Supplemental Material for:

Characterization of a novel plasmid-borne thiopeptide gene cluster in *Staphylococcus epidermidis* strain 115

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Contents:

Supplemental material for: *S. epidermidis* 115 thiopeptide plasmid

SUPPLEMENTAL METHODS

I. Transformation of *Bacillus subtilis* 168 by starvation-induced competence

- modified from (1)

a) Media preparation

Starvation medium 1 (SM1) contains 0.2% ammonium sulfate, 1.2% dipostassium hydrogen phosphate, 0.6% potassium dihydrogen phosphate, 0.1% sodium citrate dihydrate, 0.2% magnesium sulfate heptahydrate, 0.2% yeast extract, 0.025% casamino acids, 0.01% L-tryptophan and 0.5% glucose.

Starvation medium 2 (SM2) differs slightly from SM1, containing 0.2% ammonium sulfate, 1.2% dipostassium hydrogen phosphate, 0.6% potassium dihydrogen phosphate, 0.1% sodium citrate dihydrate, 0.8% magnesium sulfate heptahydrate, 0.1% yeast extract, 0.0125% casamino acids, 0.01% L-tryptophan, 0.5% glucose and 0.022% calcium chloride dihydrate.

Both solutions were mixed fresh on the day of transformation from stock solutions prepared ahead of time. The ammonium sulfate, dipostassium hydrogen phosphate, potassium dihydrogen phosphate and sodium citrate dihydrate can be combined and autoclaved to provide the base (ST Base) for both media. All other solutions were maintained as separate stocks, sterilized by autoclaving (magnesium sulfate heptahydrate and calcium chloride) or filter sterilization (yeast extract, casamino acids, tryptophan and glucose).

b) Preparation of competent cells

Pure cultures of *B. subtilis* 168 cells were grown on LB agar without antibiotics, then used to inoculate a 4-ml liquid culture of LB. Cells were grown overnight at 37°C, 225rpm for 14-18 h. 1 ml of overnight culture was combined with 15 ml of warm, freshly prepared SM1 (final OD600 ≈ 0.4-0.6) and incubated at 37°C, 225rpm for 5 h. Following incubation in SM1, an equal volume of pre-warmed SM2 was added and cells were further incubated for 2 h under the same conditions. At this stage and for at least 60-90 min after, cells are competent and are ready for transformation. Cells may be combined with glycerol (final conc. 10%) and frozen at -80^{o}C at this stage, however subsequent transformations may be at least two-fold less efficient than those performed with fresh competent cells.

c) Transformation of competent cells

500 µl of competent *B. subtilis* cells were combined with 100-500 ng plasmid DNA (<5 µl volume) and incubated at 37°C (with rotation) for 30 min. 200 µl of fresh LB was then added to cells and incubated for an additional 60 min. Following this final incubation step, the desired volume of cells was plated on antibiotic-containing LB (LB-Cm_5) and grown overnight at 37°C. Transformation efficiencies in the range of 2 x 10^4-2 x 10^5 transformants per µg DNA are routinely observed using this procedure.

I. Transformation of *Bacillus subtilis* SCK6 by xylose induced competence

B. subtilis strain SCK6 (2) harbors a Pxyl-*comK* insertion for rapid induction of competence in the presence of xylose. Strains were transformed by diluting overnight cultures of strain SCK6 into LB-xyl (1% w/v) for 2 hours at 37°C to induce competence. At this stage cells were mixed with 100-500 ng plasmid DNA and incubated for 60 min prior to selection. Following this final incubation step, the desired volume of cells was plated on antibiotic-containing LB (LB-Cm5) and grown overnight at 37°C. High efficiency transformations are readily obtained in this manner.

Supplemental material for: *S. epidermidis* 115 thiopeptide plasmid

LIST OF SUPPLEMENTAL TABLES AND FIGURES

SUPPLEMENTAL TABLES S1-S5

Table S1. Strains and Assay Conditions Used in Inhibition Studies

Indicator (Test) Strain	Gram Stain	Assay Method[a]	Sensitive	Culture Media[b]
B. anthracis Sterne strain 1043	Positive	Patch	+	TSA
B. brevis ATCC 8246	Positive	Spot	+	NB
B. cereus ATCC 14579	Positive	Patch, spot	-	LB, TSA
B. licheniformis ATCC 14580	Positive	Spot	+	NB
B. megaterium ATCC 15127	Positive	Patch	+	LB, TSA
B. mycoides ATCC 6462	Positive	Spot	+	NB
B. sphaericus ATCC 14577	Positive	Spot	+	NB
B. subtilis ATCC 19659	Positive	Spot	+	LB,TSA
B. subtilis ATCC 6051	Positive	Spot	+	LB
B. subtilis SCK6	Positive	Spot	+	LB
B. subtilis subsp. *spizizenii* ATCC 6633	Positive	Patch, spot	-	NB
B. subtilis 168	Positive	Spot	+	LB
B. thuringiensis ATCC 19269	Positive	Spot	+	NB
B. thuringiensis Al Hakam	Positive	Spot	+	NB
C. difficile ATCC 43598	Positive	Spot	+	LB
C. perfringens ATCC 13124	Positive	Spot	+	TSA w/ blood
E. faecalis ATCC 19433	Positive	Spot	+	LB
E. faecalis clinical isolate RL112105 (VRE)	Positive	Patch	+	TSA
L. monocytogenes ATCC 13932	Positive	Spot	+	LB,TSA
L. monocytogenes ATCC 15313	Positive	Spot	+	LB,TSA
P. polymyxa ATCC 842	Positive	Spot	+	NB
S. agalactiae ATCC 12386	Positive	Spot	+	TSA w/ blood
S. aureus ATCC 29213	Positive	Spot	+	LB
S. aureus ATCC 6538	Positive	Spot	+	LB
S. aureus ATCC 43300 (MRSA)	Positive	Spot	+	LB
S. aureus environmental isolate RL1 (MRSA)	Positive	Spot	+	LB
S. aureus environmental isolate RL2 (MRSA)	Positive	Spot	+	LB
S. aureus environmental isolate RL3 (MRSA)	Positive	Spot	+	LB
S. aureus environmental isolate RL4 (MRSA)	Positive	Spot	+	TSA
S. aureus environmental isolate RL5 (MRSA)	Positive	Spot	+	LB
S. epidermidis PB004	Positive	Patch	+	LB, TSA
S. epidermidis PB023	Positive	Spot	+	LB
S. pneumoniae ATCC 6303	Positive	Patch	+	LB
S. pyogenes RL421682	Positive	Patch	+	LB
S. pyogenes ATCC 51339	Positive	Patch	+	LB
B. thailandensis E135	Negative	Spot	-	LB
E. coli 0157:H7	Negative	Patch	-	TSA
Y. pseudotuberculosis RLPI399	Negative	Patch	-	LB
M. smegmatis ATCC 14468	Acid fast	Spot	+	7H11

[a] Patch, flanking patch assay; Spot, spot-on-lawn assay

[b] LB, Luria broth; NB, nutrient broth; TSA, tryptic soy agar; 7H11, mycobacteria 7H11 agar

Table S2: Primer pairs used in assembly of pBac115[a]

Primer pair	Primer name	Direction	Sequence (5' - 3')	Expected product (bp)	Target
1	oPB009	Forward	GGAGTATTATACGCTGCCAGC	2068	Contig 1
	oPB010	Reverse	CCACAAGGAAATGCATCCGCA		
2	oPB011	Forward	GCTGACACAACTTCTCCTGG	2493	Contig 1
	oPB012	Reverse	ATTGGACCAGGAATGGGCAG		
3	oPB015	Forward	CTTGTGTGATCTCCGGCATTCC	2397	Contig 2
	oPB016	Reverse	TGCCTGGTGGCTCATCTTTG		
4	oPB019	Forward	TGAACGTGGAGATGTTGCTGG	1895	Contig 3
	oPB020	Reverse	CAGAATGGAGGGACGAACAAGTG		
5	oPB023	Forward	TTACAAGCAAGCGTAGCGAGC	659	Contig 4
	oPB024	Reverse	ATCGCTTGAGCCCTACTCTCC		
6	oPB027	Forward	CGAATGATGTGGTTATCGCAGG	776	Contig 5
	oPB028	Reverse	TCACTTCCATATCCAGCGTCG		

[a]Primer pairs 1, 2 and 4 amplified the expected product from strain 115 but not the pBac115-deficient strain 115C. All other primer pairs amplified the expected products from both strains.

Table S3. NMR Protocols

Protocol	**Key Parameters**
^{1}H	64 scans
^{13}C	20000 scans
gradient-filtered DQ-COSY	16 scans, 400 increments
z-filtered TOCSY	32 scans, 256 increments, 90 ms DIPSI-2 spinlock
adiabatic HSQC	16 scans, 96 increments, J1=146 Hz
band-selective adiabatic HSQC	32 scans, 128 increments, 5-80 ppm
band-selective adiabatic HSQC	32 scans, 128 increments, 105-150 ppm
adiabatic HSQC-TOXY	64 scans, 96 increments, 90 ms MLEV-17 spinlock
gradient-filtered adiabatic HMBC	16 scans, 96 increments, Jn=8 Hz
band-selective grad.-filtered adiab. HMBC	64 scans, 128 increments, Jn=12 Hz, 110-180 ppm
band-selective grad.-filtered adiab. HMBC	256 scans, 128 increments, Jn=3 Hz, 110-180 ppm
adiabatic ^{15}N-HSQC	128 scans, 96 increments, J1= 90 Hz
adiabatic ^{15}N-HSQC-TOXY	128 scans, 96 increments, 80 ms MLEV-17 spinlock

Table S4. Summary of pBac115 Blastp Results

pBac115 Gene Product	Size (aa)	Thiocillin Protein Homolog[a]	Size (aa)	Identity/similarity /coverage %[b]	Top blastp Results	Predicted function[c]
Orf16 (TclE)	50	TclE	53	52/65/98 (entire product); 100/100/100 (core peptide)	none	Structural (precursor) peptide
Orf17 (TclS)	224	TclS	237	23/44/81	Short-chain dehydrogenase/reductase SDR [*Pseudomonas sp.* CFII64], oxidoreductase [*Pseudomonas syringae*], putative uncharacterized protein [*Megamonas funiformis* CAG:377]	Stochastic changes (decarboxylation) at residue 14(3)
Orf18	160	-	-	-	Hypothetical cyanophage protein [*Synechococcus* phage S-RSM4], putative regulator of cell autolysis [*Solitalea canadensis*], serine phosphatase [uncultured bacterium]	Unknown
Orf19 (TclQ)	141	TclQ/TclT	142	60/79/99	50S ribosomal protein L11 [*Leptolyngbya sp.* PCC 7376], 50S ribosomal protein L11 [*Nocardioides sp.* JS614], 50S ribosomal protein L11 [*Geitlerinema sp.* PCC 7105]	Self-immunity
Orf20 (TclI)	243	TclI	504	41/53/13	Bacterioferritin comigratory protein [*Lactobacillus gasseri*], thiazole-containing bacteriocin maturation protein [*Geobacillus stearothermophilus* NUB3621], bacteriocin maturation protein [*Bacillus sp.* UNC41MFS5]	Other modification
Orf21 (TclJ)	564	TclJ	661	35/50/87	Conserved hypothetical protein [*Lactobacillus gasseri*], SagD family biosynthesis docking scaffold protein [*Bacillus cereus*], bacteriocin biosynthesis protein SagD [*Alicyclobacillus pomorum*], cyclodehydratase [*Streptomyces sp.* NRRL 30471]	Heterocyclization (4), YcaO (DUF181) homolog(5)
Orf22 (TclK)	844	TclK	872	28/47/92	Lanthionine biosynthesis protein [*Bacillus cereus*], lantibiotic dehydratase, superfamily protein [*Lactobacillus gasseri*], NosE [*Streptomyces actuosus*], NocE [*Nocardia sp.* ATCC 202099]	Dehydratase (full length)(4)

Table S4. (continued) Summary of pBac115 Blastp Results

pBac115 Gene Product	Size (aa)	Thiocillin Protein Homolog[a]	Size (aa)	Identity/similarity /coverage %[b]	Top blastp Results	Predicted function[c]
Orf23 (TclL)	268	TclL	324	28/45/95	Lantibiotic biosynthesis protein [*Lactobacillus gasseri*], lantibiotic biosynthesis protein [*Bacillus cereus*], thiopeptide-type bacteriocin biosynthesis domain-containing protein [*Enterococcus faecalis*]	Dehydratase (truncated)(4)
Orf24 (TclM)	265	TclM	326	32/52/79	Hypothetical protein [*Bacillus cereus*], hypothetical protein [*Lactobacillus gasseri*], hypothetical protein [*Actinoalloteichus spitiensis*]	Cycloaddition of Dha1 and Dha10 to produce pyridine(3)
Orf25 (TclN)	448	TclN	523	25/43/95	NADH oxidase [*Bacillus cereus*], conserved hypothetical protein [*Lactobacillus gasseri*], SagB-type dehydrogenase domain-containing protein [*B. cereus*]	Heterocyclization(4) McbC homolog(5)
Orf26 (TclP)	233	TclP	257	38/57/99	Short-chain dehydrogenase [*Bacillus cereus*], 3-oxoacyl-[acyl-carrier-protein] reductase FabG [*Clostridium saccharobutylicum*], hypothetical protein [*Saccharibacillus kuerlensis*]	Stochastic changes (decarboxylation) at residue 14(3)
Orf27 (TclU)	114	TclU	124	29/57/58	MULTISPECIES: transcriptional activator tipA [*Lactobacillus casei* group], transcriptional activator tipA [*Lactobacillus casei*], MerR family transcriptional regulator [*Lactobacillus casei*]	Regulation(4)

[a] based upon comparisons with *B. cereus* ATCC 14579

[b] results of directed blastp between translated products of pBac115 ORFS and *B. cereus* ATCC 14579 tcl genes

[c] numbers in parentheses correspond to references which can be found in the Supplemental References section

Table S5. Chemical Shifts of Micrococcin P1 in DMSO-d6

Position	Residue	Type	δ ^{13}C (ppm)	δ ^{15}N (ppm)	δ ^{1}H (ppm)	Mult.	J (Hz)
1	Thr 1, γ	CH3	21.51	-	1.00	d	6.2
2	Thr 1, β	CH	65.55	-	3.69	m	-
2	Thr 1, OH	OH	-	-	4.63	d	*br*
3	[a]Thr 1, α	CH2	47.35	-	3.06	m	-
4	Thr 1, NH	NH	-	107.30	7.90	t	5.8
5	Thr 2, CO	C=O	164.79	-	-	-	-
6	Thr 2, α	C	131.07	-	-	-	-
7	[b]Thr 2, β	CH	128.31	-	6.50	q	7.0
8	[b]Thr 2, γ	CH3	13.99	-	1.68	d	7.0
9	Thr 2, NH	NH	-	117.20	9.51	s	-
10	[c]Cys 1, CO	C=O	159.44	-	-	-	-
11	Thiazole 1	C	150.90	-	-	-	-
12	Thiazole 1	CH	125.85	-	8.44	s	-
13	Thiazole 1	C	161.87	-	-	-	-
14	Thiazole 2	C	149.90	-	-	-	-
15	Thiazole 2	CH	122.14	-	8.57	s	-
16	Thiazole 2	C	168.81	-	-	-	-
17	Pyridine	C	150.21	-	-	-	-
18	Pyridine	CH	119.12	-	8.32	d	8.1
19	Pyridine	CH	141.14	-	8.43	d	8.1
20	Pyridine	C	129.60	-	-	-	-
21	Pyridine	C	151.53	-	-	-	-
22	Thiazole 3	C	152.95	-	-	-	-
23	Thiazole 3	CH	121.90	-	8.10	s	-
24	Thiazole 3	C	170.46	-	-	-	-
25	Thr 3, α	CH	56.59	-	5.06	dd	4.6, 8.8
26	Thr 3, β	CH	67.60	-	3.99	m	-
26	Thr 3, OH	OH	-	-	4.76	d	*br*
27	Thr 3, γ	CH3	21.07	-	1.02	d	6.4
28	Thr 3, NH	NH	-	115.40	8.21	d	8.9
29	[c]Cys 4, CO	C=O	160.91	-	-	-	-
30	Thiazole 4	C	149.56	-	-	-	-
31	Thiazole 4	CH	125.15	-	8.28	s	-
32	Thiazole 4	C	170.32	-	-	-	-
33	Val α	CH	55.85	-	5.12	dd	9.0, 9.0
34	Val β	CH	32.68	-	2.50	m	-

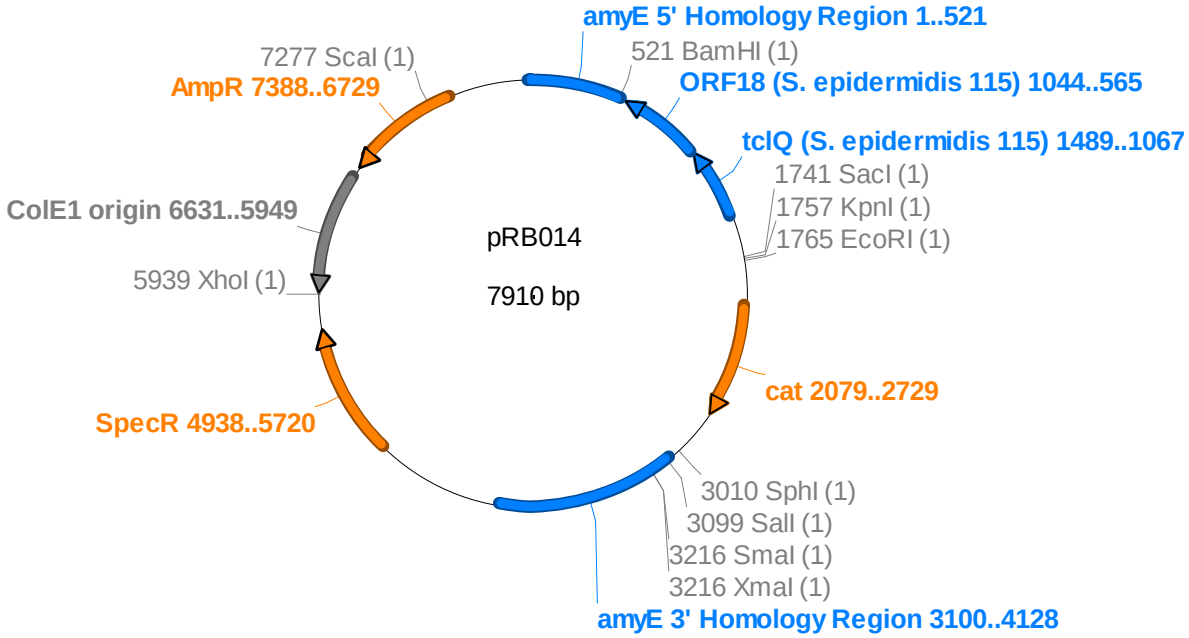
35	[d]Val γ	CH3	18.94	-	0.96	d	6.7
36	[d]Val γ	CH3	20.03	-	0.85	d	6.6
37	Val NH	NH	-	120.10	8.38	d	9.0
38	[c,e]Cys 5, CO	C=O	160.24	-	-	-	-
39	Thiazole 5	C	148.71	-	-	-	-
40	Thiazole 5	CH	124.92	-	8.19	s	-
41	Thiazole 5	C	166.81	-	-	-	-
42	Thr 4, α	C	130.09	-	-	-	-
43	[b]Thr 4, β	CH	129.07	-	6.45	q	6.9
44	[b]Thr 4, γ	CH3	14.24	-	1.74	d	6.8
45	Thr 4, NH	NH	-	120.40	9.51	s	-
46	Thr 5, CO	C=O	168.98	-	-	-	-
47	Thr 5, α	CH	58.09	-	4.67	dd	3.4, 8.0
48	Thr 5, β	CH	67.98	-	4.37	m	-
48	Thr 5, OH	OH	-	-	5.42	d	6.0
49	Thr 5, γ	CH3	20.31	-	1.36	d	6.3
50	Thr 5, NH	NH	-	109.30	7.85	d	7.9
51	[c,e]Cys 6, CO	C=O	160.27	-	-	-	-
52	Thiazole 6	C	150.12	-	-	-	-
53	Thiazole 6	CH	126.31	-	8.36	s	-
54	Thiazole 6	C	164.54	-	-	-	-

^{1}H and ^{13}C chemical shifts obtained from both 1D and 2D spectra; ^{15}N chemical shifts obtained from 2D spectra. Multiplicities and J values obtained from 1D ^{1}H spectrum. [a]Thr residue is decarboxylated. [b]Thr residue is dehydrated. [c]Remainder of the Cys is in a thiazole ring. [d]The assignment of carbons 35 and 36 is ambiguous. [e]The assignment of carbons 38 and 51 is ambiguous. *br* denotes a signal that was too broad to accurately measure the J value.

SUPPLEMENTAL FIGURES S1-S2

FIG S1. Complete maps and sequences of plasmids used to generate *B. subtilis* mutants

A Plasmid pRB014 used to generate *B. subtilis* SCK6 *amyE*::*tclQ-orf18-cat* mutant. The plasmid map shows critical features and unique restriction sites, where relevant. The complete nucleotide sequence is provided below with *tclQ* and *cat* shown in lowercase font.



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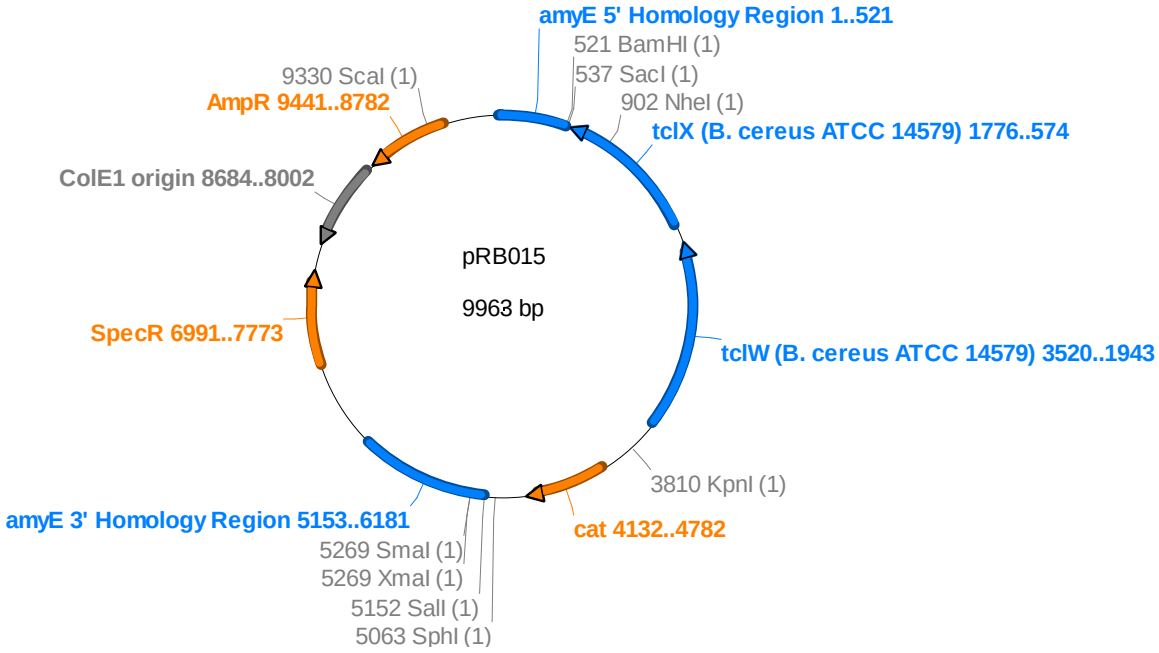
B Plasmid pRB015 used to generate *B. subtilis* SCK6 *amyE*::*tclWX-cat* mutant. The plasmid map shows critical features and unique restriction sites, where relevant. The complete nucleotide sequence is provided below with *tclW*, *tclX* and *cat* indicated in lowercase font.



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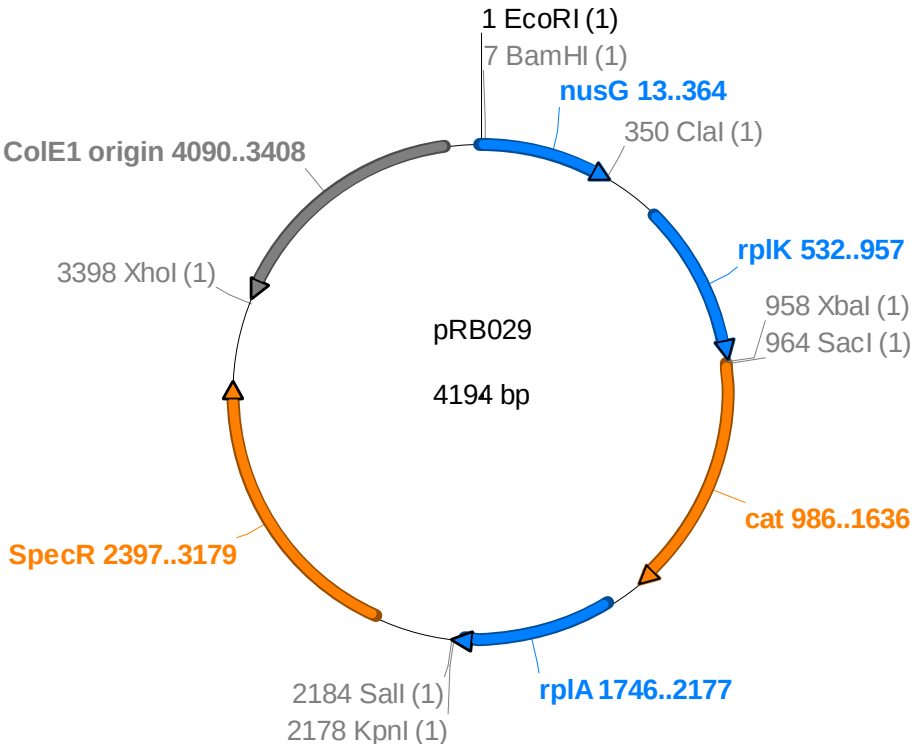
C Plasmid pRB029 used to generate *B. subtilis* 168 Δ*rplK*::*rplK-cat* mutant (PB213). The plasmid map shows critical features and unique restriction sites, where relevant. The complete nucleotide sequence is provided below with *rplK* and *cat* shown in lowercase font.



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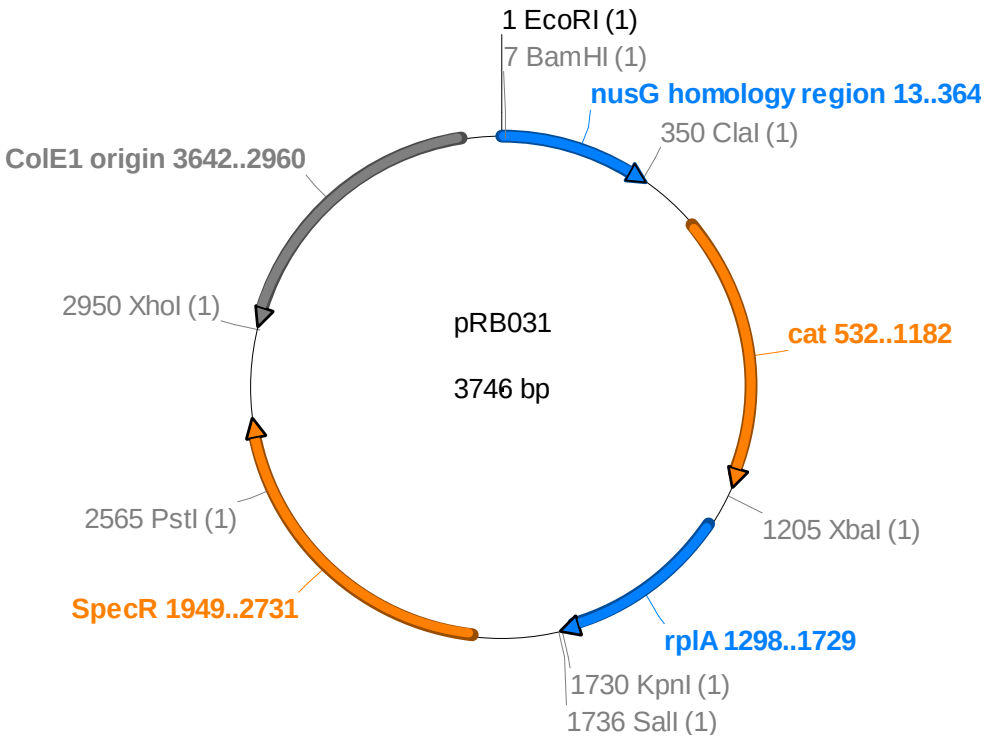
D Plasmid pRB031 used to generate *B. subtilis* 168 Δ*rplK*::*cat* mutant (PB230). The plasmid map shows critical features and unique restriction sites, where relevant. The complete nucleotide sequence is provided below with *cat* shown in lowercase font.



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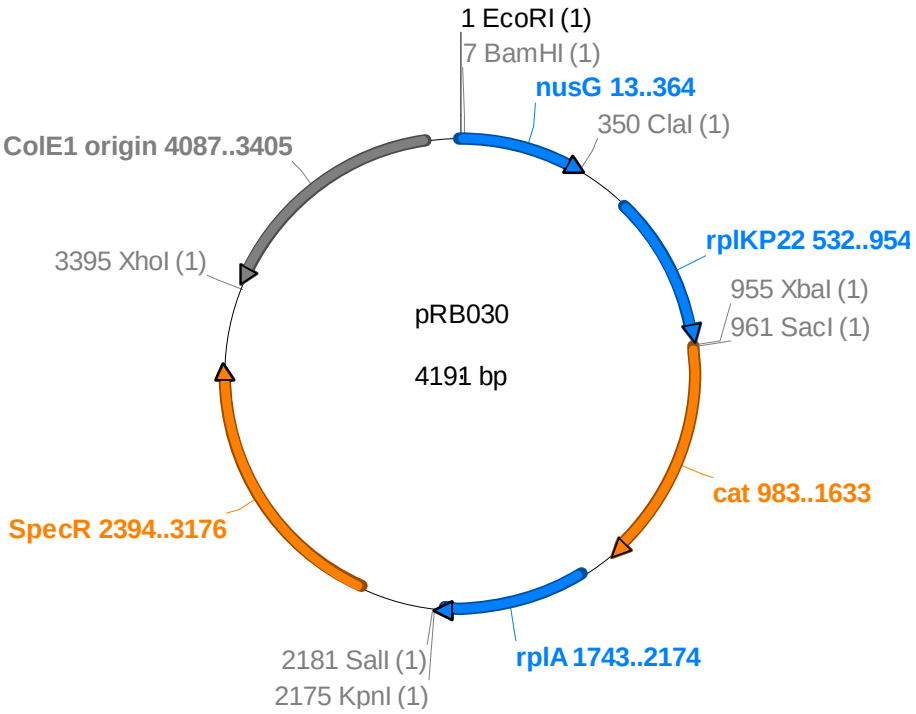
E Plasmid pRB030 used to generate *B. subtilis* 168 Δ*rplK*::*rplK*Δ*P22-cat* mutant (PB214). The plasmid map shows critical features and unique restriction sites, where relevant. The complete nucleotide sequence is provided below with *rplK*Δ*P22* and *cat* shown in lowercase font.



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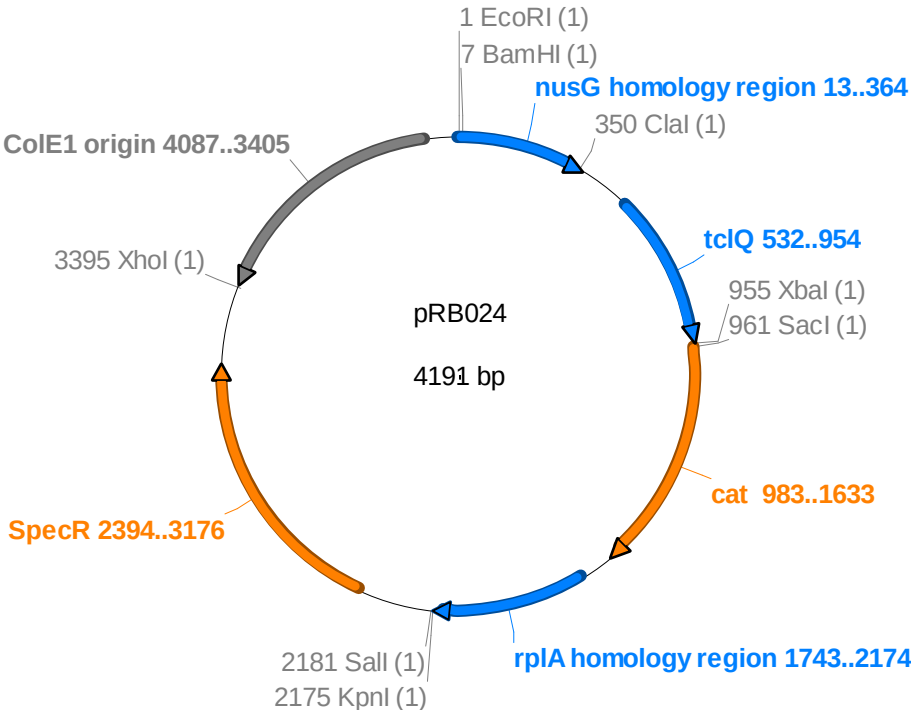
F Plasmid pRB024 used to generate *B. subtilis* 168 Δ*rplK*::*tclQ-cat* mutant (PB215). A plasmid map shows critical features and unique restriction sites, where relevant. The complete nucleotide sequence is provided below with *tclQ* and *cat* shown in lowercase font.



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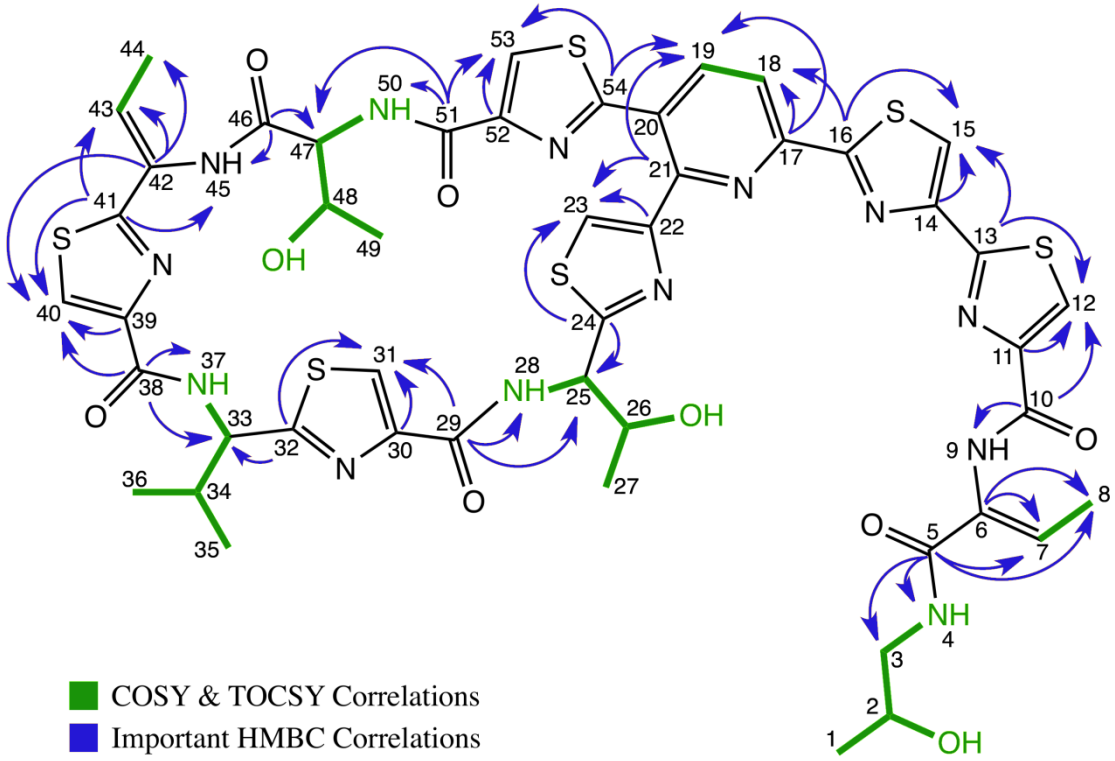

FIG S2. Summary of NMR 2D correlations. NMR analysis analysis identified a peptide-like backbone with a series of highly modified substituents, no N-terminus, and with a decarboxylated threonine at the C-terminus. The 2D NMR correlations implied a macrocyclic ring structure as well as several smaller aromatic rings, consistent with the structure of Micrococcin P1. Also, two of the threonine residues were dehydrated, leaving a double bond between the α and β carbons; the six cysteine residues were each converted into a thiazole ring; and the two serine residues were condensed into a pyridine ring, making the macrocycle.

SUPPLEMENTAL REFERENCES

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3. **Bowers AA, Walsh CT, Acker MG.** 2010. Genetic interception and structural characterization of thiopeptide cyclization precursors from *Bacillus cereus*. J Am Chem Soc **132:**12182-12184.
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