Supplemental Information for:

Characterization of Site Directed Mutations in the Lanthipeptide Mutacin 1140

Shaorong Chen [#a], Shawanda Wilson-Stanford [#a], William Cromwell [a], Jeffrey D. Hillman [b], Adam Guerrero [a], Charlotte A. Allen [a], Joseph A. Sorg [a], Leif Smith [a]*

[a] Department of Biological Sciences, Texas A&M University, College Station, TX 77843
[b] Oragenics Inc. 13700 Progress Blvd., Alachua, FL 32615

[#] The authors contribute equally.
*Corresponding author: Leif Smith, Department of Biological Sciences, Texas A&M University, College Station, TX 77843, Phone: 979-845-2417, Fax: 979-845-2891, Email: jsmith@bio.tamu.edu

Contents:

Supplemental Table 1. Primers Used for Mutagenesis of MU1140
Supplemental Table 2. Aligned sequences of natural lanthipeptide variants
Supplemental Table 3. Bioactivity of Strains Producing Variants of Mutacin 1140 Compared to Mutacin 1140
Supplemental Figure 1. MALDI TOF mass spectra for mutacin 1140 variants
Supplemental Figure 2. RP-HPLC Chromatograms of purified lanthipeptides
Supplemental References

Supplemental Table 1. Primers Used for Mutagenesis of MU1140

Primers	**Sequence (5' – 3')**
SRWmutA_F	AGAATTCAGGATGCTATCGCTGCTTTTTTTGTG
SRWmutA_R	AGAATTCAGGAAAGTTGCCATATGGTTTTGTG
Phe1Gly_F	GATCCAGATACTCGT**GGC**AAAAGTTGGAGCCTTTGTACG
Phe1Gly_R	CAACTTTT**GCC**ACGAGTATCTGGATCGTCGTTGC
Phe1Ile_F	GATCCAGATACTCGT**ATC**AAAAGTTGGAGCCTTTGTACG
Phe1Ile_R	CAACTTTT**GAT**ACGAGTATCTGGATCGTCGTTGC
Phe1Thr_F	GATCCAGATACTCGT**ACC**AAAAGTTGGAGCCTTTGTACG
Phe1Thr_R	CAACTTTT**GGT**ACGAGTATCTGGATCGTCGTTGC
Phe1Ser_F	GATCCAGATACTCGT**TCC**AAAAGTTGGAGCCTTTGTACG
Phe1Ser_R	CAACTTTT**GGA**ACGAGTATCTGGATCGTCGTTGC
Phe1Trp_F	GATCCAGATACTCGT**TGG**AAAAGTTGGAGCCTTTGTACG
Phe1Trp_R	CAACTTTT**CCA**ACGAGTATCTGGATCGTCGTTGC
Trp4Ala_F	**GCA**AGCCTTTGTACGCCTGGTTG
Trp4Ala_R	ACAAAGGCT**TGC**ACTTTTGAAACG
Trp4insAla_F	**GCA**AGCCTTTGTACGCCTGGTTG
Trp4insAla_R	CAAAGGCT**TGC**CCAACTTTTGAAACG
ΔTrp4_F	---AGCCTTTGTACGCCTGGTTG
ΔTrp4_R	CGTACAAAGGCT---ACTTTTGAAACG
Dha5Ala_F	**GCA**CTTTGTACGCCTGGTTGTGC
Dha5Ala_R	GGCGTACAAAG**TGC**CCAACTTTTGAA
Alas7insAla_F	**GCA**ACGCCTGGTTGTGCAAGGAC
Alas7insAla_R	ACCAGGCGT**TGC**ACAAAGGCTCC
Arg13Asp_F	**GAC**ACAGGTAGTTTCAATAGTTAC
Arg13Asp_R	GAAACTACCTGT**GTC**TGCACAACCAG
Phe17insAla_F	**GCA**AATAGTTACTGTTGCTG
Phe17insAla_R	GTAACTATT**TGC**GAAACTCCATG
Asn18Ala_F	**GCA**AGTTACTGTTGCTGATTG
Asn18Ala_R	ACAGTAACT**TGC**GAAACTACCTG
Outside primers are SRWmutA_F and SRWmutA_R and are homologous to the 5' and 3' flanking DNA. Underlined section represents the engineered EcoRI site. Mutations are either bolded or dashes.	

Supplemental Table 2. Aligned sequences of natural lanthipeptide variants

Type-A1 Lanthipeptide	Name	Core Peptide Sequence
Nisin Group	Nisin A(*1*)	ITSISLCTPG-CKTGALMGCNMKTATCHCSIHVSK
	Nisin Z(*2*)	ITSISLCTPG-CKTGALMGCNMKTATCNCSIHVSK
	Nisin Q(*3*)	ITSISLCTPG-CKTGALQGCNLKTATCNCSVHVSK
	Nisin F(*4*)	ITSISLCTPG-CKTGALMGCNMKTATCNCSVHVSK
	Nisin U(*5*)	ITSKSLCTPG-CKTGILMTCPLKTATCGCHFG
	Subtilin(*6*)	WKSESLCTPG-CVTGALQTCFLQTLTCNC--KISK
	Ericin S(*7*)	WKSESVCTPG-CVTGVLQTCFCQTITCNC--HISK
	Ericin A(*7*)	VLSESLCTPG-CITGPLQTCYLCFPTFAKC
	Streptin(*8*)	VGSRYLCTPGSCWKLVCFTTTTVK
Epidermin Group	Epidermin(*9*)	IASKFICTPG-CAKTGS--FNSYCC
	Epidermin [leu6](*10*)	IASKFLCTPG-CAKTGS--FNSYCC
	Gallidermin(*11*)	VASKFLCTPG-CAKTGS--FNSYCC
	Staphylococcin T(*12*)	VASKFLCTPG-CAKTGS--FNSYCC
	Mutacin B-Ny266(*13*)	FKSWSFCTPG-CAKTGS--FNSYCC
	Mutacin 1140/III(*14, 15*)	FKSWSLCTPG-CARTGS--FNSYCC
	Mutacin 1(*16*)	FSSLSLCSLG-CTGVKNP-FNSYCC
	Microbisporicin(*17*)	VTSWSLCTPG-CTSPGGGSNCSFCC
Pep5 Group	Epilancin K7(*18*)	SASV-LKTSIKVSKKYCKGV---TLTCGCNITGGK
	Epilancin 15X(*19*)	SASI-VKTTIKASKKLCRGF---SLSCGCHFSGKK
	Pep5(*20*)	TAGPAIRASVKQCQKTLKATRLFTVSCKGKN-GCK
	Epicidin 280(*21*)	SLGPAIKAT-RQVCP--KATRFVTVSCKKSDCQ

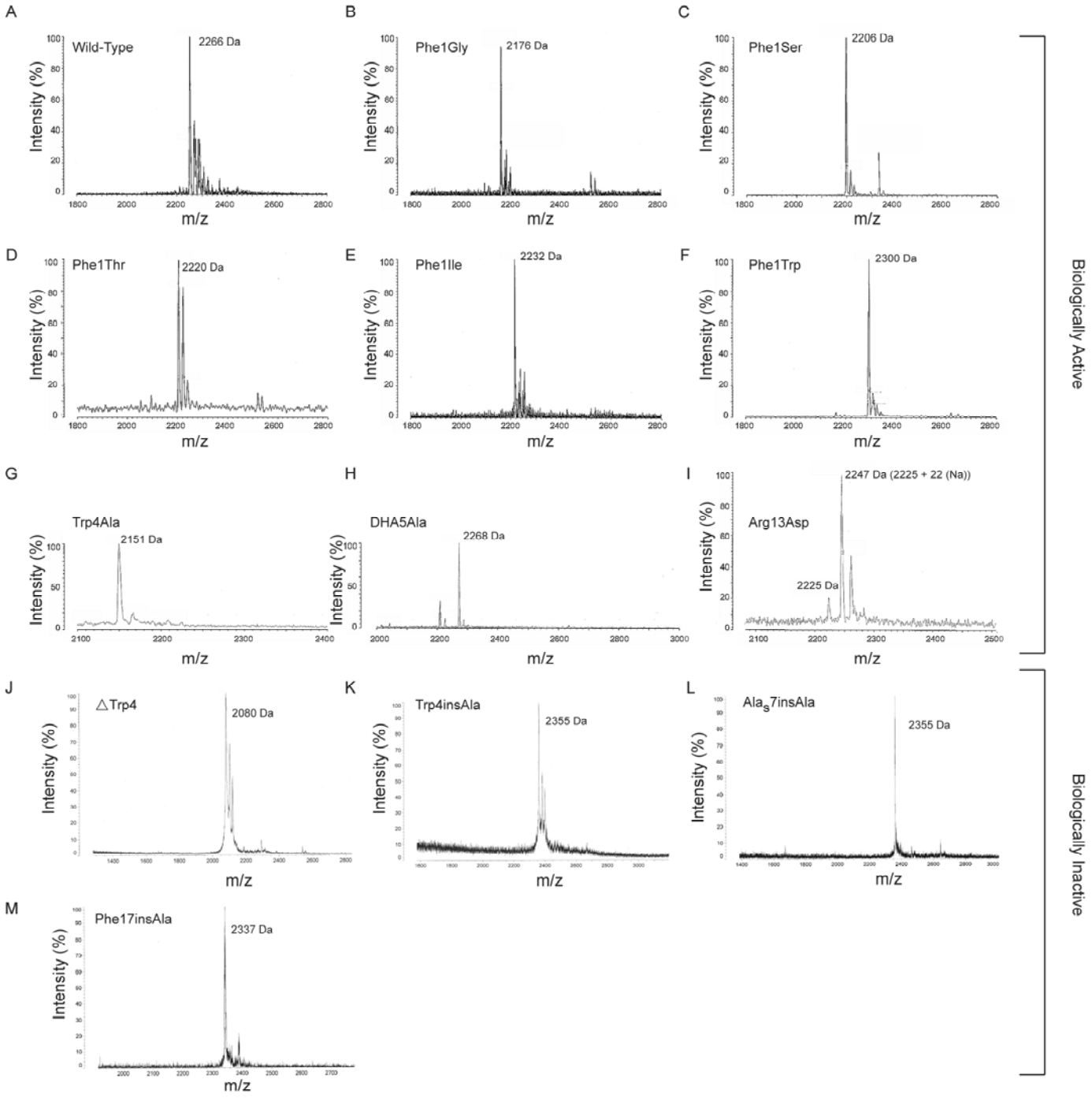
*Amino acid residues are highlighted in grey that differ from the representative sequence highlighted in yellow.

Supplemental Table 3. Bioactivity of Strains Producing Variants of Mutacin 1140 Compared to Wild-Type Mutacin 1140

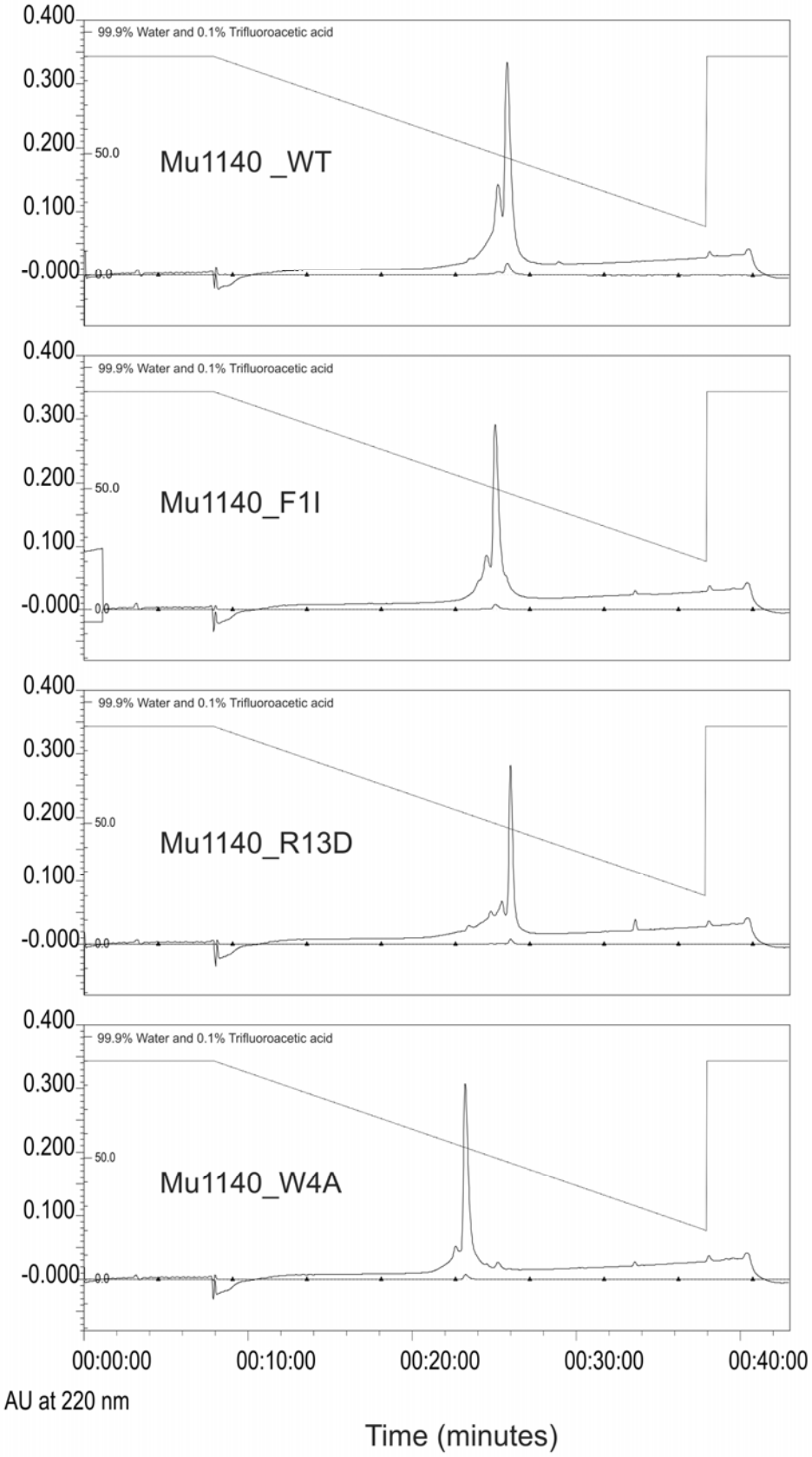
Variant Produced	Mean Area* (mm^2)	Standard Error of the Mean (SEM)	Ratio of Variant to Wild-Type Activities	Statistical Significance (*p* value)[#]
Mutacin 1140 (wild-type)	299.29	59.58	-	-
Phe1Gly	290.49	35.07	0.970577	>.05
Phe1Ser	308.87	35.78	1.031981	>.05
Phe1Thr	394.76	50.52	1.318951	<.05
Phe1Ile	550.04	114.86	1.837763	<.05
Phe1Trp	229.49	50.61	0.766769	>.05
Trp4insAla	0	0	0	<.05
ΔTrp4	0	0	0	<.05
Trp4Ala	576.43	54.43	1.925944	<.05
DHA5Ala	357.01	42.85	1.192853	>.05
AlaS7insAla	0	0	0	<.05
Arg13Asp	655.65	107.99	2.19062	<.05
Phe17insAla	0	0	0	<.05
Asn18Ala	0	0	0	<.05
Trp4Ala-Arg13Asp	0	0	0	<.05

* Based on 10 independent samples.

[#] Student's t Test



Supplemental Figure 1. MALDI TOF mass spectra for mutacin 1140 variants. Panel A. Mutacin 1140, Panel B. Mutacin 1140_Phe1Gly variant; Panel C. Mutacin 1140_Phe1Ser variant; Panel D. Mutacin 1140_Phe1Thr variant; Panel E. Mutacin 1140_Phe1Ile variant; Panel F. Mutacin 1140_Phe1Trp variant; Panel G. Mutacin 1140_Trp4Ala variant; Panel H. Mutacin 1140_DHA5Ala variant; Panel I. Mutacin 1140_Arg13Asp variant, Panel J. Mutacin 1140_ΔTrp4 variant, Panel K. Mutacin 1140_Trp4insAla variant, Panel L. Mutacin 1140_AlaS7insAla variant, and Panel M. Mutacin 1140_Phe17insAla variant.



Supplemental Figure 2. RP-HPLC Chromatograms of purified mutacin 1140 and variants.

Supplemental References

1. **Gross, E., and Morell, J. L.** (1971) Structure of Nisin, *JACS* **93**: 4634-&.
2. **Mulders, J. W. M., Boerrigter, I. J., Rollema, H. S., Siezen, R. J., and Devos, W. M.** (1991) Identification and Characterization of the Lantibiotic Nisin-Z, a Natural Nisin Variant, *Eur. J. Biochem.* **201**: 581-584.
3. **Zendo, T., Fukao, M., Ueda, K., Higuchi, T., Nakayama, J., and Sonomoto, K.** (2003) Identification of the lantibiotic Nisin Q, a new natural nisin variant produced by Lactococcus lactis 61-14 isolated from a river in Japan, *Biosci. Biotechnol. Biochem.* **67**: 1616-1619.
4. **De Kwaadsteniet, M., Doeschate, K. T., and Dicks, L. M. T.** (2009) Nisin F in the treatment of respiratory tract infections caused by Staphylococcus aureus, *Lett. Appl. Microbiol.* **48**: 65-70.
5. **Wirawan, R. E., Kleese, N. A., Jack, R. W., and Tagg, J. R.** (2006) Molecular and genetic characterization of a novel nisin variant produced by Streptococcus uberis, *Appl. Environ. Microbiol.* **72**: 1148-1156.
6. **Gross, E., and Nebelin, E.** (1973) Structural Elucidation of Subtilin, *Fed. Proc.* **32:** 577-&.
7. **Stein, T., Borchert, S., Conrad, B., Feesche, J., Hofemeister, B., Hofemeister, J., and Entian, K. D.** (2002) Two different lantibiotic-like peptides originate from the ericin gene cluster of Bacillus subtilis A1/3, *J. Bacteriol.* **184**: 1703-1711.
8. **Wescombe, P. A., and Tagg, J. R.** (2003) Purification and characterization of streptin, a type A1 lantibiotic produced by Streptococcus pyogenes, *Appl. Environ. Microbiol.* **69**: 2737-2747.
9. **Allgaier, H., Jung, G., Werner, R. G., Schneider, U., and Zahner, H.** (1986) Epidermin - Sequencing of a Heterodet Tetracyclic 21-Peptide Amide Antibiotic, *Eur. J. Biochem.* **160**: 9-22.
10. **Israil, A. M., Jack, R. W., Jung, G., and Sahl, H. G.** (1996) Isolation of a new epidermin variant from two strains of Staphylococcus epidermidis--frequency of lantibiotic production in coagulase-negative staphylococci, *Zentralblatt FÃ¼r Bakteriologie: Int. J. Med. Microbiol.* **284**: 285.
11. **Kellner, R., Jung, G., Horner, T., Zahner, H., Schnell, N., Entian, K. D., and Gotz, F.** (1988) Gallidermin - a New Lanthionine-Containing Polypeptide Antibiotic, *Eur. J. Biochem.* **177**: 53-59.
12. **Furmanek, B., Kaczorowski, T., Bugalski, R., Bielawski, K., Bogdanowicz, J., and Podhajska, A. J.** (1999) Identification, characterization and purification of the lantibiotic staphylococcin T, a natural gallidermin variant, *J. Appl. Microbiol.* **87**: 856-866.
13. **MotaMeira, M., Lacroix, C., LaPointe, G., and Lavoie, M. C.** (1997) Purification and structure of mutacin B-Ny266: A new lantibiotic produced by Streptococcus mutans, *Febs Lett.* **410**: 275-279.
14. **Qi, F. X., Chen, P., and Caufield, P. W.** (1999) Purification of mutacin III from group III Streptococcus mutans UA787 and genetic analyses of mutacin III biosynthesis genes, *Appl. Environ. Microbiol.* **65**: 3880-3887.
15. **Smith, L., Novak, J., Rocca, J., McClung, S., Hillman, J. D., and Edison, A. S.** (2000) Covalent structure of mutacin 1140 and a novel method for the rapid identification of lantibiotics, *Eur. J. Biochem.* **267**: 6810-6816.
16. **Qi, F. X., Chen, P., and Caufield, P. W.** (2000) 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.* **66**: 3221-3229.
17. **Castiglione, F., Lazzarini, A., Carrano, L., Corti, E., Ciciliato, I., Gastaldo, L., Candiani, P., Losi, D., Marinelli, F., Selva, E., and Parenti, F.** (2008) Determining the structure and mode of action of microbisporicin, a potent lantibiotic active against multiresistant pathogens, *Chem. Biol.* **15**: 22-31.
18. **Vandekamp, M., Vandenhooven, H. W., Konings, R. N. H., Bierbaum, G., Sahl, H. G., Kuipers, O. P., Siezen, R. J., Devos, W. M., Hilbers, C. W., and Vandeven, F. J. M.** (1995) Elucidation of the Primary Structure of the Lantibiotic Epilancin K7 From Staphylococcus-Epidermidis K7 - Cloning and Characterization of the Epilancin-K7-Encoding Gene and Nmr Analysis of Mature Epilancin K7, *Eur. J. Biochem.* **230**: 587-600.
19. **Ekkelenkamp, M. B., Hanssen, M., Hsu, S. T. D., de Jong, A., Milatovic, D., Verhoef, J., and van Nuland, N. A. J.** (2005) Isolation and structural characterization of epilancin 15X, a novel lantibiotic from a clinical strain of Staphylococcus epidermidis, *Febs Lett.* **579**: 1917-1922.

20. Kaletta, C., Entian, K. D., Kellner, R., Jung, G., Reis, M., and Sahl, H. G. (1989) Pep5, a New Lantibiotic - Structural Gene Isolation and Prepeptide Sequence, *Arch. Microbiol.* **152**, 16-19.
21. **Heidrich, C., Pag, U., Josten, M., Metzger, J., Jack, R. W., Bierbaum, G., Jung, G., and Sahl, H. G.** (1998) Isolation, characterization, and heterologous expression of the novel lantibiotic epicidin 280 and analysis of its biosynthetic gene cluster, *Appl. Environ. Microbiol.* **64**: 3140-3146.