi.org/10.1128/aem.69.5.2737-2747.2003.

(41) Mohr, K. I.; Volz, C.; Jansen, R.; Wray, V.; Hoffmann, J.; Bernecker, S.; Wink, J.; Gerth, K.; Stadler, M.; Müller, R. Pinensins: The First Antifungal Lantibiotics. *Angew. Chem. Int. Ed. Engl.* **2015**, *54* (38), 11254–11258. https://doi.org/10.1002/anie.201500927.

(42) van den Hooven, H. W.; Lagerwerf, F. M.; Heerma, W.; Haverkamp, J.; Piard, J. C.; Hilbers, C. W.; Siezen, R. J.; Kuipers, O. P.; Rollema, H. S. The Structure of the Lantibiotic Lacticin 481 Produced by Lactococcus Lactis: Location of the Thioether Bridges. *FEBS Lett.* **1996**, *391* (3), 317–322. https://doi.org/10.1016/0014-5793(96)00771-5.

(43) Krull, R. E.; Chen, P.; Novak, J.; Kirk, M.; Barnes, S.; Baker, J.; Krishna, N. R.; Caufield, P. W. Biochemical Structural Analysis of the Lantibiotic Mutacin II. *J. Biol. Chem.* **2000**, *275* (21), 15845–15850. https://doi.org/10.1074/jbc.275.21.15845.

(44) Fujinami, D.; Mahin, A. Al; Elsayed, K. M.; Islam, M. R.; Nagao, J. ichi; Roy, U.; Momin, S.; Zendo, T.; Kohda, D.; Sonomoto, K. The Lantibiotic Nukacin ISK-1 Exists in an Equilibrium between Active and Inactive Lipid-II Binding States. *Commun. Biol.* **2018**, *1* (1), 1–9. https://doi.org/10.1038/s42003-018-0150-3.

(45) Jack, R. W.; Carne, A.; Metzger, J.; Stefanović, S.; Sahl, H. G.; Jung, G.; Tagg, J. Elucidation of the Structure of SA-FF22, a Lanthionine-Containing Antibacterial Peptide Produced by Streptococcus Pyogenes Strain FF22. *Eur. J. Biochem.* **1994**, *220* (2), 455–462. https://doi.org/10.1111/j.1432-1033.1994.tb18643.x.

(46) Georgalaki, M. D.; Van den Berghe, E.; Kritikos, D.; Devreese, B.; Van Beeumen, J.;

Kalantzopoulos, G.; De Vuyst, L.; Tsakalidou, E. Macedocin, a Food-Grade Lantibiotic Produced by Streptococcus Macedonicus ACA-DC 198. *Appl. Environ. Microbiol.* **2002**, *68* (12), 5891–5903. https://doi.org/10.1128/AEM.68.12.5891-5903.2002.

(47) Kalmokoff, M. L.; Lu, D.; Whitford, M. F.; Teather, R. M. Evidence for Production of a New Lantibiotic (Butyrivibriocin OR79A) by the Ruminal Anaerobe Butyrivibrio Fibrisolvens OR79: Characterization of the Structural Gene Encoding Butyrivibriocin OR79A. *Appl. Environ. Microbiol.* **1999**, *65* (5), 2128–2135. https://doi.org/10.1128/AEM.65.5.2128-2135.1999.

(48) Wescombe, P. A.; Upton, M.; Renault, P.; Wirawan, R. E.; Power, D.; Burton, J. P.; Chilcott, C. N.; Tagg, J. R. Salivaricin 9, a New Lantibiotic Produced by Streptococcus Salivarius. *Microbiology* **2011**, *157* (5), 1290–1299. https://doi.org/10.1099/mic.0.044719-0.

(49) Hyink, O.; Wescombe, P. A.; Upton, M.; Ragland, N.; Burton, J. P.; Tagg, J. R. Salivaricin A2 and the Novel Lantibiotic Salivaricin B Are Encoded at Adjacent Loci on a 190-Kilobase Transmissible Megaplasmid in the Oral Probiotic Strain Streptococcus Salivanus K12. *Appl. Environ. Microbiol.* **2007**, *73* (4), 1107–1113. https://doi.org/10.1128/AEM.02265-06.

(50) Wescombe, P. A.; Dyet, K. H.; Dierksen, K. P.; Power, D. A.; Jack, R. W.; Burton, J. P.; Inglis, M. A.; Wescombe, A. L.; Tagg, J. R. Salivaricin G32, a Homolog of the Prototype Streptococcus Pyogenes Nisin-Like Lantibiotic SA-FF22, Produced by the Commensal Species Streptococcus Salivarius. *Int. J. Microbiol.* **2012**, *2012*, 738503. https://doi.org/10.1155/2012/738503.

(51) Pridmore, D.; Rekhif, N.; Pittet, A. C.; Suri, B.; Mollet, B. Variacin, a New Lanthionine-Containing Bacteriocin Produced by Micrococcus Varians: Comparison to Lacticin 481 of Lactococcus Lactis. *Appl. Environ. Microbiol.* **1996**, *62* (5), 1799–1802. https://doi.org/10.1128/aem.62.5.1799-1802.1996.

(52) Ongey, E. L.; Giessmann, R. T.; Fons, M.; Rappsilber, J.; Adrian, L.; Neubauer, P. Heterologous Biosynthesis, Modifications and Structural Characterization of Ruminococcin-A, a Lanthipeptide From the Gut Bacterium Ruminococcus Gnavus E1, in Escherichia Coli. *Front. Microbiol.* **2018**, *9*.

https://doi.org/10.3389/fmicb.2018.01688.

(53) Ross, K. F.; Ronson, C. W.; Tagg, J. R. Isolation and Characterization of the Lantibiotic Salivaricin A and Its Structural Gene SalA from Streptococcus Salivarius 20P3. *Appl. Environ. Microbiol.* **1993**, *59* (7), 2014–2021. https://doi.org/10.1128/aem.59.7.2014-2021.1993.

(54) Wescombe, P. A.; Upton, M.; Dierksen, K. P.; Ragland, N. L.; Sivabalan, S.; Wirawan, R. E.; Inglis, M. A.; Moore, C. J.; Walker, G. V.; Chilcott, C. N.; Jenkinson, H. F.; Tagg, J. R. Production of the Lantibiotic Salivaricin A and Its Variants by Oral Streptococci and Use of a Specific Induction Assay to Detect Their Presence in Human Saliva. *Appl. Environ. Microbiol.* **2006**, *72* (2), 1459–1466. https://doi.org/10.1128/AEM.72.2.1459-1466.2006.

(55) Geng, M.; Austin, F.; Shin, R.; Smith, L. Covalent Structure and Bioactivity of the Type All Lantibiotic Salivaricin A2. *Appl. Environ. Microbiol.* **2018**, *84* (5), e02528-17. https://doi.org/10.1128/AEM.02528-17.

(56) Prasch, T.; Naumann, T.; Markert, R. L. M.; Sattler, M.; Schubert, W.; Schaal, S.; Bauch, M.; Kogler, H.; Griesinger, C. Constitution and Solution Conformation of the Antibiotic Mersacidin Determined by NMR and Molecular Dynamics. *Eur. J. Biochem.* **1997**, *244* (2), 501–512. https://doi.org/10.1111/j.1432-1033.1997.00501.x.

(57) Zimmermann, N.; Jung, G. The Three-Dimensional Solution Structure of the Lantibiotic Murein-Biosynthesis-Inhibitor Actagardine Determined by NMR. *Eur. J. Biochem.* **1997**, *246* (3), 809–819. https://doi.org/10.1111/j.1432-1033.1997.00809.x.

(58) Simone, M.; Monciardini, P.; Gaspari, E.; Donadio, S.; Maffioli, S. I. Isolation and Characterization of NAI-802, a New Lantibiotic Produced by Two Different Actinoplanes Strains. *J. Antibiot. (Tokyo).* **2013**, *66* (2), 73–78. https://doi.org/10.1038/ja.2012.92.

(59) Holtsmark, I.; Mantzilas, D.; Eijsink, V. G. H.; Brurberg, M. B. Purification, Characterization, and Gene Sequence of Michiganin A, an Actagardine-like Lantibiotic Produced by the Tomato Pathogen Clavibacter Michiganensis Subsp. Michiganensis. *Appl. Environ. Microbiol.* **2006**, *72* (9), 5814–5821. https://doi.org/10.1128/AEM.00639-06.

(60) Arguelles Arias, A.; Ongena, M.; Devreese, B.; Terrak, M.; Joris, B.; Fickers, P. Characterization of Amylolysin, a Novel Lantibiotic from Bacillus Amyloliquefaciens GA1. *PLoS One* **2013**, *8* (12), e83037. https://doi.org/10.1371/journal.pone.0083037.

(61) Turner, D. L.; Brennan, L.; Meyer, H. E.; Lohaus, C.; Siethoff, C.; Costa, H. S.; Gonzalez, B.; Santos, H.; Suarez, J. E. Solution Structure of Plantaricin C, a Novel Lantibiotic. *Eur. J. Biochem.* **1999**, *264* (3), 833–839. https://doi.org/10.1046/j.1432-1327.1999.00674.x.

(62) Basi-Chipalu, S.; Dischinger, J.; Josten, M.; Szekat, C.; Zweynert, A.; Sahl, H. G.; Bierbaum, G. Pseudomycoicidin, a Class II Lantibiotic from Bacillus Pseudomycoides. *Appl. Environ. Microbiol.* **2015**, *81* (10), 3419–3429. https://doi.org/10.1128/AEM.00299-15.

(63) McClerren, A. L.; Cooper, L. E.; Quan, C.; Thomas, P. M.; Kelleher, N. L.; van der Donk, W. A. Discovery and in Vitro Biosynthesis of Haloduracin, a Two-Component Lantibiotic. *Proc. Natl. Acad. Sci.* **2006**, *103* (46), 17243–17248. https://doi.org/10.1073/pnas.0606088103.

(64) Holo, H.; Jeknic, Z.; Daeschel, M.; Stevanovic, S.; Nes, I. F. Plantaricin W from Lactobacillus Plantarum Belongs to a New Family of Two-Peptide Lantibiotics. *Microbiology* **2001**, *147* (Pt 3), 643–651. https://doi.org/10.1099/00221287-147-3-643.

(65) Sawa, N.; Wilaipun, P.; Kinoshita, S.; Zendo, T.; Leelawatcharamas, V.; Nakayama, J.; Sonomoto, K. Isolation and Characterization of Enterocin W, a Novel Two-Peptide Lantibiotic Produced by Enterococcus Faecalis NKR-4-1. *Appl. Environ. Microbiol.* **2012**, *78* (3), 900–903. https://doi.org/10.1128/AEM.06497-11.

(66) van Staden, A. D. P.; Heunis, T.; Smith, C.; Deane, S.; Dicks, L. M. T. Efficacy of Lantibiotic Treatment of Staphylococcus Aureus-Induced Skin Infections, Monitored by In Vivo Bioluminescent Imaging. *Antimicrob. Agents Chemother.* **2016**, *60* (7), 3948–3955. https://doi.org/10.1128/AAC.02938-15.

(67) Shenkarev, Z. O.; Finkina, E. I.; Nurmukhamedova, E. K.; Balandin, S. V; Mineev, K. S.; Nadezhdin, K. D.; Yakimenko, Z. a; Tagaev, A. a; Temirov, Y. V; Arseniev, A. S.; Ovchinnikova, T. V. Isolation, Structure Elucidation, and Synergistic Antibacterial Activity of a Novel Two-Component Lantibiotic Lichenicidin from Bacillus

Licheniformis VK21. *Biochemistry* **2010**, *49* (30), 6462–6472. https://doi.org/10.1021/bi100871b.

(68) Martin, N. I.; Sprules, T.; Carpenter, M. R.; Cotter, P. D.; Hill, C.; Ross, R. P.; Vederas, J. C. Structural Characterization of Lacticin 3147, a Two-Peptide Lantibiotic with Synergistic Activity. *Biochemistry* **2004**, *43* (11), 3049–3056. https://doi.org/10.1021/bi0362065.

(69) Navaratna, M. A.; Sahl, H. G.; Tagg, J. R. Two-Component Anti-Staphylococcus Aureus Lantibiotic Activity Produced by Staphylococcus Aureus C55. *Appl. Environ. Microbiol.* **1998**, *64* (12), 4803–4808. https://doi.org/10.1128/AEM.64.12.4803-4808.1998.

(70) Zhao, X.; Van Der Donk, W. A. Structural Characterization and Bioactivity Analysis of the Two-Component Lantibiotic Flv System from a Ruminant Bacterium. *Cell Chem. Biol.* **2016**, *23* (2), 246–256. https://doi.org/10.1016/j.chembiol.2015.11.014.

(71) Hyink, O.; Balakrishnan, M.; Tagg, J. R. Streptococcus Rattus Strain BHT Produces Both a Class I Two-Component Lantibiotic and a Class II Bacteriocin. *FEMS Microbiol. Lett.* **2005**, *252* (2), 235–241. https://doi.org/10.1016/j.femsle.2005.09.003.

(72) Yonezawa, H.; Kuramitsu, H. K. Genetic Analysis of a Unique Bacteriocin, Smb, Produced by Streptococcus Mutans GS5. *Antimicrob. Agents Chemother.* **2005**, *49* (2), 541–548. https://doi.org/10.1128/AAC.49.2.541-548.2005.

(73) Xin, B.; Zheng, J.; Liu, H.; Li, J.; Ruan, L.; Peng, D.; Sajid, M.; Sun, M. Thusin, a Novel Two-Component Lantibiotic with Potent Antimicrobial Activity against Several Gram-Positive Pathogens. *Front. Microbiol.* **2016**, *7*, 1115. https://doi.org/10.3389/fmicb.2016.01115.

(74) Singh, M.; Chaudhary, S.; Sareen, D. Roseocin, a Novel Two-component Lantibiotic from an Actinomycete. *Mol. Microbiol.* **2020**, *113* (2), 326–337. https://doi.org/10.1111/mmi.14419.

(75) Huo, L.; Van Der Donk, W. A. Discovery and Characterization of Bicereucin, an Unusual d -Amino Acid-Containing Mixed Two-Component Lantibiotic. *J. Am. Chem. Soc.* **2016**, *138* (16), 5254–5257. https://doi.org/10.1021/jacs.6b02513.

(76) Tang, W.; van der Donk, W. A. The Sequence of the Enterococcal Cytolysin Imparts Unusual Lanthionine Stereochemistry. *Nat. Chem. Biol.* **2013**, *9* (3), 157–159. https://doi.org/10.1038/nchembio.1162.

(77) Lohans, C. T.; Li, J. L.; Vederas, J. C. Structure and Biosynthesis of Carnolysin, a Homologue of Enterococcal Cytolysin with D-Amino Acids. *J. Am. Chem. Soc.* **2014**, *136* (38), 13150–13153. https://doi.org/10.1021/ja5070813.

(78) Wang, J.; Zhang, L.; Teng, K.; Sun, S.; Sun, Z.; Zhong, J. Cerecidins, Novel Lantibiotics from Bacillus Cereus with Potent Antimicrobial Activity. *Appl. Environ. Microbiol.* **2014**, *80* (8), 2633–2643. https://doi.org/10.1128/AEM.03751-13.

(79) Skaugen, M.; Nissen-Meyer, J.; Jung, G.; Stevanovic, S.; Sletten, K.; Inger, C.; Abildgaard, M.; Nes, I. F. In Vivo Conversion of L-Serine to D-Alanine in a Ribosomally Synthesized Polypeptide. *J. Biol. Chem.* **1994**, *269* (44), 27183–27185. https://doi.org/10.1016/S0021-9258(18)46966-9.

(80) Kessler, H.; Steuernagel, S.; Will, M.; Jung, G.; Kellner, R.; Gillessen, D.; Kamiyama, T. The Structure of the Polycyclic Nonadecapeptide Ro 09-0198. *Helv. Chim. Acta* **1988**, *71* (8), 1924–1929. https://doi.org/10.1002/hlca.19880710811.

(81) Kodani, S.; Komaki, H.; Ishimura, S.; Hemmi, H.; Ohnishi-Kameyama, M. Isolation and Structure Determination of a New Lantibiotic Cinnamycin B from Actinomadura Atramentaria Based on Genome Mining. *J. Ind. Microbiol. Biotechnol.* **2016**, *43* (8), 1159–1165. https://doi.org/10.1007/s10295-016-1788-9.

(82) Hayashi, F.; Nagashima, K.; Terui, Y.; Kawamura, Y.; Matsumoto, K.; Itazaki, H. The Structure of PA48009: The Revised Structure of Duramycin. *J. Antibiot. (Tokyo).* **1990**, *43* (11), 1421–1430. https://doi.org/10.7164/antibiotics.43.1421.

(83) Zimmermann, N.; Freund, S.; Fredenhagen, A.; Jung, G. Solution Structures of the Lantibiotics Duramycin B and C. *Eur. J. Biochem.* **1993**, *216* (2), 419–428. https://doi.org/10.1111/j.1432-1033.1993.tb18159.x.

(84) Wakamiya, T.; Ueki, Y.; Shiba, T.; Kido, Y.; Motoki, Y. The Structure of Ancovenin, a New Peptide Inhibitor of Angiotensin I Converting Enzyme. *Tetrahedron Lett.* **1985**, *26* (5), 665–668. https://doi.org/10.1016/S0040-4039(00)89174-1.

(85) Chen, E.; Chen, Q.; Chen, S.; Xu, B.; Ju, J.; Wang, H. Mathermycin, a Lantibiotic from the Marine Actinomycete Marinactinospora Thermotolerans SCSIO 00652. *Appl. Environ. Microbiol.* **2017**, *83* (15), e00926-17. https://doi.org/10.1128/AEM.00926-17.

(86) Vikeli, E.; Widdick, D. A.; Batey, S. F. D.; Heine, D.; Holmes, N. A.; Bibb, M. J.; Martins, D. J.; Pierce, N. E.; Hutchings, M. I.; Wilkinson, B. In Situ Activation and Heterologous Production of a Cryptic Lantibiotic from an African Plant Ant-Derived Saccharopolyspora Species. *Appl. Environ. Microbiol.* **2020**, *86* (3), e01876-19. https://doi.org/10.1128/AEM.01876-19.

(87) Zhang, J.; Feng, Y.; Teng, K.; Lin, Y.; Gao, Y.; Wang, J.; Zhong, J. Type AII Lantibiotic Bovicin HJ50 with a Rare Disulfide Bond: Structure, Structure-Activity Relationships and Mode of Action. *Biochem. J.* **2014**, *461* (3), 497–508. https://doi.org/10.1042/BJ20131524.

(88) Wang, J.; Ma, H.; Ge, X.; Zhang, J.; Teng, K.; Sun, Z.; Zhong, J. Bovicin HJ50-like Lantibiotics, a Novel Subgroup of Lantibiotics Featured by an Indispensable Disulfide Bridge. *PLoS One* **2014**, *9* (5), e97121. https://doi.org/10.1371/journal.pone.0097121.

(89) Meindl, K.; Schmiederer, T.; Schneider, K.; Reicke, A.; Butz, D.; Keller, S.; Gühring, H.; Vértesy, L.; Wink, J.; Hoffmann, H.; Brönstrup, M.; Sheldrick, G. M.; Süssmuth, R. D. Labyrinthopeptins: A New Class of Carbacyclic Lantibiotics. *Angew. Chemie Int. Ed.* **2010**, *49* (6), 1151–1154. https://doi.org/10.1002/anie.200905773.

(90) Völler, G. H.; Krawczyk, J. M.; Pesic, A.; Krawczyk, B.; Nachtigall, J.; Süssmuth, R. D. Characterization of New Class III Lantibiotics--Erythreapeptin, Avermipeptin and Griseopeptin from Saccharopolyspora Erythraea, Streptomyces Avermitilis and Streptomyces Griseus Demonstrates Stepwise N-Terminal Leader Processing. *Chembiochem* **2012**, *13* (8), 1174–1183. https://doi.org/10.1002/cbic.201200118.

(91) Wang, H.; van der Donk, W. A. Biosynthesis of the Class III Lantipeptide Catenulipeptin. *ACS Chem. Biol.* **2012**, *7* (9), 1529–1535. https://doi.org/10.1021/cb3002446.

(92) Völler, G. H.; Krawczyk, B.; Ensle, P.; Süssmuth, R. D. Involvement and Unusual Substrate Specificity of a Prolyl Oligopeptidase in Class III Lanthipeptide Maturation. *J. Am. Chem. Soc.* **2013**, *135* (20), 7426–7429. https://doi.org/10.1021/ja402296m.

(93) Iorio, M.; Sasso, O.; Maffioli, S. I.; Bertorelli, R.; Monciardini, P.; Sosio, M.; Bonezzi, F.; Summa, M.; Brunati, C.; Bordoni, R.; Corti, G.; Tarozzo, G.; Piomelli, D.; Reggiani, A.; Donadio, S. A Glycosylated, Labionin-Containing Lanthipeptide with Marked Antinociceptive Activity. *ACS Chem. Biol.* **2014**, *9* (2), 398–404. https://doi.org/10.1021/cb400692w.

(94) Jungmann, N. A.; Van Herwerden, E. F.; Hügelland, M.; Süssmuth, R. D. The Supersized Class III Lanthipeptide Stackepeptin Displays Motif Multiplication in the Core Peptide. *ACS Chem. Biol.* **2016**, *11* (1), 69–76. https://doi.org/10.1021/acschembio.5b00651.

(95) Krawczyk, B.; Völler, G. H.; Völler, J.; Ensle, P.; Süssmuth, R. D. Curvopeptin: A New Lanthionine-Containing Class III Lantibiotic and Its Co-Substrate Promiscuous Synthetase. *ChemBioChem* **2012**, *13* (14), 2065–2071. https://doi.org/10.1002/cbic.201200417.

(96) Kodani, S.; Hudson, M. E.; Durrant, M. C.; Buttner, M. J.; Nodwell, J. R.; Willey, J. M. The SapB Morphogen Is a Lantibiotic-like Peptide Derived from the Product of the Developmental Gene RamS in Streptomyces Coelicolor. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (31), 11448–11453. https://doi.org/10.1073/pnas.0404220101.

(97) Goto, Y.; Li, B.; Claesen, J.; Shi, Y.; Bibb, M. J.; van der Donk, W. A. Discovery of Unique Lanthionine Synthetases Reveals New Mechanistic and Evolutionary Insights. *PLoS Biol.* **2010**, *8* (3), e1000339. https://doi.org/10.1371/journal.pbio.1000339.

(98) Iftime, D.; Jasyk, M.; Kulik, A.; Imhoff, J. F.; Stegmann, E.; Wohlleben, W.; Süssmuth, R. D.; Weber, T. Streptocollin, a Type IV Lanthipeptide Produced by Streptomyces Collinus Tü 365. *ChemBioChem* **2015**, *16* (18), 2615–2623. https://doi.org/10.1002/cbic.201500377.

(99) Hegemann, J. D.; van der Donk, W. A. Investigation of Substrate Recognition and Biosynthesis in Class IV Lanthipeptide Systems. *J. Am. Chem. Soc.* **2018**, *140* (17), 5743–5754. https://doi.org/10.1021/jacs.8b01323.

(100) Ren, H.; Shi, C.; Bothwell, I. R.; van der Donk, W. A.; Zhao, H. Discovery and Characterization of a Class IV Lanthipeptide with a Nonoverlapping Ring Pattern. *ACS Chem. Biol.* **2020**, *15* (6), 1642–1649. https://doi.org/10.1021/acschembio.0c00267.