

Supplementary Materials: Micellar Antibiotics of *Bacillus*

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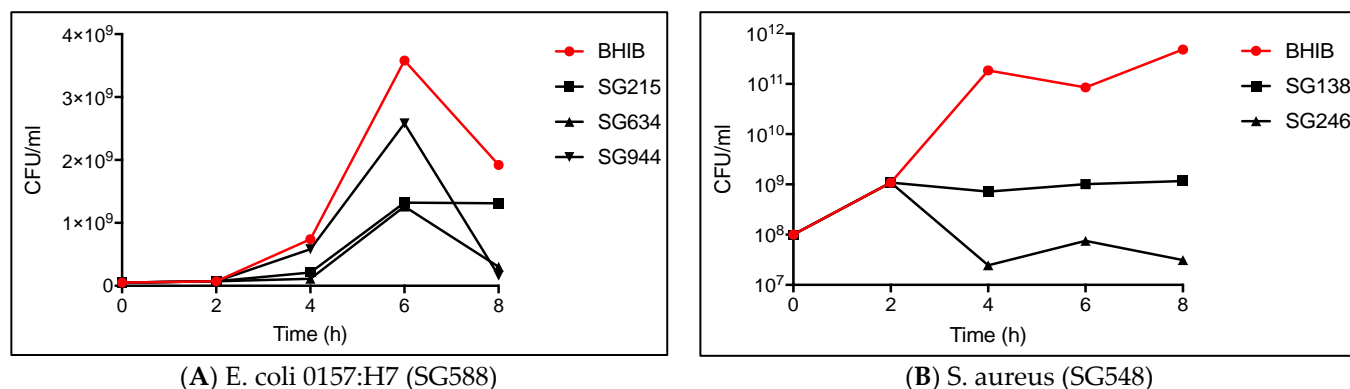


Figure S1. Pathogen growth kinetics using CFU counts. Cell free supernatants from *Bacillus* strain cultures (SGx) were added (1:10 *v/v*, indicated by red arrow) to mid-logarithmic cultures of Gram negative and Gram-positive pathogens. Growth was monitored by determination of CFU/ml in cultures with or without (untreated) addition of supernatants.

Table S1. Genome analysis of lipopeptide, polyketide and bacilysin genes present in *B. velezensis* 'active' strains. Operons identified from genome sequencing of *B. velezensis* strains with activity to *C. difficile*. Activities determined to CD630 using a microdilution assay are shown as the dilution factor. ANI values (two-way) compared to *B. velezensis* (NCBI: NZ_CP018133.1) are given as a % and were determined using the Enveomics program [1]. The order of ORFs in the fengycin operon differs between strains as indicated.

Antimicrobial	SG57	SG137	SG185	SG277	SG297
a-CD630 activity	1:80	1:80	1:80	1:160	1:160
ANI value	97.25	98.00	97.99	97.59	98.90
surfactin	<i>srfAA-D</i>	<i>srfAA-D</i>	<i>srfAA-D</i>	<i>srfAA-D</i>	<i>srfAA-D</i>
fengycin	<i>yng-fenBAEDC</i>	<i>yng-fenBAEDC</i>	<i>yng-fenBAEDC</i>	<i>yng-fenED-/-CBA</i>	<i>yng-EDCBA</i>
iturins	<i>fenF, mycA-C</i>	<i>fenF, mycA-C</i>	<i>fenF, mycA-C</i>	<i>fenF, mycA-C</i>	<i>fenF, mycA-C</i>
bacilysin	<i>bacA-E</i>	<i>bacA-E</i>	<i>bacA-E</i>	<i>bacA-E</i>	<i>bacA-E</i>
bacillaene (<i>pks1</i>)	<i>pksA-S</i>	<i>pksA-S</i>	<i>pksA-S</i>	<i>pksA-S</i>	<i>pksA-S</i>
macrolactin (<i>pks2</i>)	<i>mlnA-H</i>	<i>mlnA-H</i>	<i>mlnA-H</i>	<i>mlnA-H</i>	<i>mlnA-H</i>
difficidin (<i>pks3</i>)	<i>dfnA-O</i>	<i>dfnA-O</i>	<i>dfnA-O</i>	<i>dfnA-O</i>	<i>dfnA-O</i>

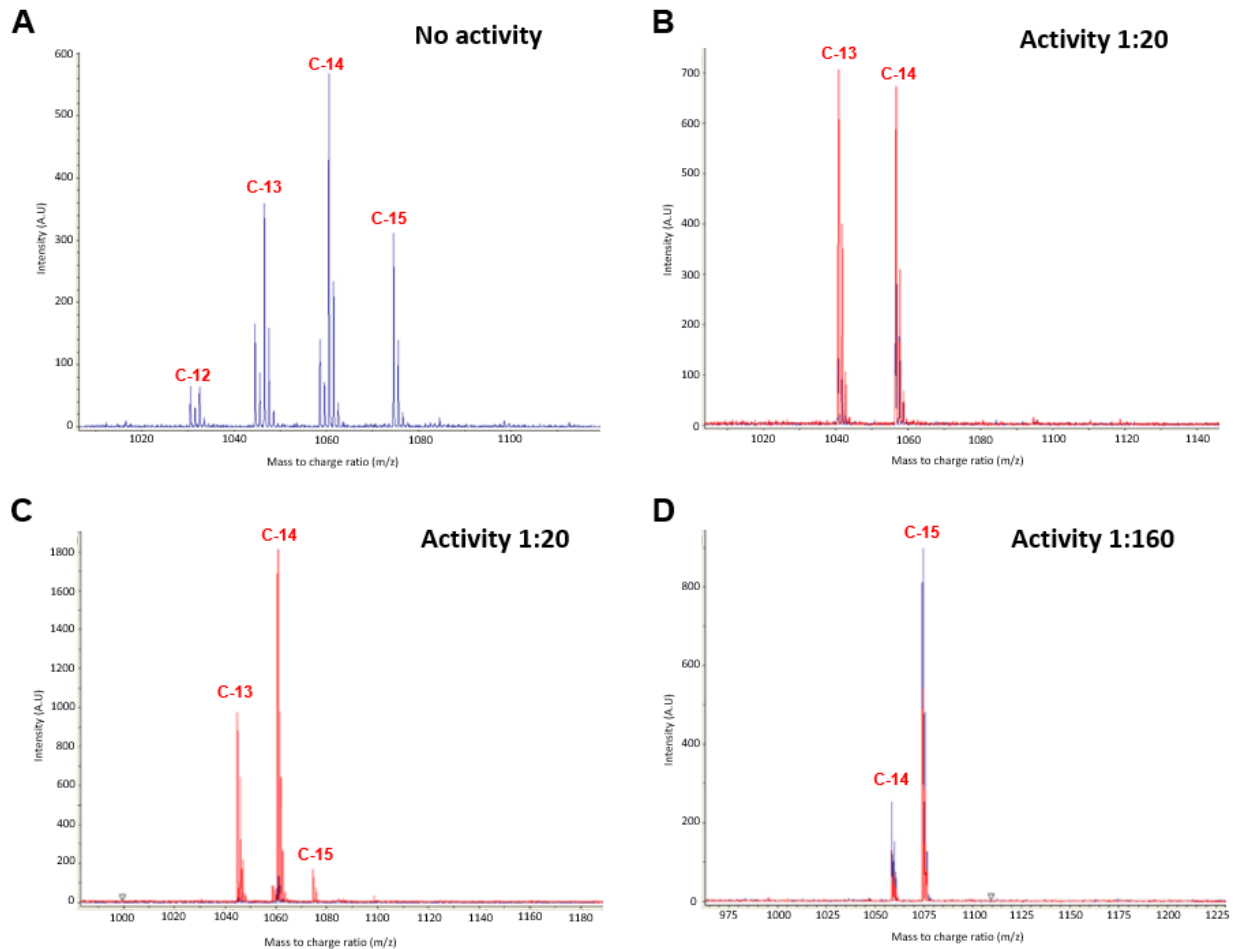


Figure S2. MALDI-TOF analysis of commercial vs *Bacillus* surfactins. MALDI-TOF analysis of commercially acquired surfactin (Sigma S3523) (**A**) and surfactin from three distinct 'active' fractions collected after RP-HPLC separation of the SG277 SEC material; active fraction exhibiting activity against CD630 of 1:20 (**B**), active fraction exhibiting activity against CD630 of 1:20 (**C**), active fraction exhibiting activity against CD630 of 1:160 (**D**). Activity against CD630 (1/dilution factor) and surfactin species are indicated in the figures. Activity was greatest in the fraction containing C-15 surfactin (**D**).

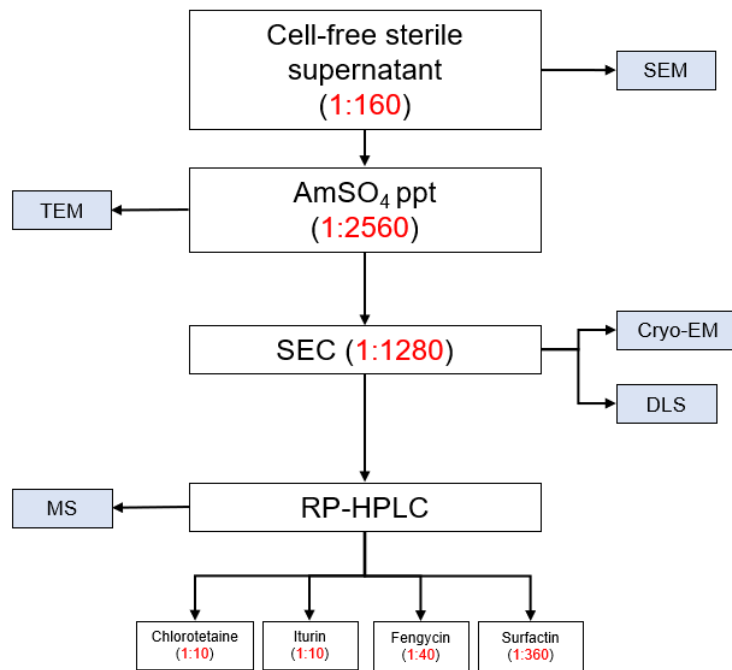


Figure S3. Purification steps. Flow diagram showing purification steps of culture supernatants and analyses performed. TEM: transmission electron microscopy, MS: MALDI-TOF analysis, DLS: dynamic light scattering, SEC: size exclusion chromatography, RP-HPLC: Reversed-phase high performance liquid chromatography. Activity against CD630 using a microdilution assay is indicated in brackets.

Table S2. Antimicrobial screening^a.

Group ^b	Strain No.	Species ^c	Biosurfactant activity ^d	Heat resistance ^e	Gram-negative			Gram-positive				
					Salmonella			B. cereus (SG584)	C. difficile (630)	S. hominis (ATCC 27844)	S. aureus (SG548)	L. monocytogenes (NCTC 19115)
					E. coli (0157:H7; 078:K80)	enterica (NCTC 13349:SG576)	K. aerogenes (NCTC 1006)					
"B. subtilis"	SG634	B. velezensis	+++	+	BS						BS	
	SG113	B. velezensis	+	+	BS							
	SG661	B. velezensis	+++	+	BS							
	SG630	B. velezensis	+	+			BS					
	SG184	B. velezensis	+++	+		BS				BS		
	SG545	B. velezensis	+	+							BC	
	SG91	B. velezensis	++	+				BC		BS	BC	
	SG309	B. velezensis	+	+							BC	
	SG541	B. velezensis	+	+				BC				
	SG944	B. velezensis		+	BS					BS		
	SG71	B. velezensis	+	+		BS				BS		
	SG660	B. velezensis	++	+		BS				BS		
	SG677	B. velezensis	+++	+							BS	
	SG57	B. velezensis	++	+					BL			
	SG137	B. velezensis	++	+					BL			
	SG185	B. velezensis	++	+					BL			
	SG277	B. velezensis	+++	+					BL			
	SG297	B. velezensis	+++	+					BL		BC	
	SG202	B. velezensis	+	+		BS					BC	
	SG943	B. velezensis		+							BS	
	SG633	B. velezensis	+	+							BC	
	SG629	B. velezensis	+	+		BS					BC	
	SG695	B. velezensis	+	+							BC	
	SG138	B. velezensis	++	+							BS	
	SG18	B. amyloliquefaciens	+	+	BS							
	SG246	B. amyloliquefaciens	+	+		BS					BC	
	SG531	B. subtilis	+	+				BS				
	SG308	B. subtilis	++	+		BS						
	SG281	B. subtilis	+	+		BS						
	SG691	B. subtilis	+++	+	BS							
	SG3	B. subtilis	++	+	BS							
	SG310	B. mojavensis	+++	+			BS					
Other	SG215	B. infantis	+	+	BS							
	SG836	B. pseudomycoides	-	+			BS					
	SG655	Viridibacillus arvi	++	+			BS					
	SG224	Mesobacillus subterraneus	++	+							BS	

^a cultures from strains were tested for inhibitory activity to a panel of Gram negative and Gram positive pathogens. Initial screening was by well diffusion and secondary screening using co-culture where activity is defined as BS, bacteriostatic, BC, bactericidal or BL, bacteriolytic. Strains were obtained from strain collections or were lab stocks.^b operational group of related species, [2].^c species assigned by *gyrA* sequence analysis [3] for members of the *B. subtilis* group and 16S rRNA [4,5] for remaining strains. ^d biosurfactant activity defined as zone of oil displacement; +, <10mm; ++, 10–20mm; +++, >20mm.^e inhibitory activity present after heating of cell-free supernatant for 15 min. at 90°C.

References:

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