Supporting Information

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Supplementary Methods

Stereochemistry of the Amino Acids

Amino acid chirality was determined by reacting acid hydrolysates of the locillomycins with o-phthaldialdehyde (OPA) together with N-isobutyryl-L-cysteine (IBLC) as chiral reagents.[1] Briefly, locillomycins were hydrolyzed with 6 M HCl containing 1% phenol at 110 ℃ for 24 h. After hydrolysis, the samples were cooled and dried for further analysis. 260 mM IBLC and 170 mM OPA in 1 M potassium borate buffer of pH 10.4 were used as derivatization reagent. 50 μL 0.4 M sodium borate buffer of pH 10.4, 10 μL OPA/IBLC reagent, 22 μL samples or amino acid standard solution before injections on a reversed-phase column (RP-18, 5 μM, 4 × 250 mm; Merck). Mobile phase: eluent A, 23 mM sodium acetate, adjusted to pH 6.0 by addition of 10% acetic acid; eluent B, methanol/acetonitrile (600 mL + 50 mL); linear gradient , 0% B to 53.5% B in 90 min at a flow rate at 0.5 mL/min; then 100% B for 10 min followed by 100% A for 5 min.

Assay of the antifungal activities against mycelium growth of *F. oxysporum*.

The fungal strains were cultured at 28 ℃ on potato dextrose agar (PDA) medium containing (per liter) 200 g of potato infusion, 20 g of glucose, and 20 g of agar (pH 7.0). The wild-type and mutant strains antifungal activities against mycelium growth of *F. oxysporum* was performed as follows. Mycelia plugs (5 mm) of *F. oxysporum* were deposited in the center of the plates at equal distances from the wells. 5 μL portions of culture strains were deposited on both sides at a distance 2.5 cm from the rims of mycelia plugs. The plates were inoculated at 28 ℃, and inhibition zones were measured after 3 to 7 days.

Antibacterial and Antiviral Activities

The antibacterial activities of the purified antibiotics were determined by MIC which takes into account the higher dilution of antibiotics at which no growth of the test bacteria was visible.[2] The bacteria used for the test were Staphylococcus aureus and Xanthomonas oryzae pv. oryzae. The antiviral activities of the purified antibiotics against porcine epidemic diarrhea (PED) virus were performed according to a previous report.[3] Briefly, Vero cells seeded with complete confluent condition were infected with PED virus at a multiplicity of infection (0.01 MOI) with or without locillomycins extract at 0, 24, 40, and 48 h post infection. The number of viruses was

determined by quantitative Real-Time PCR. Total RNA was isolated and used for cDNA synthesis. Real-time PCR was performed by subjecting the reaction mixtures to initial denaturation at 94 ℃ for 3 min, followed by 40 cycles of 94 ℃ for 20 s, 65 ℃ for 20 s, and 72 ℃ for 30 s. The primers PEDF and PEDR specific for Nucleocapsid gene of PED virus are used for PCR (Table S7).

Evaluation of hemolytic activities. To evaluate the hemolytic activities of the wild-type *B. subtilis* 916 and the mutants, strains were streaking inoculated on the blood agar plates with 5% defibrinated sheep blood. Hemolytic activities were visualized by development of a clear halo around the growth of the strains after incubation at 37 ℃ for 1 – 4 days. In all cases, three replicate plates were used for each strain and the experiment was repeated once.

(1) Words, K. *Chromatographia* **1991**, *32*, 383–388.

(2) Leclere, V.; Bechet, M.; Adam, A.; Guez, J.-S.; Wathelet, B.; Ongena, M.; Thonart, P.; Gancel, F.; Chollet-Imbert, M.; Jacques, P. *Applied Environ. Microbiol.* **2005**, *71*, 4577–4584.

(3) Cho, W.-K.; Kim, H.; Choi, Y. J.; Yim, N.-H.; Yang, H. J.; Ma, J. Y. *Evid. Based Complement. Alternat. Med.* **2012**, *2012*, 985151.

Supplementary Tables

Table S1: Summary of the open reading frames of the locillomycin gene cluster

ORF	No. of aa	Protein homology (NCBI No.)	Percentage of aa identity
ybbR	479	*Bacillus amyloliquefaciens* FZB42 conserved hypothetical protein YbbR (ABS72628.1)(479 aa)	100
ybbT	455	*Bacillus amyloliquefaciens* FZB42 putative phosphoglucomutase YbbT (ABS72629.1)(448 aa)	96
glmS	600	*Bacillus amyloliquefaciens* FZB42 l-glutamine-D-fructose-6-phosphate amidotransferase （ABS72631.1）（600 aa）	100
Orf1	291	*Bacillus amyloliquefaciens* FZB42 putative ATP binding protein of ABC transporter （ABS72632.1）（290 aa）	98
locD	1293	*Paenibacillus polymyxa* E681 polyketide synthase module containing protein (ADM70188.1)(2029 aa)	40
LocA	2325	*Aneurinibacillus migulanus* Gramicidin S synthase II(P0C063.2) (4451 aa)	40
LocB	3868	*Paenibacillus mucilaginosus* K02 fusaricidin synthetase (YP_006190135.2)(6737 aa)	48
LocC	2126	*Brevibacillus brevis* NBRC 100599 tyrocidine synthetase III (YP_002772270.1)(6487 aa)	42
Orf3	227	*Bacillus amyloliquefaciens* FZB42 member of the two componenent signal transduction system containing the sensor IM histidine kinase, RBAM_002380 (ABS72637.1)(227 aa)	100
Orf4	340	*Bacillus amyloliquefaciens* FZB42 membrane bound sensor kinase; probably linked with the response regulator RBAM002380 (ABS72638.1)(340)	98
Orf5	254	*Bacillus amyloliquefaciens* FZB42 putative ABC-type antimicrobial peptidetransport system, ATPase (ABS72639.1)(254 aa)	98
Orf6	642	*Bacillus amyloliquefaciens* FZB42 putative ABC-type transport system,permease(ABS72640.1)(642 aa)	97
Orf7	1010	*Bacillus methanolicus* Multidrug transporter AcrB (WP 004433265.1)(1001 aa)	61
kdgR1	281	*Bacillus amyloliquefaciens* FZB42 putative 2,5-diketo-D-gluconic acid reductase KdgR1 (ABS72642.1) (281 aa)	98

Note: aa, amino acid.

[a]The closest homologs were based on NCBI searches conducted July 8, 2013.

Table S2: Adenylation domain substrate specificity predicted and determined

A domain	Selectivity-confering amino acid at position										Amino acids	
	235	236	239	278	299	301	322	330	331	517	Predicted[a]	Proved[b]
LocA1	D	F	W	N	I	G	M	V	H	K	Thr	Thr
LocB1	D	A	V	Q	M	D	C	V	D	K	Asp/Asn/Glu/Gln	Gln/Asn
LocB2	D	L	T	K	I	G	H	I	G	K	Asp/Asn/Glu/Gln	Asp
LocB3	D	I	L	Q	Y	G	M	I	W	K	Gly/Ala	Gly
LocC1	D	G	L	F	T	V	R	V	E	K	tyr	tyr
LocC2	D	A	F	W	I	G	A	T	F	K	val/leu/ile	val

[a] Comparison of the adenylation domain specificity with known nonribosomal peptide natural products.

[b] The internal denylation domain were investigated in terms of activity in the ATP-PPi exchange reaction with the amino acids of locillomycin and a control without amino acid.

Table S3: MS characteristics of the fragment of Locillomycin-A

Molecule Weight	Relative Abundance	Molecule Formula	Sequence
212.1	53	$C_{13}H_{26}O_1N_1$	$CH_3(CH_2)_{11}CONH$
221.1	105	$C_{11}H_{13}N_2O_3$	Gly-Tyr
244.1	74	$C_9H_{14}N_3O_5$	Gln-Asp
280.3	268	$C_{14}H_{22}N_3O_3$	Tyr-Val
297.1	51	$C_{17}H_{33}N_2O_2$	Thr(Link)
301.3	50	$C_{11}H_{17}N_4O_6$	Gln-Asp-Gly
336.3	60	$C_{15}H_{18}N_3O_6$	Asp-Gly-Tyr
397.4	55	$C_{22}H_{41}N_2O_4$	Val-Thr(Link)
398.2	63	$C_{21}H_{40}N_3O_4$	Thr(Link)-Gln
402.1	54	$C_{14}H_{20}N_5O_9$	Asp-Gly-Asn-Asp
415.4	81	$C_{15}H_{23}N_6O_8$	Gln-Asp-Gly-Asn
459.2	47	$C_{16}H_{23}N_6O_{10}$	Asp-Gly-Asn-Asp-Gly
507.1	72	$C_{21}H_{27}N_6O_9$	Gly-Asn-Asp-Gly-Thr
530.2	277	$C_{19}H_{28}N_7O_{11}$	Gln-Asp-Gly-Asn-Asp
587.1	106	$C_{21}H_{31}N_8O_{12}$	Gln-Asp-Gly-Asn-Asp-Gly
622.2	97	$C_{25}H_{32}N_7O_{12}$	Asp-Gly-Asn-Asp-Gly-Tyr
640.4	74	$C_{31}H_{54}N_5O_9$	Val-Thr(Link)-Gln-Asp
688	69	$C_{36}H_{58}N_5O_8$	Tyr-Val-Thr(Link)-Gln
721.2	83	$C_{30}H_{41}N_8O_{13}$	Asp-Gly-Asn-Asp-Gly-Tyr-Val
732.1	49	$C_{37}H_{58}N_5O_{10}$	Asp-Gly-Tyr-Val-Thr(Link)
750.3	51	$C_{30}H_{40}N_9O_{14}$	Gln-Asp-Gly-Asn-Asp-Gly-Thr
849.4	193	$C_{35}H_{49}N_{10}O_{15}$	Gln-Asp-Gly-Asn-Asp-Gly-Tyr-Val
860.5	52	$C_{42}H_{66}N_7O_{12}$	Tyr-Val-Thr(Link)-Gln-Asp-Gly
884.4	50	$C_{38}H_{62}N_9O_{15}$	Thr(Link)-Gln-Asp-Gly-Asn-Asp-Gly3
975.6	41	$C_{46}H_{71}N_8O_{15}$	Asp-Gly-Tyr-Val-Thr(Link)-Gln-Asp
983.6	47	$C_{43}H_{71}N_{10}O_{16}$	Val-Thr(Link)-Gln-Asp-Gly-Asn-Asp
1146.6	404	$C_{52}H_{80}N_{11}O_{18}$	locillomycin A+H

Thr(link): represents the amino of a Thr reacting with the fatty acid ($CH_3(CH_2)_{11}COOH$) to form an amide bond.

Table S4: MS fragment characteristics of Locillomycin B

Molecule Weight	Relative Abundance	Molecule Formula	Sequence
221	47	$C_{11}H_{13}N_2O_3$	Gly-Tyr
244	87	$C_9H_{14}N_3O_5$	Gln-Asp
287.1	48	$C_{10}H_{15}N_4O_6$	Asn-Asp-Gly
336.1	81	$C_{15}H_{18}N_3O_6$	Asp-Gly-Tyr
402.4	56	$C_{14}H_{20}N_5O_9$	Asp-Gly-Tyr-Asp
415	54	$C_{15}H_{23}N_6O_8$	Gln-Asp-Gly-Asn
507.3	61	$C_{21}H_{27}N_6O_9$	Gly-Asn-Asp-Gly-Tyr
587.2	99	$C_{21}H_{31}N_8O_{12}$	Gln-Asp-Gly-Asn-Asp-Gly
603.9	76	$C_{33}H_{55}N_4O_6$	Gly-Tyr-Val-Thr(Link)
622	130	$C_{25}H_{32}N_7O_{12}$	Asp-Gly-Asn-Asp-Gly-Tyr
721.2	94	$C_{30}H_{41}N_8O_{13}$	Asp-Gly-Asn-Asp-Gly-Tyr-Val
750.1	45	$C_{30}H_{40}N_9O_{14}$	Gln-Asp-Gly-Asn-Asp-Gly-Tyr
832.1	63	$C_{41}H_{66}N_7O_{11}$	Asn-Asp-Gly-Tyr-Val-Thr(Link)
849.3	233	$C_{35}H_{49}N_{10}O_{15}$	Gln-Asp-Gly-Asn-Asp-Gly-Tyr-Val
898.7	89	$C_{39}H_{64}N_9O_{15}$	Thr(Link)-Gln-Asp-Gly-Asn-Asp-Gly
917.5	80	$C_{44}H_{69}N_8O_{13}$	Gly-Asn-Asp-Gly-Tyr-Val-Thr(Link)
940.1	50	$C_{42}H_{70}N_9O_{15}$	Val-Thr(Link)-Gln-Asp-Gly-Asn-Asp
1160.6	366	$C_{53}H_{82}N_{11}O_{18}$	locillomycin B+H

Thr(link): represent the amino of a Thr reacted with the fatty acid ($CH_3(CH_2)_{12}COOH$) to form amide bond.

Table S5: MS fragment characteristics of Locillomycin C

Molecule Weight	Relative Abundance	Molecule Formula	Sequence
172.2	62	$C_6H_{10}N_3O_3$	Gly-Asn
221	190	$C_{11}H_{13}N_2O_3$	Gly-Tyr
230.1	49	$C_8H_{12}N_3O_5$	Asn-Asp
244	303	$C_9H_{14}N_3O_5$	Gln-Asp
287.1	181	$C_{10}H_{15}N_4O_6$	Asn-Asp-Gly
301.2	106	$C_{11}H_{17}N_4O_6$	Gln-Asp-Gly
326	87	$C_{19}H_{36}N_1O_3$	Thr(Link)
336	94	$C_{15}H_{18}N_3O_6$	Asp-Gly-Tyr
344.1	50	$C_{12}H_{18}N_5O_7$	Gly-Asn-Asp-Gly
402.2	69	$C_{14}H_{20}N_5O_9$	Asp-Gly-Asn-Asp
415.2	100	$C_{15}H_{23}N_6O_8$	Gln-Asp-Gly-Asn
425.2	79	$C_{24}H_{45}N_2O_4$	Val-Thr(Link)
450.1	65	$C_{19}H_{24}N_5O_8$	Asn-Asp-Gly-Tyr
459.1	51	$C_{16}H_{23}N_6O_{10}$	Asp-Gly-Asn-Asp
507.1	59	$C_{21}H_{27}N_6O_9$	Gly-Asn-Asp-Gly-Tyr
530	124	$C_{19}H_{28}N_7O_{11}$	Gln-Asp-Gly-Asn-Asp
553	192	$C_{29}H_{53}N_4O_6$	Val-Thr(Link)-Gln
569.3	99	$C_{28}H_{49}N_4O_8$	Thr(Link)-Gln-Asp
587	79	$C_{21}H_{31}N_8O_{12}$	Gln-Asp-Gly-Asn-Asp-Gly
588.4	72	$C_{33}H_{54}N_3O_6$	Tyr-Val-Thr(Link)
622.1	72	$C_{25}H_{32}N_7O_{12}$	Asp-Gly-Asn-Asp-Gly
716.1	52	$C_{38}H_{62}N_5O_8$	Tyr-Val-Thr(Link)-Gln
849.2	165	$C_{35}H_{49}N_{10}O_{15}$	Gln-Asp-Gly-Asn-Asp-Gly-Tyr-Val
912.2	61	$C_{40}H_{66}N_9O_{15}$	Thr(Link)-Gln-Asp-Gly-Asn-Asp-Gly
931.8	60	$C_{45}H_{71}N_8O_{13}$	Gly-Asn-Asp-Gly-Tyr-Val-Thr(Link)
1174.6	69	$C_{54}H_{84}N_{11}O_{18}$	LocillomycinC+H

Thr (link): represents the amino of a Thr reacted with the fatty acid ($CH_3(CH_2)_{13}COOH$) to form amide bond.

Table S6: ^{1}H and ^{13}C data of locillomycin C in CD_3OH

		δ_H (ppm)	δ_C (ppm)	HMBC
Cyclic peptide				
Thr 1	α	4.75	57.43	1’; Thr1-C_β, C’
	β	5.48	72.14	Val9-C’
	γ	1.19	16.79	Val9-C_α, C_β
	H_N	8.33	--	1’; Thr1-C_α
	C’	--	171.08	
Gln 2	α	4.30	54.68	Thr1-C’; Gln2-C_β, C_γ, C’
	β	1.98, 2.04	29.09	Gln2-C_α, C_γ, C_δ, C’
	γ	2.25	32.07	Gln2-C_α, C_β, C_δ
	δ	--	177.74	
	ε	6.65, 7.64	--	Gln2-C_γ, C_δ,
	H_N	8.01	--	Thr1-C’
	C’	--	173.24	
Asp 3	α	4.63	53.04	Gln2-C’; Asp3-C_β, C_γ, C’
	β	2.65, 2.69	39.81	Asp3-C_α, C_γ, C’
	γ	--	177.37	
	H_N	8.56	--	Gln2-C’
	C’	--	174.97	
Gly 4	α	3.92, 3.80	43.90	Asp3-C’; Gly4-C’
	H_N	8.20	--	Asp3-C’
	C’	--	173.10	
Asn 5	α	4.51	54.14	Gly4-C’; Asn5- C_β, C’
	β	2.76	37.14	Asn5- C_α, C’
	γ	--	174.41	
	δ	6.96, 7.83	--	
	H_N	8.74	--	
	C’	--	174.36	
Asp 6	α	4.56	53.53	Asn5-C’; Asp6-C_β, C_γ, C’
	β	2.65, 2.76	38.57	Asp6-C_α, C_γ, C’
	γ	--	177.55	
	H_N	8.47	--	Asn5-C’
	C’	--	174.51	
Gly 7	α	4.09, 3.93	43.46	Asp6-C’; Gly7-C’
	H_N	8.04	--	Asp6-C’
	C’	--	172.49	
Tyr 8	α	4.41	58.05	Gly7-C’; Tyr8-C_β, C_γ, C’
	β	2.98, 3.12	37.43	Tyr8-C_α, C_γ, C_δ, C’
	γ	--	129.49	
	δ	7.16	131.23	Tyr8-C_β, C_ε, C_ζ
	ε	6.69	116.35	Tyr 8-C_γ, C_δ, C_ζ
	ζ	--	157.24	
	H_N	7.92	--	Gly7-C’; Tyr 8-C_α, C_β
	C’	--	174.05	
Val 9	α	4.22	59.99	Tyr8-C’; Val9-C_β, $C_{\gamma1}$, $C_{\gamma2}$, C’
	β	2.07	31.19	Val9-C_α, C’
	γ1	0.84	19.18	Val9-C_α, C_β, $C_{\gamma2}$
	γ2	0.88	18.61	Val9-C_α, C_β, $C_{\gamma1}$
	H_N	7.53	--	
	C’	--	171.32	
Acyl group				
	1’	--	177.27	
	2’	2.41	36.84	1’; 3’; 4’
	3’	1.63	27.14	1’; 2’; 4’
	4’-13’	1.2~1.4	30.3~31.0	
	14’	1.28	23.69	15’; 13’
	15’	0.86	11.71	14’; 13’

Note: --: no such nucleus; N/A: assignment not available

Table S7 ^{1}H and ^{13}C data of locillomycin A in 90% H_2O/10%D_2O and locillomycin B in CD_3OH

		Locillomycin A			Locillomycin B	
		δ_H (ppm)	δ_C (ppm)		δ_H (ppm)	δ_C (ppm)
Cyclic peptide						
Thr 1	α	N/A	N/A	α	4.75	57.63
	β	5.50	74.34	β	5.44	71.93
	γ	1.15	18.78	γ	1.21	16.73
	H_N	8.34	--	H_N	8.38	--
	C’	--	N/A	C’	--	171.26
Gln 2	α	4.36	55.73	α	4.36	54.06
	β	1.91, 2.05	30.52	β	1.91, 2.06	29.38
	γ	2.25	33.53	γ	2.23	31.87
	δ	--	N/A	δ	--	177.69
	ε	6.73, 7.50	--	ε	6.82, 5.54	--
	H_N	8.03	--	H_N	8.06	--
	C’	--	N/A	C’	--	173.43
Asp 3	α	4.64	N/A	α	4.71	52.00
	β	2.62, 2.69	41.28	β	2.77, 2.86	36.40
	γ	--	N/A	γ	--	174.13
	H_N	8.62	--	H_N	8.70	--
	C’	--	N/A	C’	--	173.97
Gly 4	α	3.90, 3.95	45.53	α	3.93, 3.83	43.94
	H_N	8.35	--	H_N	8.17	--
	C’	--	N/A	C’	--	172.70
Asn 5	α	4.54	55.18	α	4.55	53.58
	β	2.76	38.68	β	2.74, 2.84	36.95
	γ	--	N/A	γ	--	174.29
	δ	6.93, 7.68	--	δ	6.99, 7.67	--
	H_N	8.63	--	H_N	8.64	--
	C’	--	N/A	C’	--	174.39
Asp 6	α	4.64	N/A	α	4.67	52.76
	β	2.60, 2.72	40.73	β	2.89	35.78
	γ	--	N/A	γ	--	174.00
	H_N	8.51	--	H_N	8.49	--
	C’	--	N/A	C’	--	173.52
Gly 7	α	3.90, 3.99	45.06	α	3.96	43.61
	H_N	7.89	--	H_N	8.13	--
	C’	--	N/A	C’	--	172.22
Tyr 8	α	4.52	58.44	α	4.47	57.69
	β	2.93, 3.08	39.00	β	2.94, 3.14	37.29
	γ	--	N/A	γ	--	129.44
	δ	7.11	N/A	δ	7.16	131.17
	ε	6.79	N/A	ε	6.70	116.25
	ζ	--	N/A	ζ	--	157.25
	H_N	7.82	--	H_N	7.87	--
	C’	--	N/A	C’	--	174.08
Val 9	α	4.23	61.46	α	4.17	60.41
	β	1.99	33.07	β	2.07	30.87
	γ1	0.77	20.22	γ1	0.84	19.18
	γ2	0.75	20.71	γ2	0.89	18.71
	H_N	7.76	--	H_N	7.57	--
	C’	--	N/A	C’	--	171.44
Acyl group						
	1’	--	N/A	1’	--	177.48
	2’	2.49	38.37	2’	2.41	36.81
	3’	1.62	28.16	3’	1.64	27.14
	4’-11’	0.98~1.20	30.7~32.7	4’-12’	1.23~1.35	30.3~31.3
	12’	N/A	N/A	13’	1.30	23.75
	13’	0.77	13.69	14’	0.89	14.47

Note: --: no such nucleus; N/A: assignment not available

Supplementary Figures

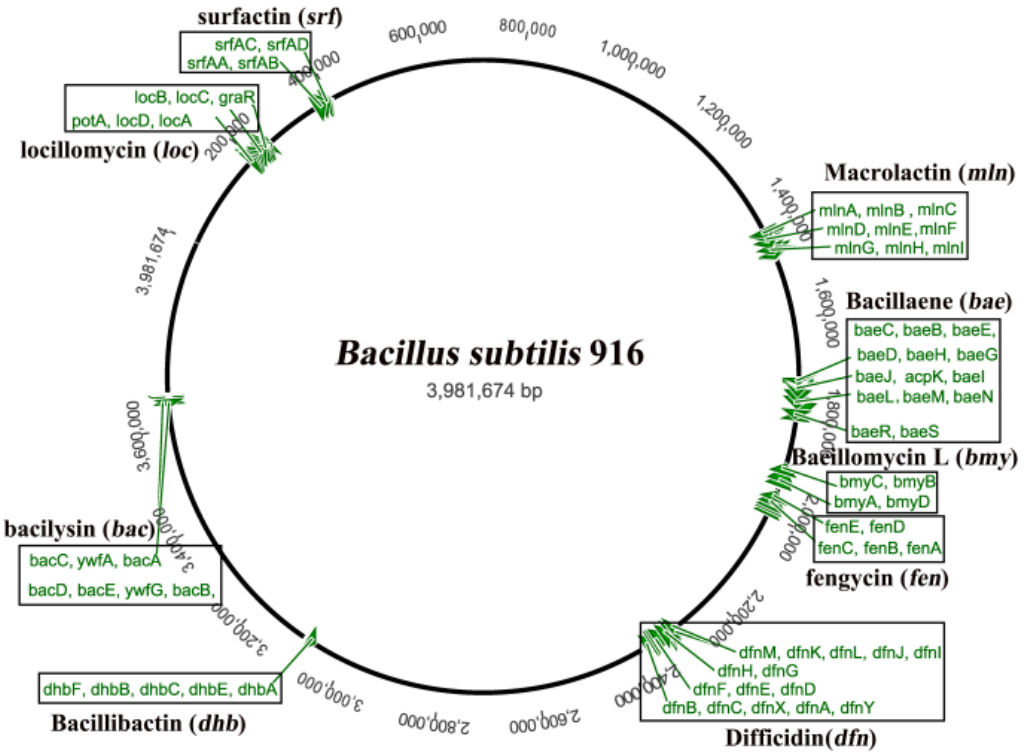

Fig. S1. The locations of five PKS gene clusters and four NRPS gene clusters in the *B. subtilis* 916 genome. The five PKS gene clusters *mln*, *bae*, *dfn*, *dhb* and *bac* are responsible for the biosynthesis of macrolactin, bacillaene, difficidin, bacillibactin and bacilysin respectively. The four NRPS gene cluster *srf*, *bmy*, *loc* and *fen* are responsible for biosynthesis of four family LPs-surfactins, bacillomycin Ls, locillomycins and fengycins respectively.

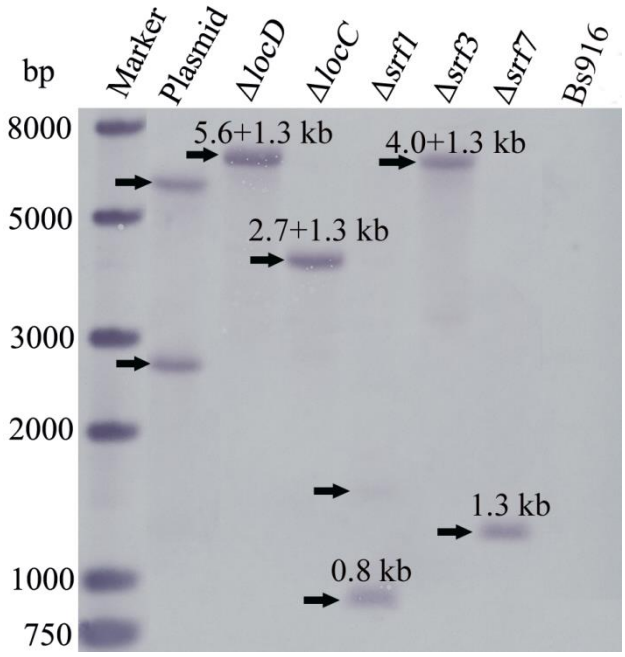

Figure S2. Southern blot analysis of neomycin resistance genes in *B. subtilis* 916 and its mutants

Genomic DNA from *B. subtilis* 916 (partially digested with Sau 3AI) and its mutants (Δ*locD* digested with *kpn*I and *Af*lII, 6.9 kb; Δ*loc* digested with *Hin*dIII, 4.0 kb; Δ*srf1* partially digested with Sau 3AI; Δ*srf3* digested with *Hin*dIII, 5.3 kb; and Δ*srf7* digested with kpnI, 1.3 kb) and subjected to Southern blotting. The plasmid was the pBEST501 from BGSC. The blot was hybridized with DIG-labelled 0.5 kb PCR product of neomycin resistance gene and probed by detection starter kit II (Roche). Pre-hybridization, hybridization and membrane washing procedures were conducted at 65 ^{o}C. The membrane was washed using stringent conditions (twice in 0.2 × SSC with 0.1% w/v SDS for 20 min) before exposure. Approximate sizes of the observed fragments are indicated on the left of the figure. Two bands of the plasmid lane represented open circular plasmid and super coiled plasmid respectively. The bands of Δ*locD,* Δ*locC* and Δ*srf3* lanes represented the sums of respective genome DNA fragments (5.6, 2.7 or 4.0 kb) and inserted neomycin cassette (1.3 kb). The band of Δ*srf7* lane represented the neomycin cassette (1.3 kb). The band of Δ*srf1* lane represented the neomycin cassette and around genome DNA sequences partially digested by Sau 3AI. No band was observed in lane of Bs916 (*B. subtilis* 916).

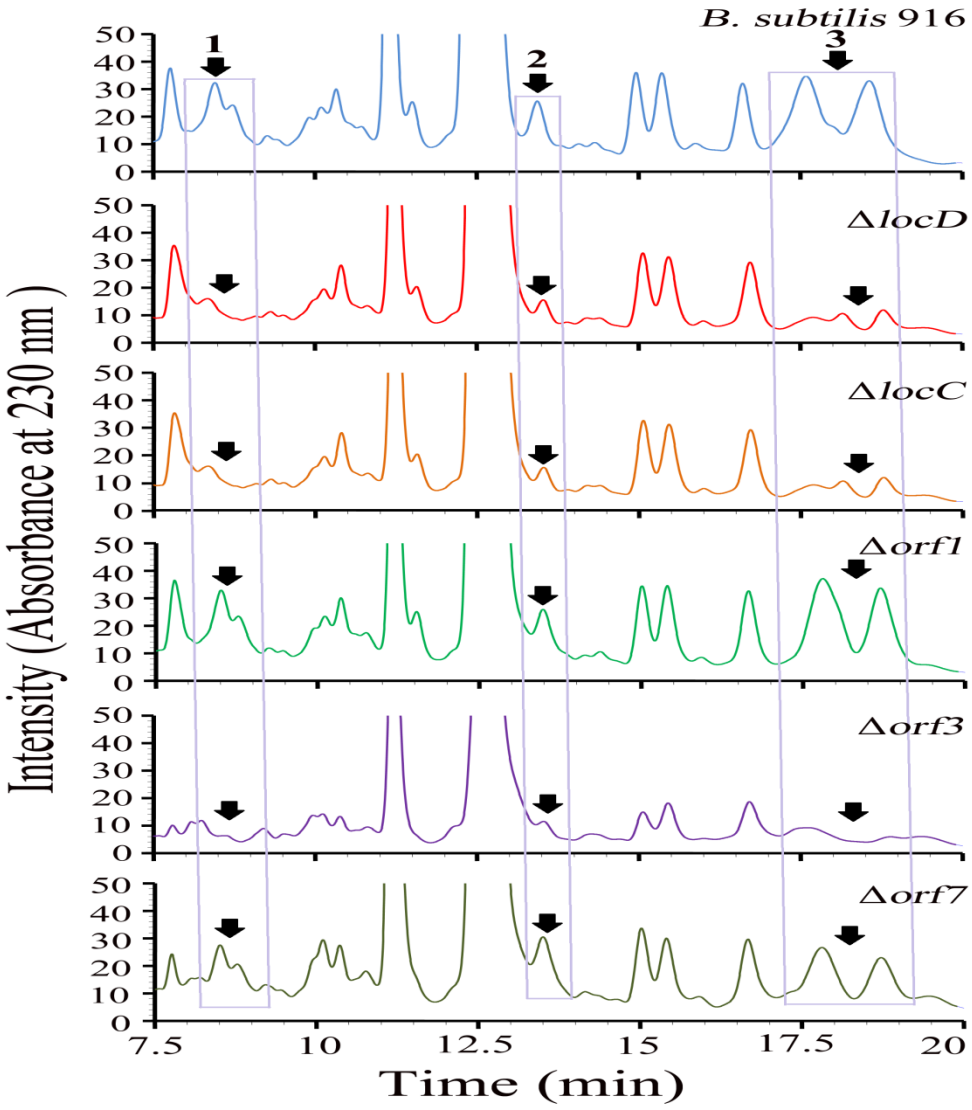

Figure S3. HPLC chromatograms for locillomycins produced by wild-type *B. subtilis* 916 and its mutants. locillomycins A-C were detected in *B. subtilis* 916 broth cultures, but were below detectable level in Δ*locD*, Δ*locC* and Δ*orf3* broth cultures. The Δ*orf1* and Δ*orf7* mutants showed no significant difference in the production of locillomycin-A, -B and -C when compared to wild-type *B. subtilis* 916.

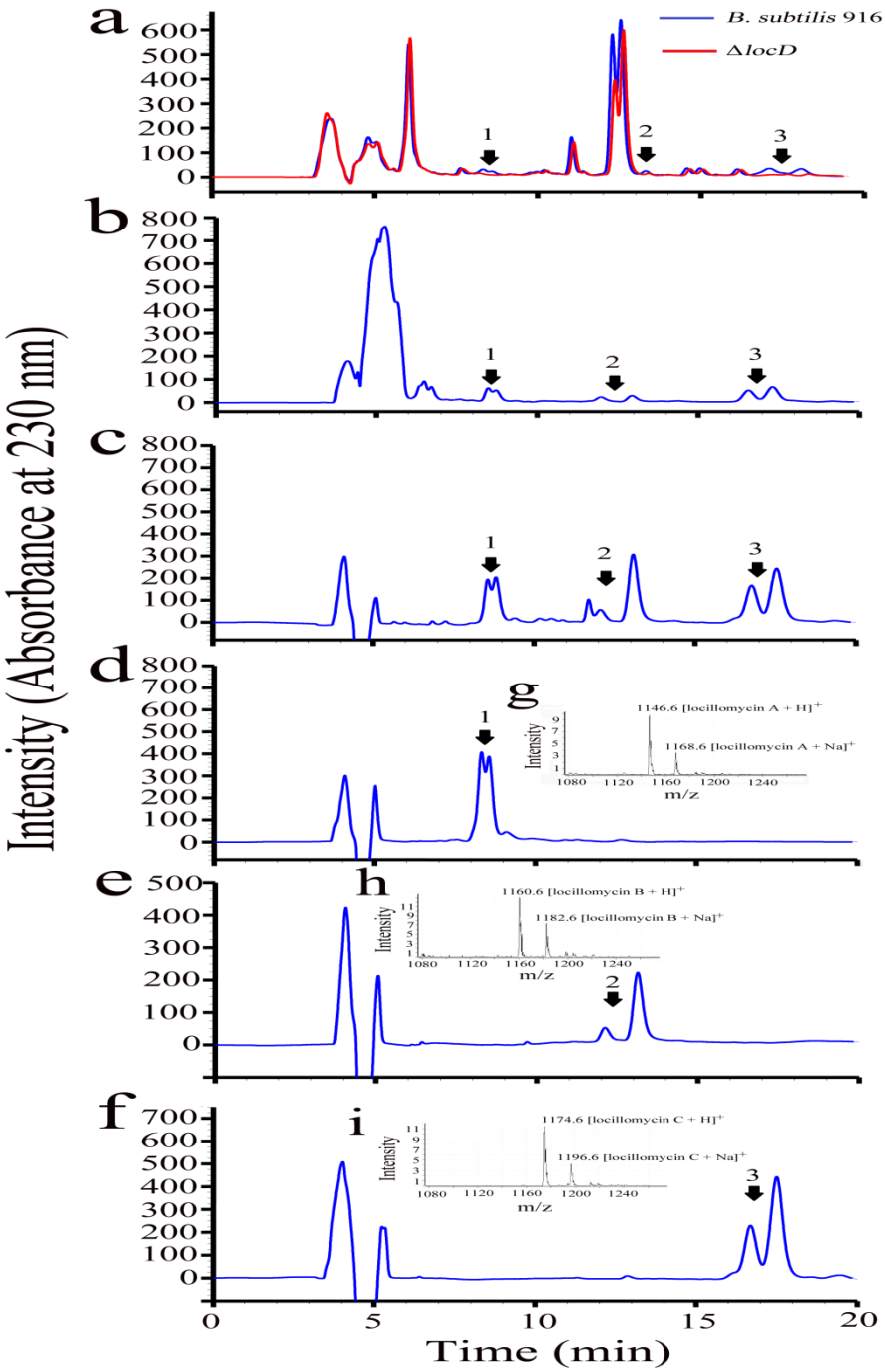

Figure S4. Isolation and purification of locillomycins. (a) HPLC analysis of the locillomycins produced by wild-type *B.subtilis* 916 (------) and its mutant ΔlocD (------). (b) locillomycins purified by Agilent amino solid phase extraction column. (c) locillomycins purified by Agilent C18 solid phase extraction column. (d～f) locillomycin A, B and C further purified by HPLC step by repetitive on a reversed-phase column (RP-18, 5μM, 4×250 mm; Merk). (g ～ i) The MS spectra of the purified locillomycin A, B and C.

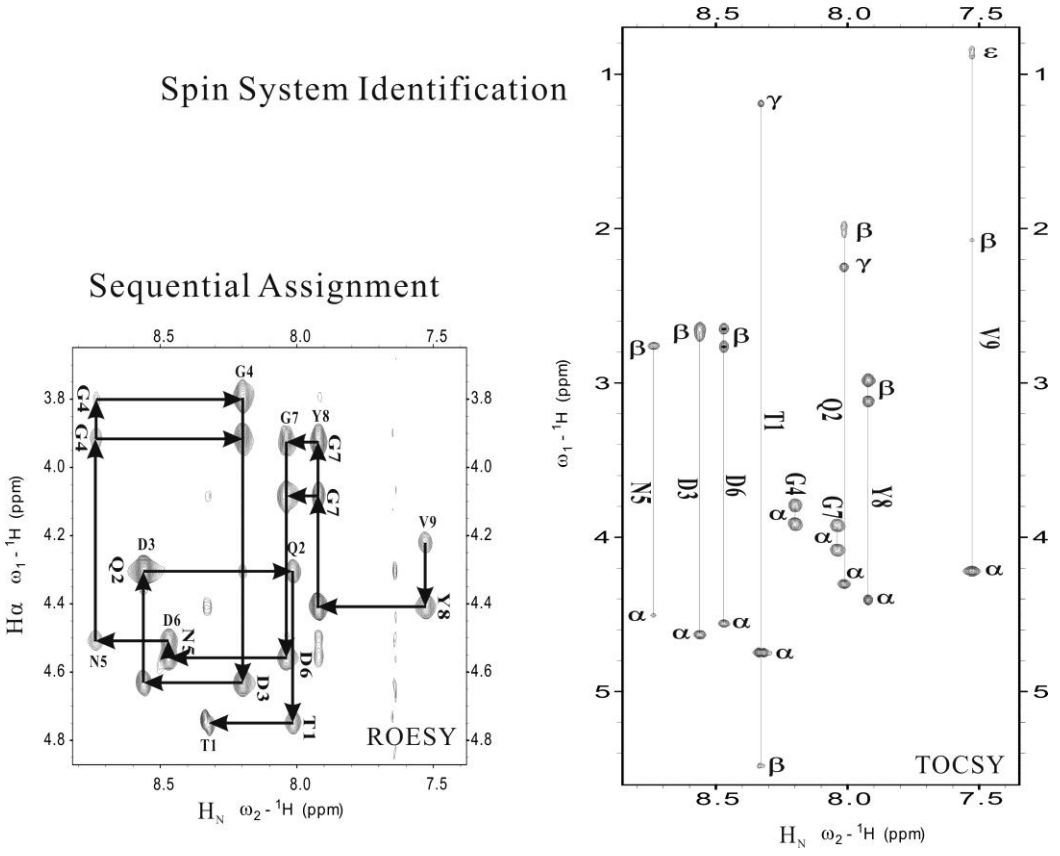

Figure S5. Sequential assignment and spin system identification of the cyclic peptide. Left panel shows the fingerprint region of the ROESY spectrum of the locillomycin C peptide moiety. Arrows show the sequential connectivity from residue V9 to T1. Intra-residues H_{Ni}-$H_{\alpha i}$ interactions are labeled with vertical characters while the inter-residue H_{Ni}-$H_{\alpha i-1}$interactions are labeled with horizontal characters. Right panel shows the amide region of the TOCSY spectrum of locillomycin C. This was used to identify amino acid types through different spin system pattern. Side chain signals from each residue was connected with solid line and labeled with Greek letter and the type and number of the residue was labeled with horizontal characters.

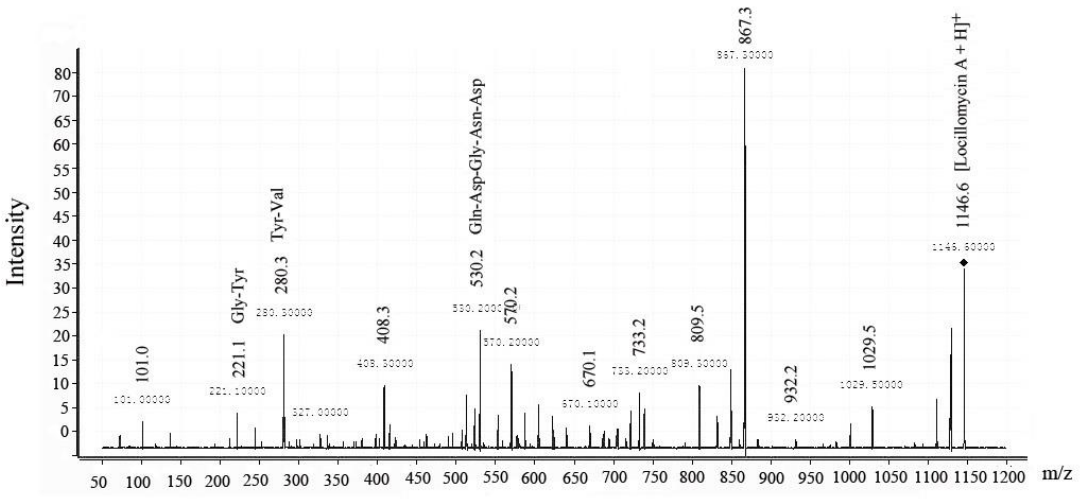

Figure S6. Tandem mass spectrum of Locillomycin A

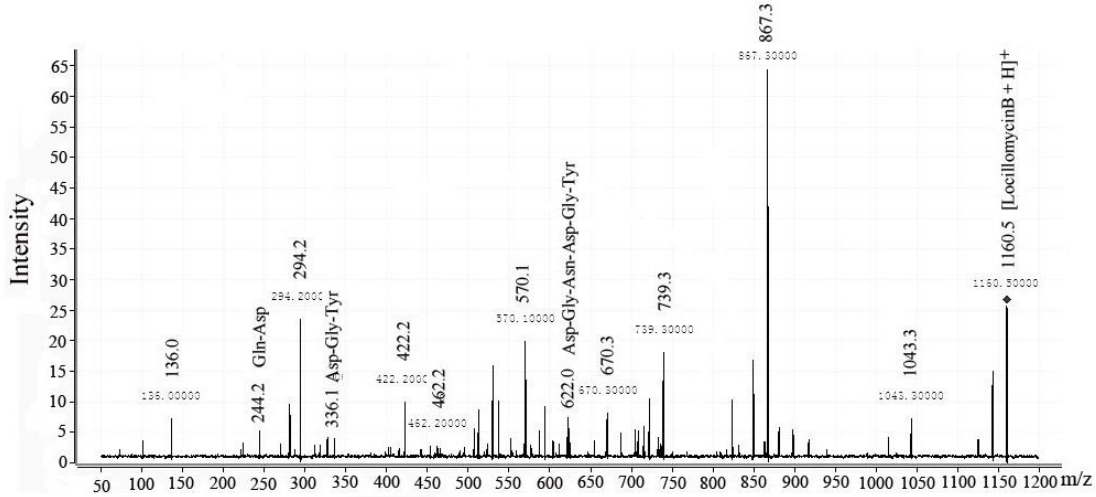

Figure S7. Tandem mass spectrum of Locillomycin B

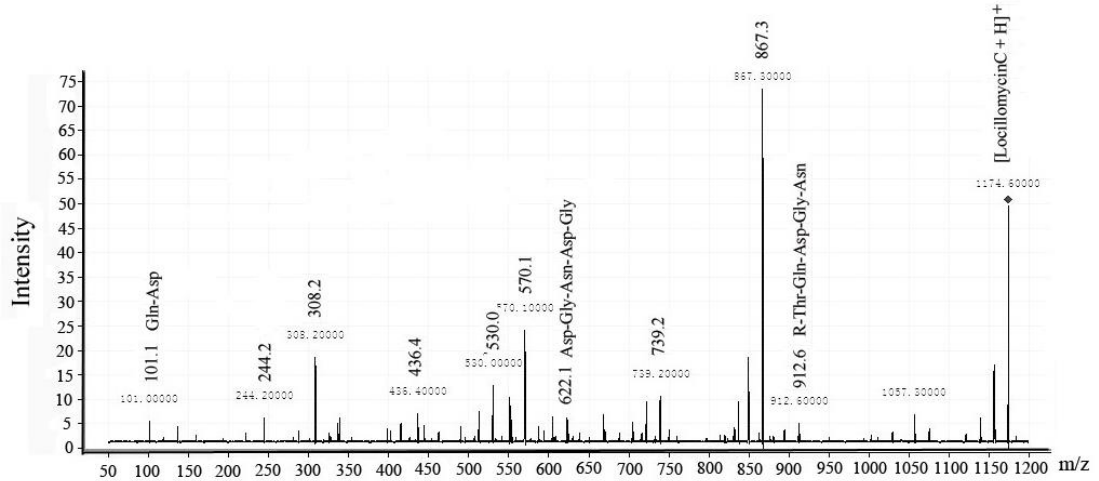

Figure S8. Tandem mass spectrum of Locillomycin C

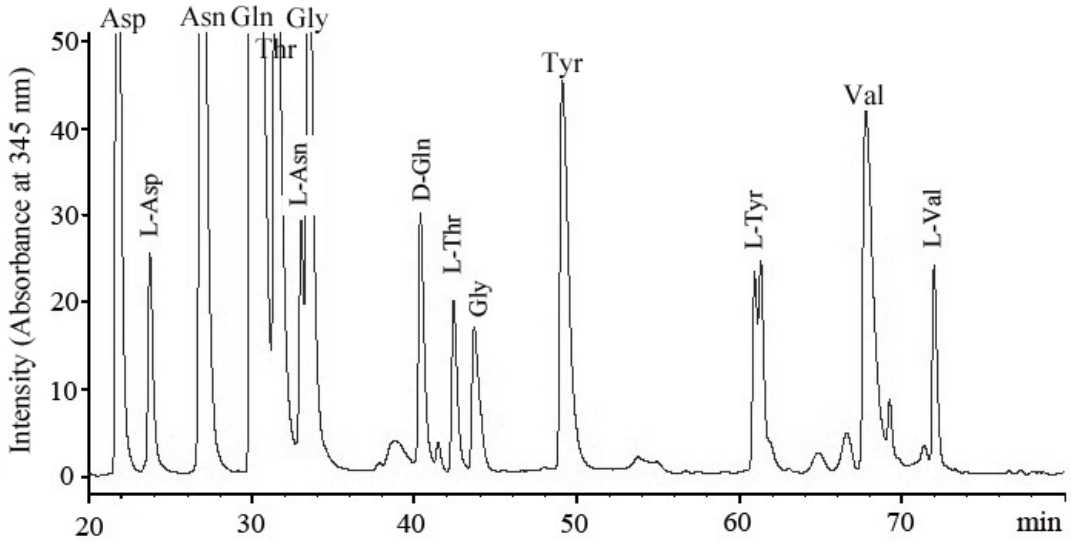

Figure S9. DL-amino acid analysis of Locillomycins by HPLC

Amino acid chirality was determined by reacting acid hydrolysates of the locillomycins with o-phthaldialdehyde (OPA) together with N-isobutyryl-L-cysteine (IBLC) as chiral reagents (detail description in "Material and Methods"). The higher peaks representing the amino acid residues reacting with OPA were labeled with a three-letter code of the corresponding amino acid. The lower peaks representing the amino acid residues reacting with both OPA and IBLC were labeled L- or D- pre three-letter code (37).

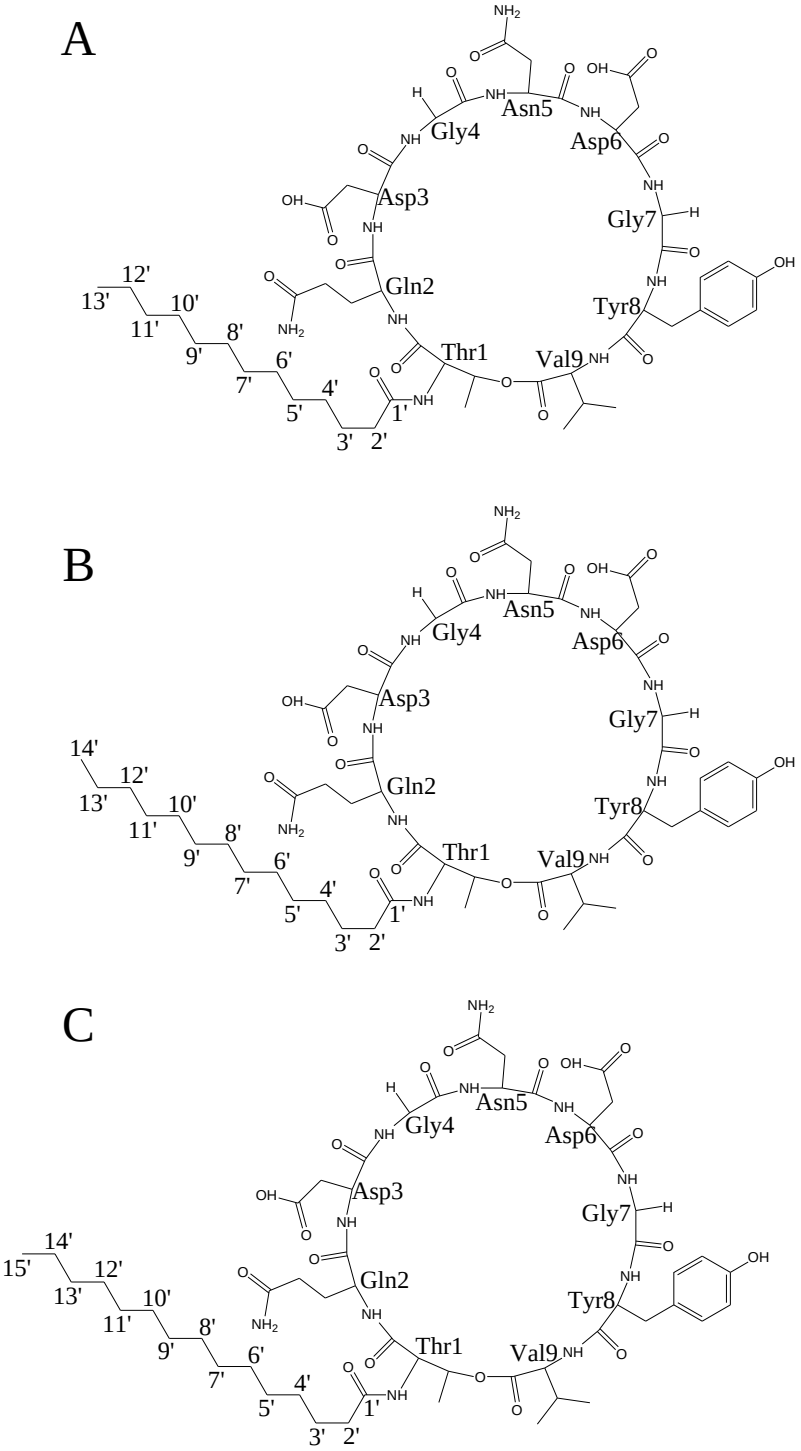

Figure S10. Chemical structures of locillomycins A, B and C

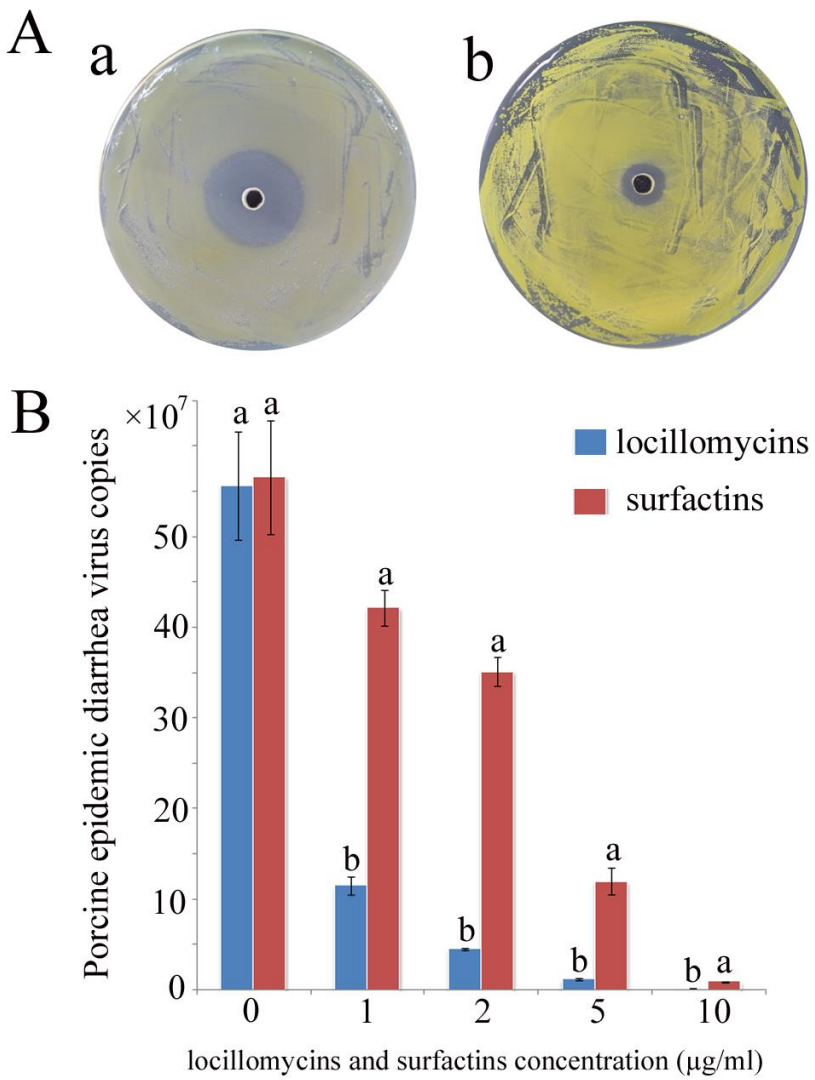

Figure S11. Antibacterial and antiviral activities of 1ocillomycins. (A) Antibacterial activities of locillomycins against *Staphylococcus aureus* (Aa) and *Xanthomonas oryzae pv. Oryzae* (Ab). locillomycin, 1 mg/mL 100 μL; the plates were incubated at 37 ^{o}C for 24 h. (B) Antiviral activities of 1ocillomycins against Porcine epidemic diarrhea virus (PEDV). The anti-virus experiment was designed using Porcine Epidemic Diarrhea Virus (PEDV) as an indicator; conducted on a 24-well plate containing a single layer of Vero cell in each well, which was infected with PEDV at a multiplicity of infection (MOI), and incubated at 37 ^{o}C. After 1 h, different concentrations of Locillomycins (blue column) or surfactins (red column) were introduced to treatments, and continuously incubated at 37 ^{o}C for 36 h. At the end of the experiment, RNA of the virus was extracted from the wells, and quantified with fluorescent qPCR. The results revealed that the PEDV infection could be effectively inhibited by locillomycins especially that of concentration at the 10 μg/mL, which reduced the virus copy by 300 fold (blue column). In contrast, the PEDV infection could also be inhibited by surfactins but that of concentration at the 10 μg/mL, which only reduced the virus copy by 30 fold (red column).

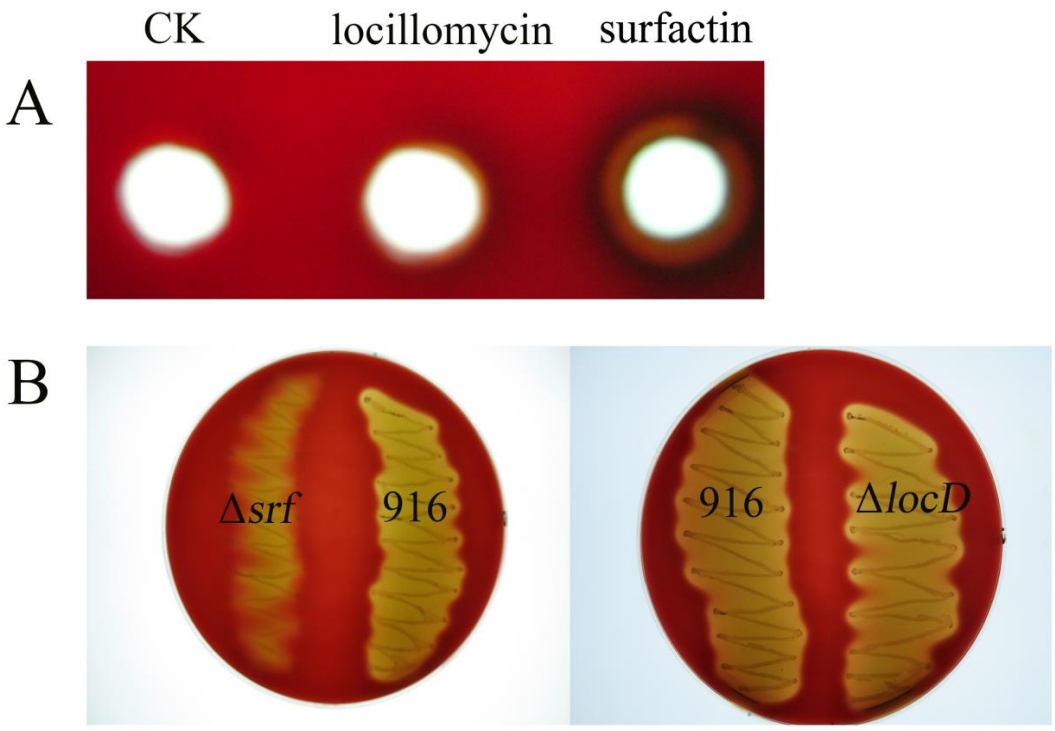

Figure S12. Haemolytic activities of locillomycins and surfactins. (A) CK, 100 μL; locillomycin, 1 mg/mL, 100 μL; surfactin, 1 mg/mL, 100 μL; the plates were incubated at 37 ℃ for 1 – 3 days. (B) *B. subtilis* 916 and its mutants Δ*srf*, Δ*LocD* were grown on blood agar plates after incubation at 37 ℃ for 1 – 3 days.