

Supplemental material for

**A Transmembrane Protein Responsible for Subtilomycin Immunity, ApnI, Unveils a Novel Model for Lantibiotic Immunity**

Yun Deng, Cong-Zhi Li, Yi-Guang Zhu, Peng-Xia Wang, Jing-Jing, Fu, Qing-Dong Qi, Dong-Hai Peng, Li-Fang Ruan, and Ming Sun\*

\*Correspondence should be addressed to Ming Sun at

State Key Laboratory of Agricultural Microbiology,

College of Life Science and Technology,

Huazhong Agricultural University,

Wuhan 430070, People's Republic of China

E-mail: [m98sun@mail.hzau.edu.cn](mailto:m98sun@mail.hzau.edu.cn)

Tel: (+86) 27 87283455, Fax: (+86) 27 87280670

**This file includes:**

**Supporting Table S1 to S2**

**Supporting Figure S1 to Figure S4 and Legends**

**Supporting References**

Table S1 Comparison of the fragments obtained from MS/MS of the 3234.48 Da peptide and the theoretical mass of predicted subtilomycin\*

Residues of core peptide	Measured m/z	Calculated mass	Theoretical mass	Difference (Da)
1-32	1079.1658 y32(3+)	3234.474	3234.4806	0.0066
1-4	425.1804 b4(+)	425.1804	425.1828	0.0024
1-5	538.2646 b5(+)	538.2646	538.2668	0.0022
6-32	1349.6148 y27(2+)	2697.214	2697.2217	0.0077
1-2	271.1079 b2(+)	271.1079	271.1086	0.0007
1-4	342.1439 b3(+)	342.1439	342.1457	0.0018
32-32	133.0598 y1(+)	132.052	132.0536	0.0016
10-32	1158.9991 y23(2+)	2315.9826	2315.9841	0.0015
1-8	806.4182 b8(+)	806.4182	806.4203	0.0021
4-32	965.4426 y29(3+)	2893.3044	2893.3428	0.0384
8-32	1257.0624 y25(2+)	2512.1092	2512.1053	-0.0039
1-9	919.5036 b9(+)	919.5036	919.5044	0.0008
9-32	1215.5418 y24(2+)	2429.068	2429.0682	0.0002
5-32	1406.1535 y28(2+)	2810.2914	2810.3058	0.0144
2-32	1051.151 y31(3+)	3150.4296	3150.4593	0.0297
1-31	1552.1953 b31(2+)	3103.3828	3103.4350	0.0522

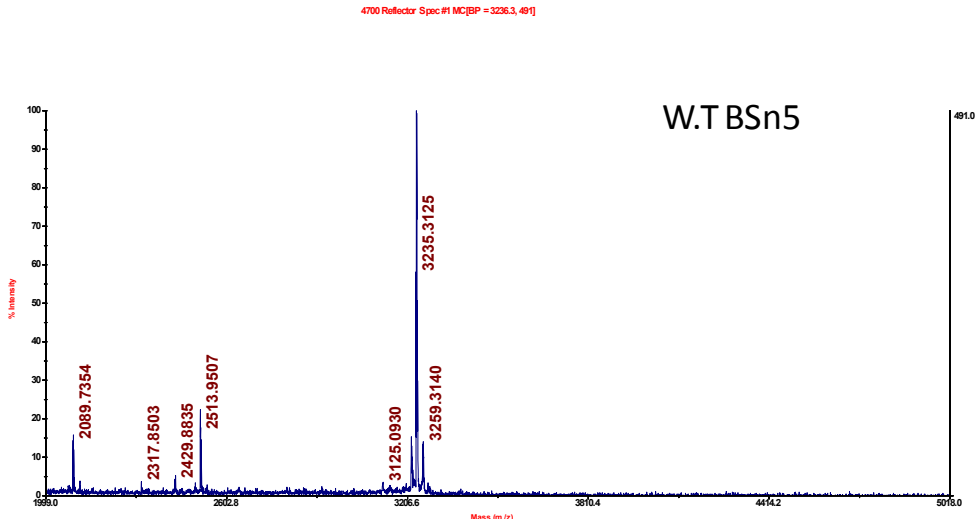
\*The fragments are listed as ions in descending order of intensity

Table S2 The location of ApnI-like immunity proteins-encoding genes in the genome of the corresponding produce strains

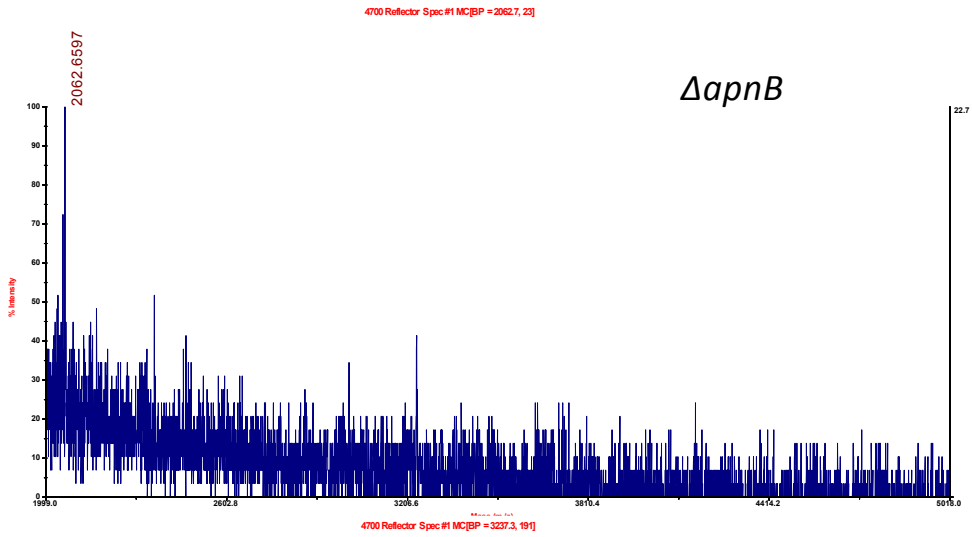
Gene cluster	Location in available genome (GenBank Accession <sup>a</sup> )	Produce strain	ApnI-like proteins (GenBank Accession)	Coverage and Similarity to ApnI (%)	Identity to ApnI (%)	Identity to ApnA (%)
subtilomycin	202,000-211,369 bp ( CP007409.1 )	<i>B.subtilis</i> OH 131.1	SubI AID00391.1	100, 100	100	100
subtilomycin	1-11,100 bp (JX912247.1)	<i>B.subtilis</i> MMA7	SubI AGL93175.1	100, 100	100	100
Putative paenibacillin	125,106-139,135 bp in contig129 (AIPP01000129.1)	<i>P. polymyxa</i> OSY-DF	PaeI AFS60104.1	72, 55	27	47
Putative paenibacillin	123,569-136,380 bp in contig14 (AFOX01000014.1)	<i>P. polymyxa</i> ATCC 842	NA	72, 55	27	47
Putative paenibacillin	135,495-149,256 bp in contig7 (AMQU01000007.1)	<i>Paenibacillus</i> ICGEB2008	NA	72, 55	27	47
Putative lantibiotic	124,141-134,856 bp (CP004135.1)	<i>B. thuringiensis</i> IS5056	AGG04945.1	90, 48	29	56-58
Putative lantibiotic	1-7,780 bp in contig00361 (ACNA01000175.1)	<i>B. thuringiensis</i> T01001	EEM31619.1	90, 48	29	50-56
Putative lantibiotic	1-7,870 bp in contig00246 (ACNK01000131.1)	<i>B. thuringiensis</i> IBL200	EEM95177.1	90, 48	24	50-56
Putative lantibiotic	1-7,800 bp in contig00015 (ACNF01000121.1)	<i>B. thuringiensis</i> 10792	EEM62998.1	90, 48	29	54-56
Putative lantibiotic	10,000-19,500 bp in contig000035 (ARXZ02000035.1)	<i>B. thuringiensis</i> T01-328	ERH97312.1	90, 48	29	54-56
Putative lantibiotic	10,000-18,388 bp (AHCQ01000073.1)	<i>B. cereus</i> BAG1X1-1	EOO24724.1	90, 48	29	50-56
Putative lantibiotic	1-8,000 bp (AHCT01000096.1)	<i>B. cereus</i> BAG1X2-1	EOO43056.1	90, 48	29	50-56
Putative lantibiotic	1-8,800 bp (AHCU01000046.1)	<i>B. cereus</i> BAG1X2-2	EOO46413.1	90, 48	29	50-56
Putative lantibiotic	10,000-18,422 bp (AHCV01000030.1)	<i>B. cereus</i> BAG1X2-3	EOO61970.1	90, 48	29	50-56
Putative lantibiotic	10,000-19,500 bp (AHCW01000050.1)	<i>B. cereus</i> BAG2O-1	EOP01400.1	90, 48	29	50-56

a. Results are from GenBank nucleotide database in June 2014.

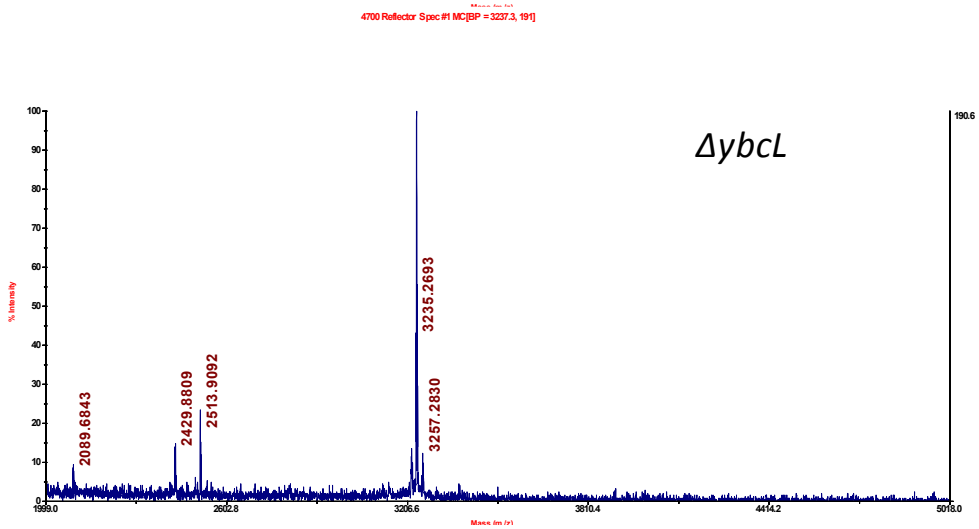
1



2



3



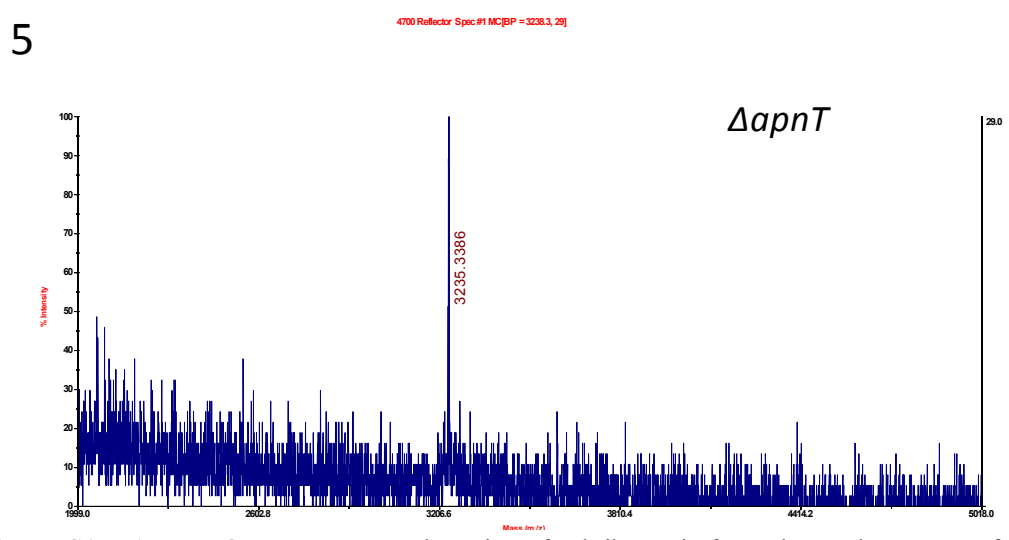
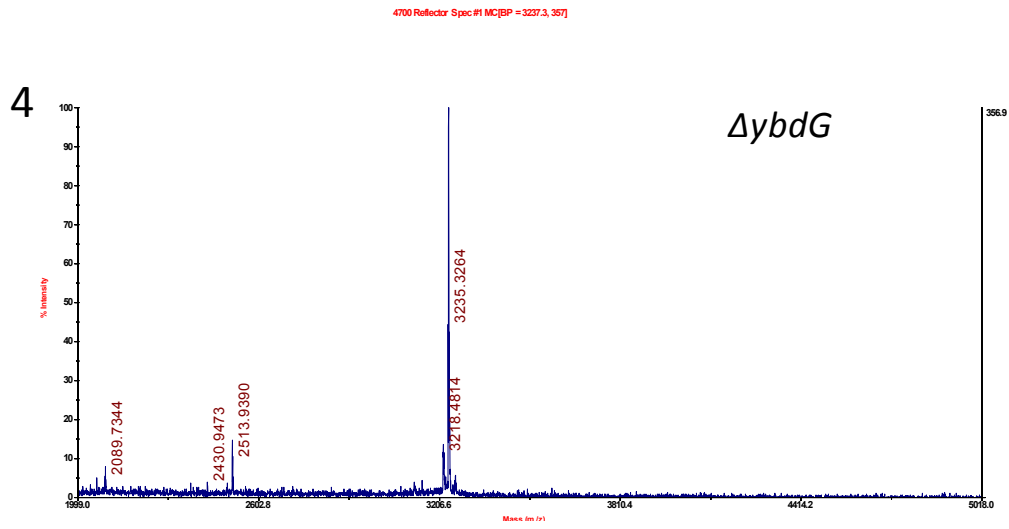


Figure. S1 MALDI-TOF mass spectra detection of subtilomycin from the crude extracts of wild type strain BSn5 and mutants. (1) Wild type strain BSn5 (2) mutant *ΔapnB* (3) mutant *ΔybcL* (4) mutant *ΔybdG* (5) mutant *ΔapnT*. The black ring refers to the respective target peaks of subtilomycin in MALDI-TOF spectrum. BP indicates Base Peak.

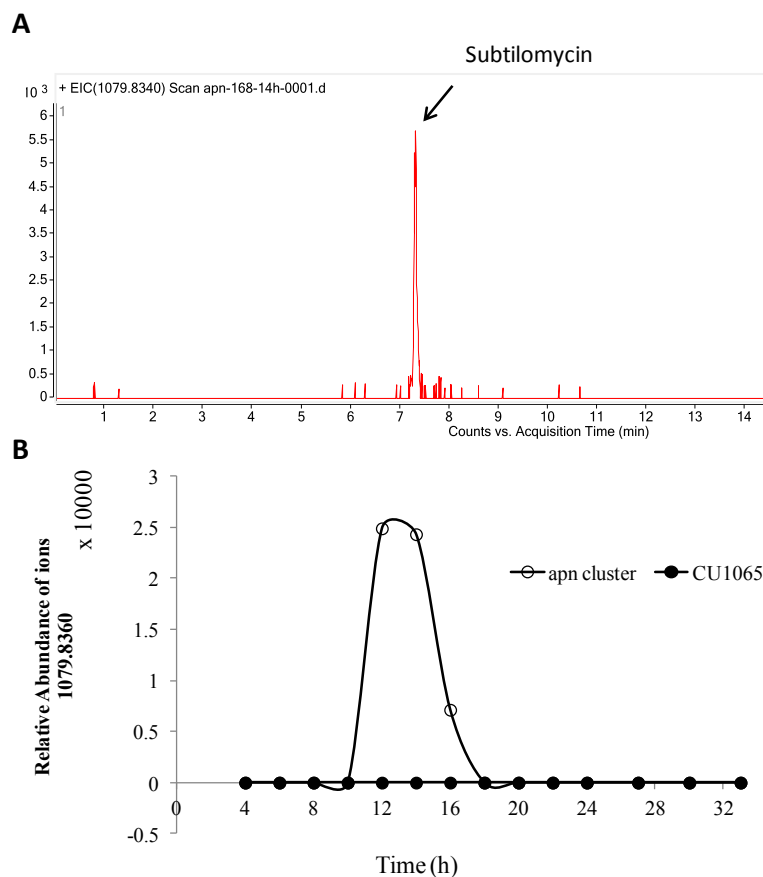


Figure. S2 Detection the production of subtilomycin from the supernatant of the recombinant with subtilomycin cluster integrated into *B.subtilis* CU1065 chromosome by high resolution LC-ESI-MS. (A) The extracted ions chromatography (EIC) of ions  $[M+3H]^{3+}$  1079.8338 m/z, which is indicated as subtilomycin. (B) Detection the ions 1079.8338 in supernatant of CU1065 and *apn* cluster recombinant during the different culture times.

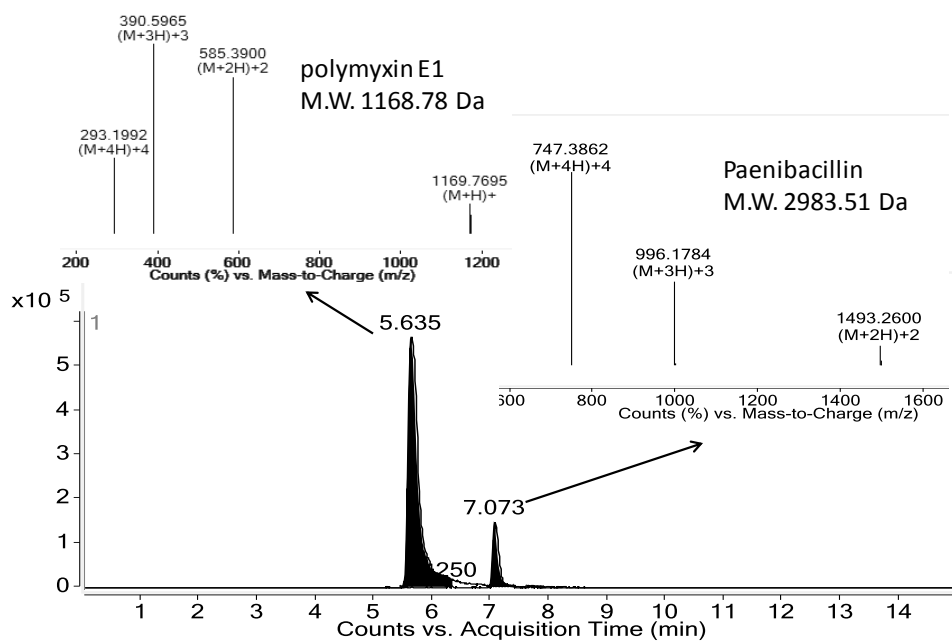


Figure. S3 The detection of paenibacillin production in crude extracts of *Paenibacillus* ATCC 842 by high resolution LC-ESI-MS. (Two compounds that have been identified from a crude extract of *Paenibacillus polymyxa* OSY-DF culture supernatant as polymyxin E1 and paenibacillin (1) were detected from a crude extract of *Paenibacillus* ATCC 842 culture supernatant. )

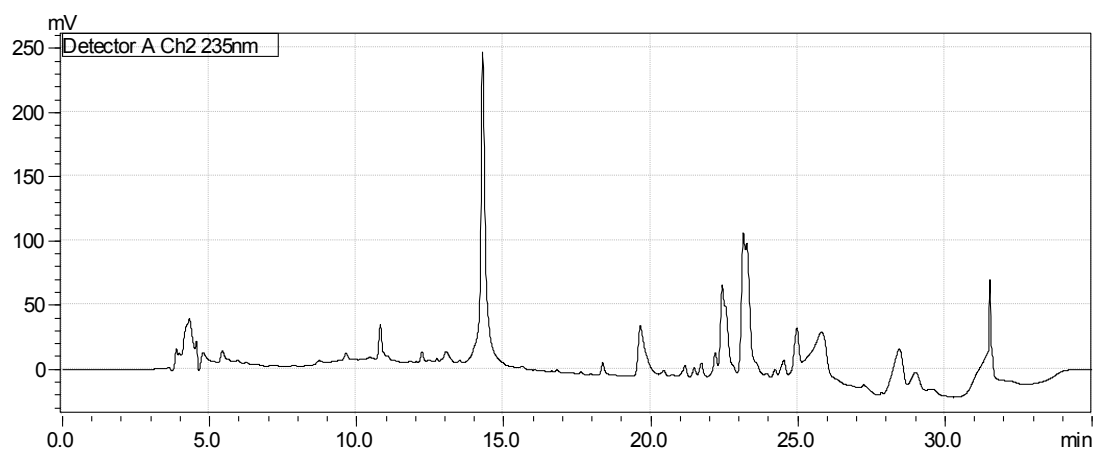


Figure. S4 Preparation RP-HPLC purification of the subtilomycin present in ammonium sulphate crude extracts (ASCE) from 12 h LB cultures of BSn5. (retention time, 14.3 min/45% acetonitrile)



1. **He Z, Kisa D, Zhang L, Yuan C, Green-Church KB, Yousef AE.** 2007. Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. *Appl Environ Microbiol* **73**:168-178.