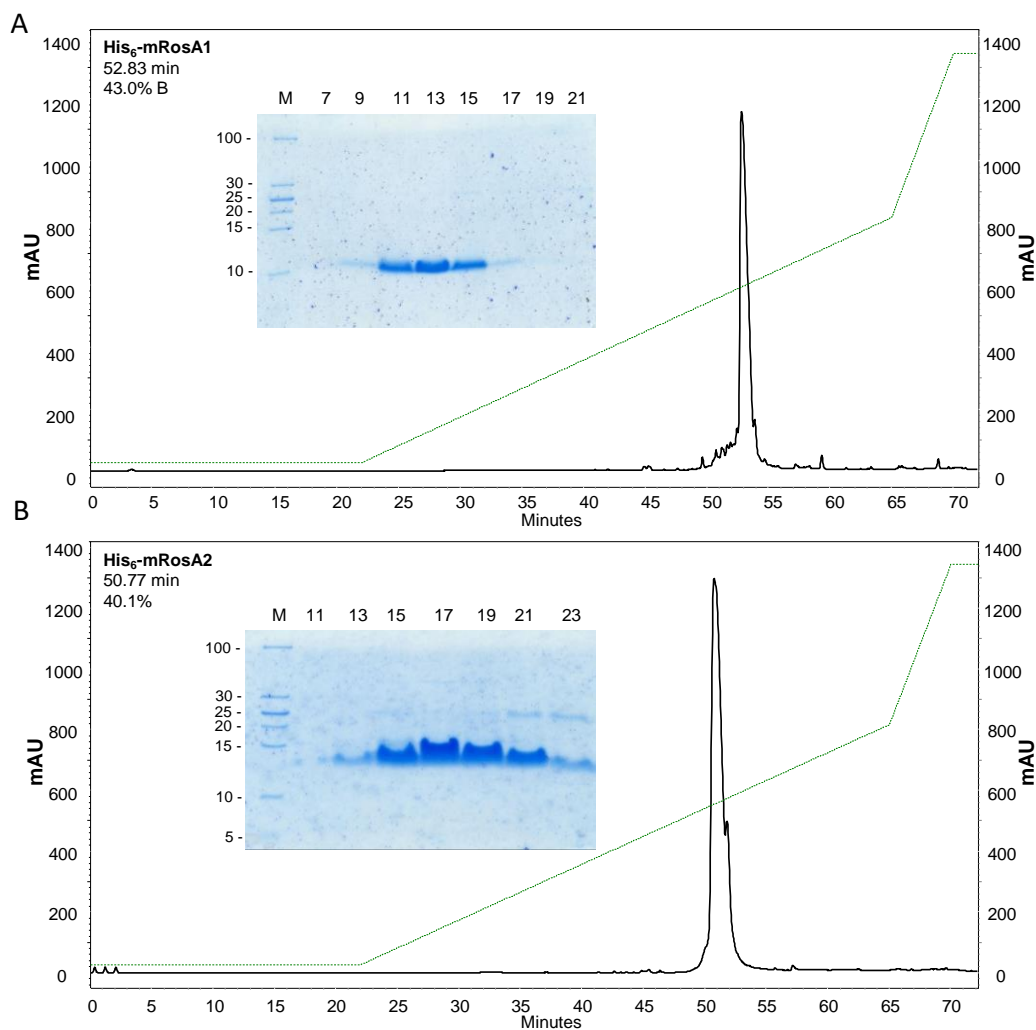
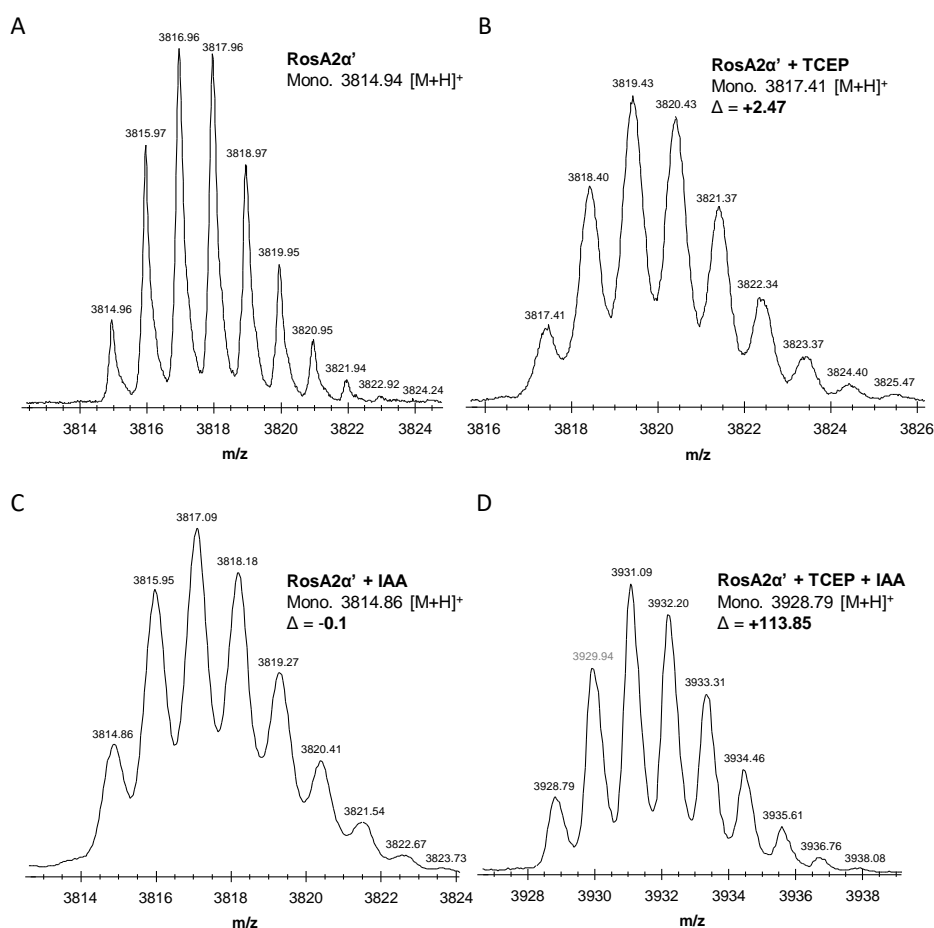


Supplemental Information

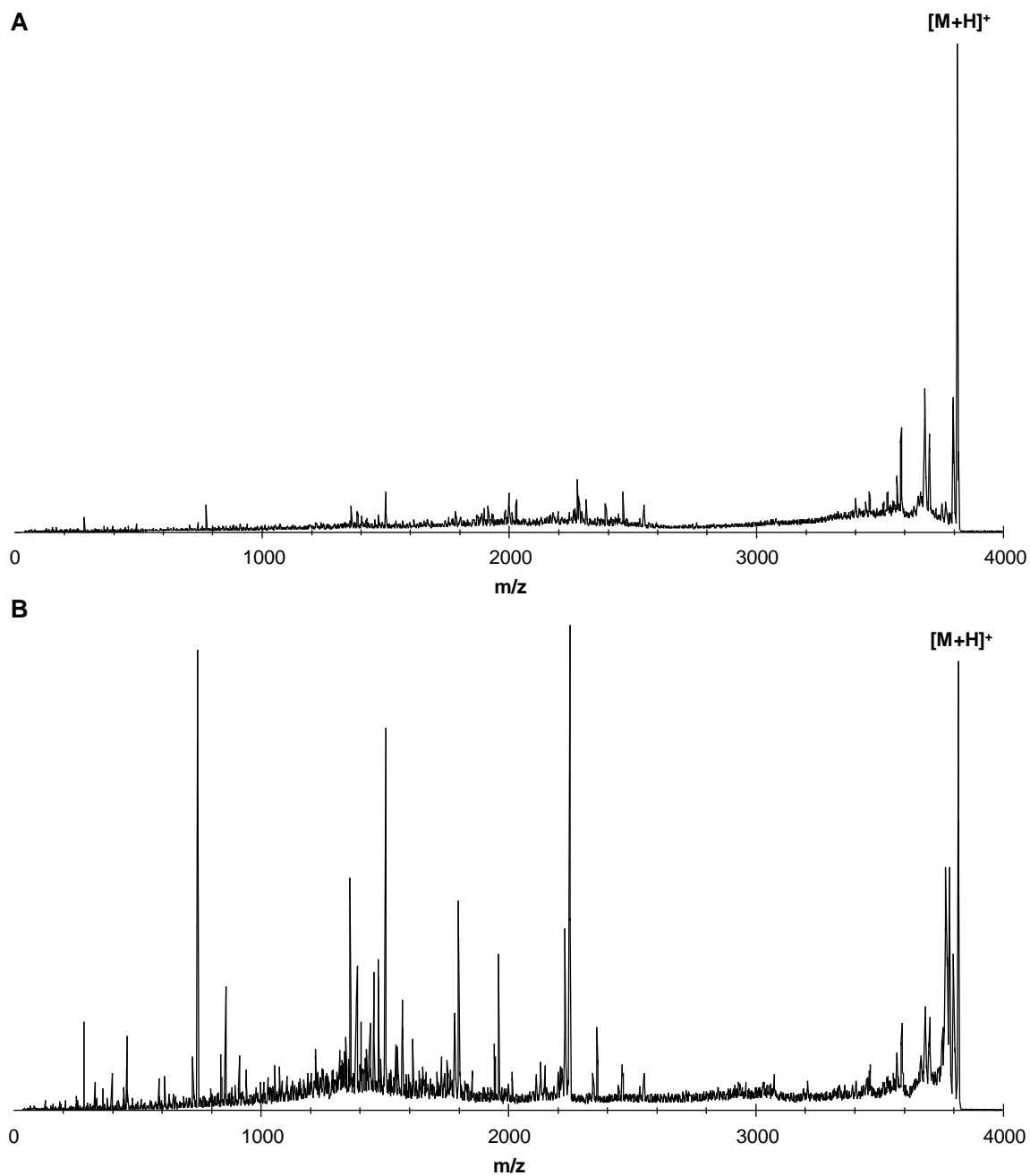
**Semi-*in vitro* Reconstitution of Roseocin, a Two-Component Lantibiotic from an Actinomycete**



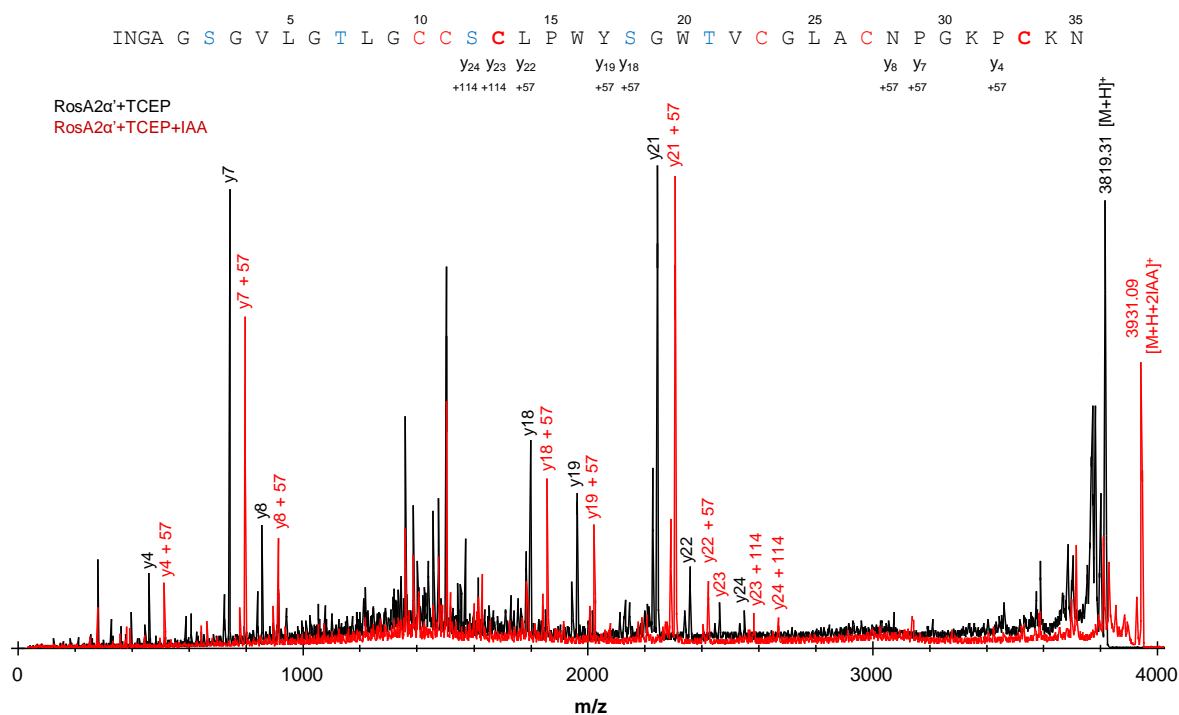
**Figure S1. Related to Figure 2. Purification of hexahistidine tagged RosA peptides with RP-HPLC.** RP-HPLC chromatogram for purification of (A) His<sub>6</sub>-RosA1 and (B) His<sub>6</sub>-RosA2. Collected fractions were lyophilized and reconstituted in MilliQ, followed by analysis on tricine SDS-PAGE (inset). Lane 1 in both the gels is low MW protein ladder and rest of the lanes are collected fractions. The spectra was noted at 280 nm.



**Figure S2. Related to Figure 3. Alkylation assay of RosA2 $\alpha'$  in high-resolution mode.** His<sub>6</sub>-mRosA2 was subjected to GluC digestion and given treatment with TCEP only, IAA only and TCEP followed by IAA. (A) RosA2 $\alpha'$  without any treatment. (B) RosA2 $\alpha'$  when treated with TCEP led to an increase of mass by  $\sim 2$  Da. (C) RosA2 $\alpha'$  treated with IAA alone did not lead to any change in mass. (D) Two carbamidomethylations (approx. +114 Da) were observed upon treatment with IAA in the presence of TCEP.



**Figure S3. Related to Figure 4. Tandem MS analysis of RosA2 $\alpha'$  with and without TCEP treatment. (A) Presence of disulfide bond prevented fragmentation. (B) Treatment with 1 mM TCEP enhanced the fragmentation.**



**Figure S4. Related to Figure 3. Tandem MS analysis of alkylated and non-alkylated RosA2α'.** Selective y ions are labelled for indicating the position of alkylation. Fragment ion y<sub>4</sub> to y<sub>22</sub> indicate one carbamidomethylation at Cys33 and y<sub>23</sub> indicate another at Cys13.

**Table S1. Bioinformatic analysis of roseocin biosynthetic gene cluster**

Locus tag	Cello v. 2.5	TMHMM Server 2.0	CDD	Predicted function
SrosN1_010100020955	Extracellular	0	TIGR03897: Type 2 lantibiotic, mersacidin/lichenicidin family	<i>rosA1</i>
SrosN1_010100020960	Extracellular	0	TIGR03897: Type 2 lantibiotic, mersacidin/lichenicidin family	<i>rosA2</i>
SrosN1_010100020965	Cytoplasmic	0	TIGR03897: type 2 lantibiotic biosynthesis protein LanM	<i>rosM</i>
SrosN1_010100020970	Cytoplasmic	1	No putative conserved domains	Hypothetical protein
SrosN1_010100020975	Membrane	6	COG2274: ABC-type bacteriocin/lantibiotic exporters, contain an N-terminal double-glycine peptidase domain	<i>rosT</i>
SrosN1_010100020980	Membrane	4	No conserved domains, 94% identity with Multidrug transporter ( <i>Streptomyces sp.</i> Ncost-T6T-1)	Immunity*
SrosN1_010100020985	Cytoplasmic	0	cd03268: ATP-binding cassette domain of the bacitracin-resistance transporter	<i>rosF</i>
SrosN1_010100020990	Membrane	6	No conserved domains,	Immunity*
SrosN1_010100020995	Cytoplasmic	0	smart00862: Transcriptional regulatory protein - C terminal, and pfam03704: Bacterial transcriptional activator domain with TPR repeats.	Transcriptional regulator

\*the role of these genes was inferred with Cello and TMHMM prediction tools. The immunity genes are often found encoded in vicinity and as these tools predict the protein encoded by these genes as transmembrane proteins.

**Table S2. b and y ions observed for RosA2 $\alpha$ ' in MALDI TOF MS/MS analysis (related to figure 4A)**

Species	Calc. mass [Da]	Obs. mass [Da]	Difference [Da]	Error
b <sub>3</sub>	285.156	285.830	0.674	0.002364
y <sub>3</sub>	364.165	364.210	0.045	0.000124
y <sub>4</sub>	461.218	462.231	1.013	0.002196
y <sub>5</sub>	589.313	590.326	1.013	0.001719
y <sub>6</sub>	646.334	647.359	1.025	0.001586
y <sub>7</sub>	743.387	744.412	1.025	0.001379
y <sub>8</sub>	857.423	858.458	1.028	0.001199
b <sub>17</sub> -2H <sub>2</sub> O	1457.654	1459.668	2.013	0.001381
b <sub>18</sub> -2H <sub>2</sub> O	1570.739	1572.752	2.013	0.001282
b <sub>19</sub> -2H <sub>2</sub> O	1667.791	1669.805	2.014	0.001208
y <sub>17</sub> -1H <sub>2</sub> O	1729.808	1731.058	1.250	0.000723
y <sub>18</sub> -2H <sub>2</sub> O	1798.84	1799.842	1.002	0.000557
b <sub>20</sub> -2H <sub>2</sub> O	1853.871	1855.884	2.013	0.001086
y <sub>19</sub> -2H <sub>2</sub> O	1961.903	1962.905	1.002	0.000511
b <sub>21</sub> -2H <sub>2</sub> O	2016.934	2018.947	2.013	0.000998
y <sub>20</sub> -2H <sub>2</sub> O	2147.982	2148.985	1.003	0.000467
y <sub>21</sub> -2H <sub>2</sub> O	2245.035	2246.037	1.002	0.000446
y <sub>22</sub> -2H <sub>2</sub> O	2358.119	2359.121	1.002	0.000425
y <sub>23</sub> -2H <sub>2</sub> O	2461.128	2463.267	2.137	0.000875
y <sub>24</sub> -2H <sub>2</sub> O	2548.160	2550.390	2.230	0.000875
b <sub>31</sub> -4H <sub>2</sub> O	2958.344	2960.489	2.145	0.000725
b <sub>32</sub> -4H <sub>2</sub> O	3072.387	3074.592	2.205	0.000718
b <sub>34</sub> -4H <sub>2</sub> O	3226.461	3228.463	2.002	0.000620
b <sub>35</sub> -4H <sub>2</sub> O	3354.556	3356.558	2.002	0.000597
y <sub>34</sub> -4H <sub>2</sub> O	3402.559	3405.040	2.481	0.000729
y <sub>35</sub> -4H <sub>2</sub> O	3459.581	3462.091	2.510	0.000726
y <sub>36</sub> -4H <sub>2</sub> O	3530.618	3533.169	2.551	0.000723
y <sub>37</sub> -4H <sub>2</sub> O	3587.640	3590.221	2.582	0.000720
y <sub>38</sub> -4H <sub>2</sub> O	3701.682	3704.323	2.641	0.000713

**Table S3. b and y ions observed for RosA1 $\beta$ ' in MALDI TOF MS/MS analysis (related to figure 4B)**

Species	Calc. [Da]	Obs. [Da]	Difference [Da]	Error
b <sub>2</sub>	<b>185.128</b>	<b>185.212</b>	<b>0.083</b>	<b>0.000448</b>
b <sub>3</sub>	300.155	301.130	0.974	0.003245
b <sub>4</sub>	415.182	416.156	0.974	0.002346
b <sub>5</sub>	543.241	544.215	0.974	0.001793
b <sub>6</sub>	656.325	657.299	0.974	0.001484
b <sub>7</sub>	771.352	771.794	0.442	0.000573
y <sub>8</sub>	796.301	796.476	0.175	0.000220
b <sub>8</sub>	828.373	828.845	0.472	0.000569
y <sub>9</sub> -1H <sub>2</sub> O	899.310	900.238	0.928	0.001031
b <sub>9</sub>	927.442	928.416	0.974	0.001050
b <sub>10</sub>	998.479	999.453	0.974	0.000975
b <sub>11</sub>	1055.500	1056.474	0.973	0.000922
b <sub>12</sub>	1112.522	1113.157	0.635	0.000571
y <sub>17</sub> -3H <sub>2</sub> O	1579.626	1580.883	1.257	0.000796
b <sub>17</sub> -2H <sub>2</sub> O	1638.743	1639.768	1.025	0.000612
y <sub>18</sub> -4H <sub>2</sub> O	1648.658	1649.865	1.207	0.000732
b <sub>18</sub> -2H <sub>2</sub> O	1709.780	1710.846	1.066	0.000610
b <sub>19</sub> -3H <sub>2</sub> O	1792.828	1793.934	1.107	0.000599
y <sub>22</sub> -4H <sub>2</sub> O	2050.852	2052.456	1.604	0.000782
y <sub>23</sub> -5H <sub>2</sub> O	2133.899	2136.106	2.207	0.001043
y <sub>24</sub> -5H <sub>2</sub> O	2246.983	2249.190	2.207	0.000990
y <sub>25</sub> -6H <sub>2</sub> O	2330.031	2332.238	2.207	0.000947
y <sub>26</sub> -6H <sub>2</sub> O	2429.100	2431.307	2.207	0.000909
y <sub>27</sub> -7H <sub>2</sub> O	2512.147	2514.012	1.864	0.000742
y <sub>28</sub> -7H <sub>2</sub> O	2583.184	2585.090	1.906	0.000738
y <sub>30</sub> -7H <sub>2</sub> O	2757.231	2759.044	1.813	0.000658
y <sub>31</sub> -7H <sub>2</sub> O	2943.310	2944.565	1.255	0.000432
y <sub>32</sub> -8H <sub>2</sub> O	3026.358	3028.611	2.253	0.000749
y <sub>33</sub> -9H <sub>2</sub> O	3109.405	3111.700	2.295	0.000738
y <sub>34</sub> -9H <sub>2</sub> O	3166.427	3168.656	2.229	0.000704
y <sub>35</sub> -9H <sub>2</sub> O	3223.448	3225.678	2.230	0.000692
y <sub>36</sub> -9H <sub>2</sub> O	3294.485	3296.715	2.230	0.000677
y <sub>37</sub> -9H <sub>2</sub> O	3393.554	3396.012	2.458	0.000724
y <sub>38</sub> -9H <sub>2</sub> O	3450.575	3453.064	2.488	0.000721
y <sub>39</sub> -9H <sub>2</sub> O	3565.602	3567.832	2.230	0.000625
y <sub>40</sub> -9H <sub>2</sub> O	3678.686	3680.916	2.230	0.000606
y <sub>41</sub> -9H <sub>2</sub> O	3806.745	3808.974	2.230	0.000586
y <sub>42</sub> -9H <sub>2</sub> O	3921.772	3924.001	2.230	0.000569
y <sub>43</sub> -9H <sub>2</sub> O	4036.799	4039.042	2.243	0.000556

**Table S4. Primers used for cloning of RosM and RosA2 $\alpha$ /RosA1 $\beta$ .**

<i>Primer</i>	<i>Sequence</i>	Source	Identifier
ACYCDuetUP1	GGATCTCGACGCTCTCCCT	Novagen	Cat#71178-3
DuetDOWN1	GATTATGCGGCCGTGTACAA	Novagen	Cat#71179-3
DuetUP2	TTGTACACGGCCGCATAATC	Novagen	Cat#71180-3
T7 Terminator	GCTAGTTATTGCTCAGCGG	Novagen	Cat#69337-3
<i>M1</i>	GAGGAACGCTTCGCCTCCTA	This paper	N/A
<i>M2</i>	AGGACGGCACCTTCGGCCTG	This paper	N/A
<i>M3</i>	GACCCCGGCGACTTCGCGGA	This paper	N/A

**Table S5. Locus tag of protein sequences used in this study**

lacticin 3147	LtnA1, O87236 LtnA2, O87237
staphylococcin C55	SacA1, BAB78438 SacA2, BAB78439
plantaricin W	PlwA1, AAG02567 PlwA2, AAG02566
BhtA	BhtA1, AAZ76603 BhtA2, AAZ76602
lichenicidin	BliA1, P86475 BliA2, P86476
haloduracin	HalA1, Q9KFM5 HalA2, Q9KFM6
BhtA	BhtA1, Q3YB75 BhtA2, Q3YB76
enterocin W	EnwA, H3JSS9 EnwB, H3JST0