

## **Supplementary materials**

### **Sustainable surfactin production by *Bacillus subtilis* using crude glycerol from different wastes**

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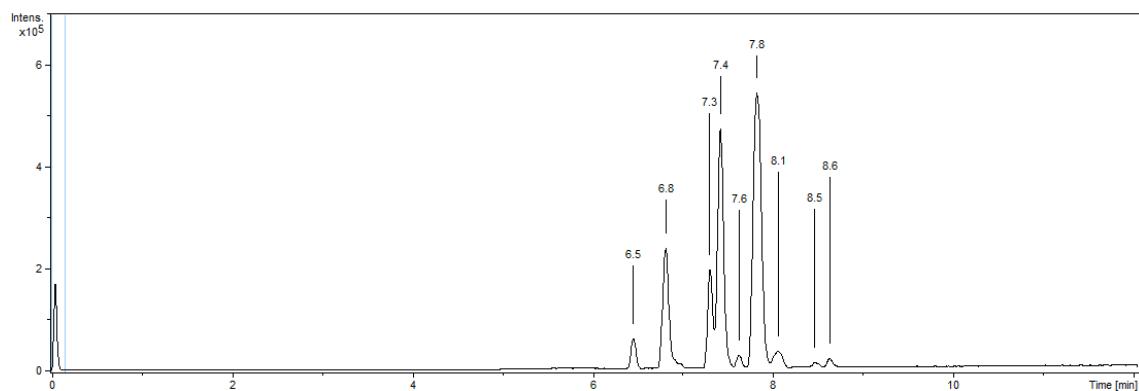
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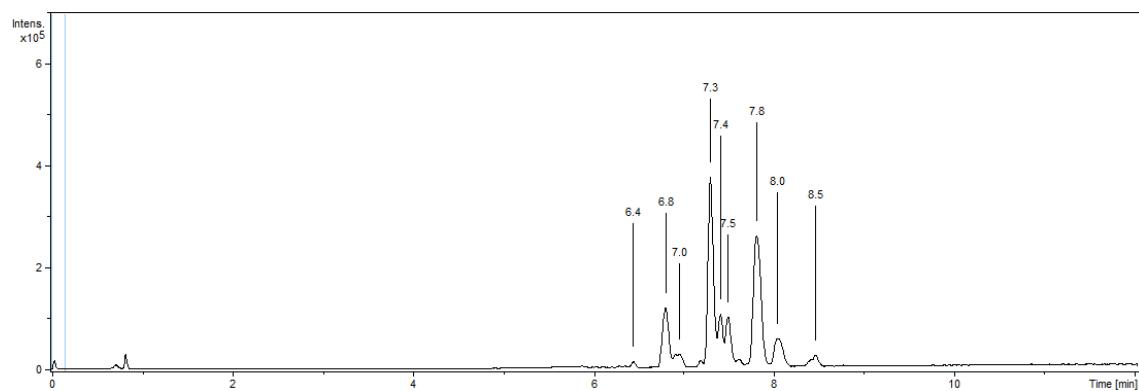
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(a)



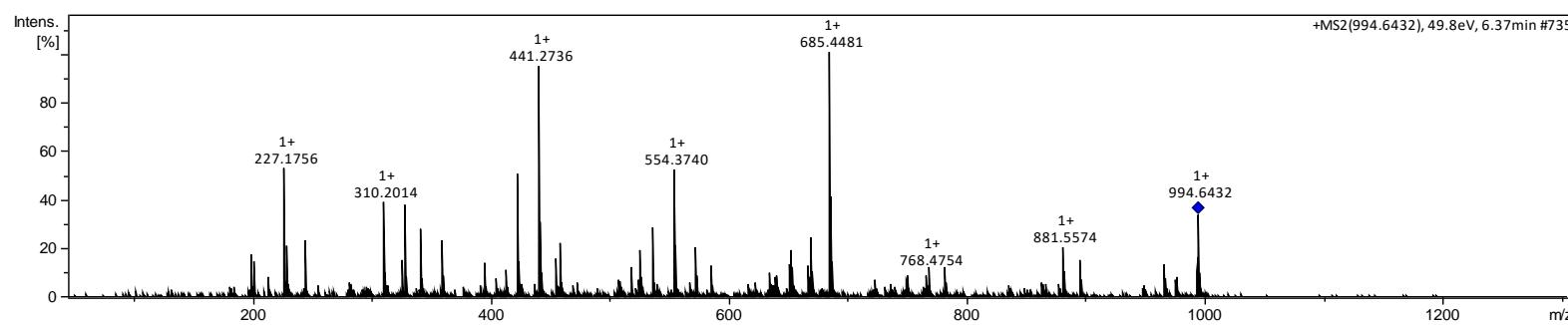
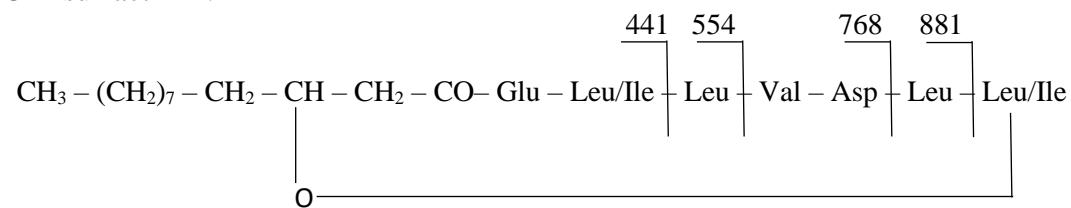
(b)



**Figure S1.** Chromatograms of standard surfactin (50  $\mu\text{g}/\text{mL}$ ) (a) and *Bacillus subtilis* #309 cell-free supernatant diluted 100 times with methanol (b). Retention times of selected surfactin peaks are marked. Data collected for  $m/z 900.0 \pm 0.5 - 1200.0 \pm 0.5$  are shown.

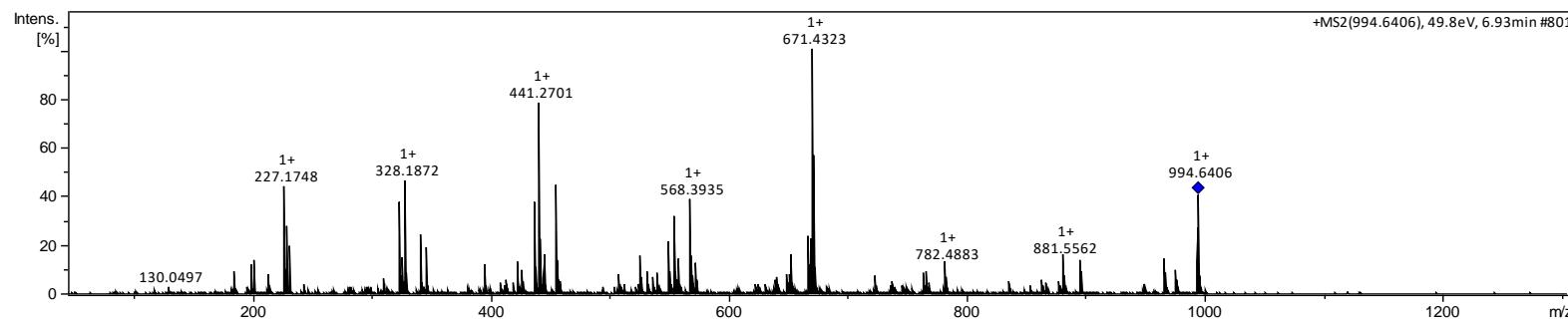
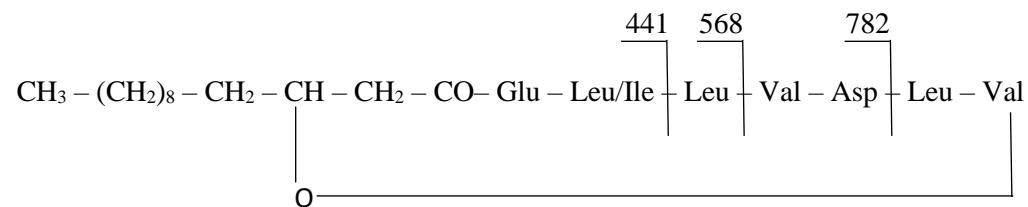
MS/MS spectrum of the  $[M+H]^+$  994.64 m/z ion at Rt=6.50 min.

C12 surfactin A:



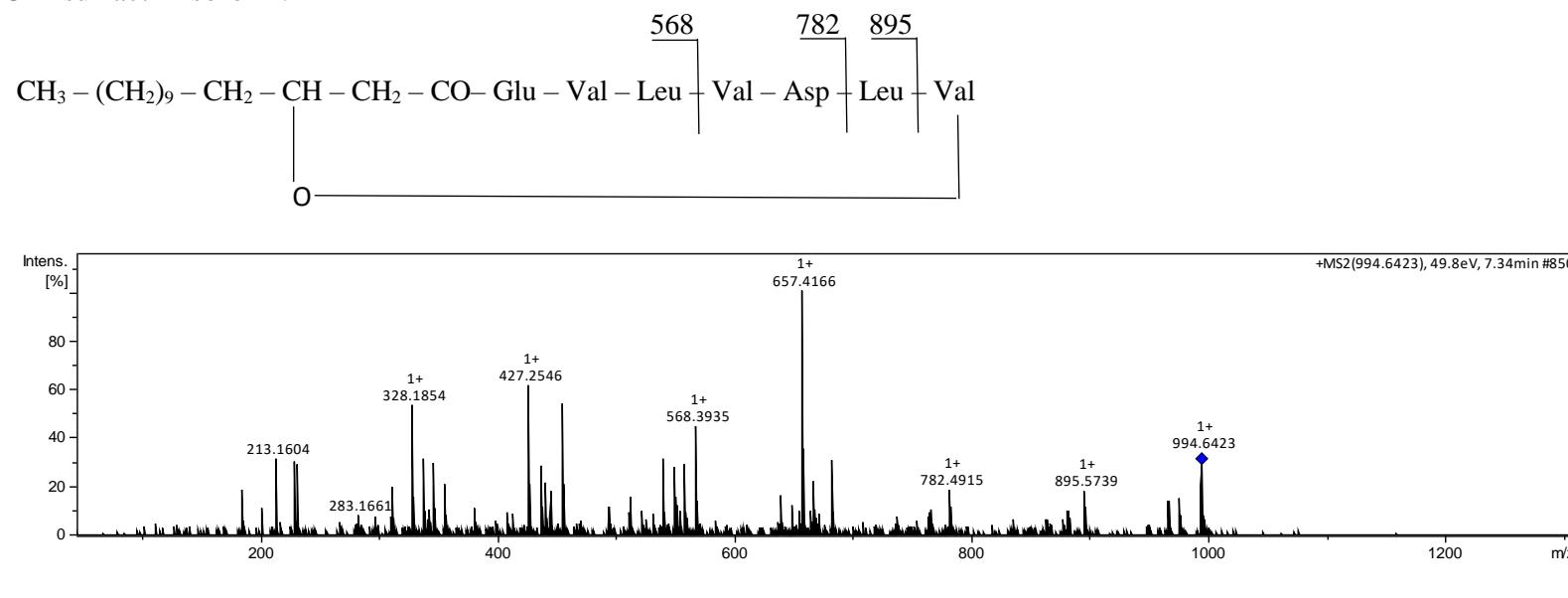
MS/MS spectrum of the  $[M+H]^+$  994.64 m/z ion at Rt=7.00 min.

C13 surfactin B:



MS/MS spectrum of the  $[M+H]^+$  994.64 m/z ion at Rt=7.50 min.

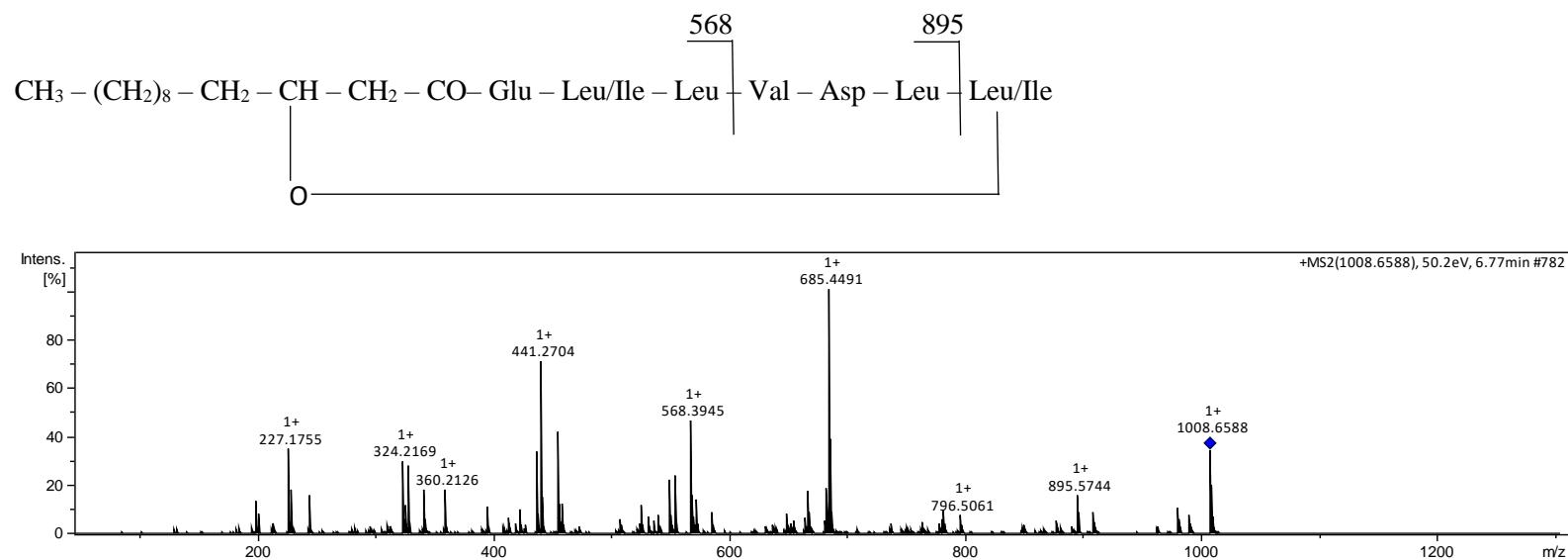
C14 surfactin isoform:



**Figure S2.** MS/MS spectra of  $[M+H]^+$  994.64 m/z ions detected in culture supernatants of *B. subtilis* #309.

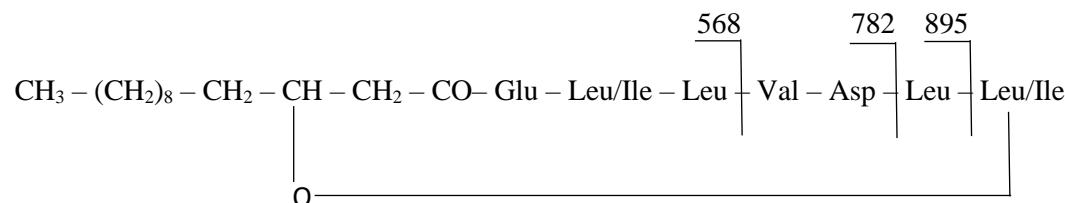
MS/MS spectrum of the  $[M+H]^+$  1008.66 m/z ion at Rt=6.82 min.

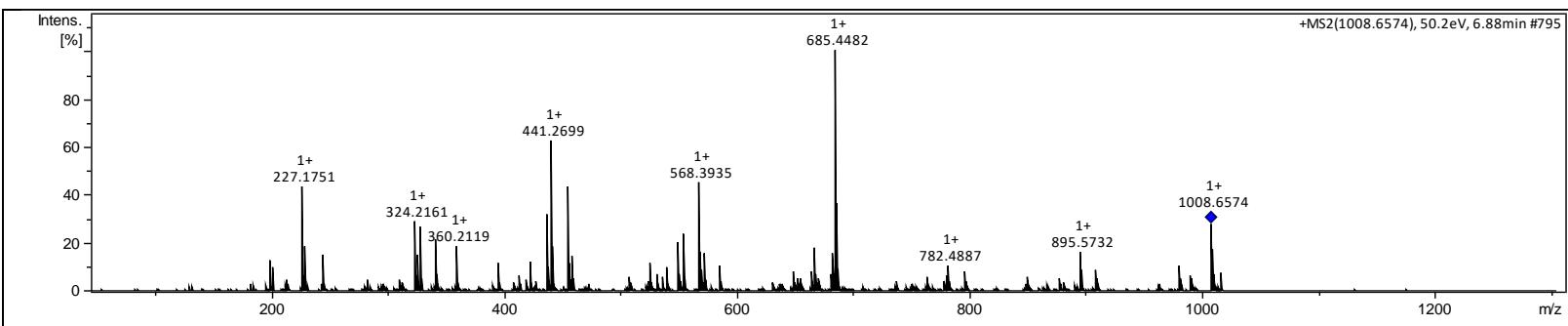
C13 surfactin A:



MS/MS spectrum of the  $[M+H]^+$  1008.66 m/z ion at Rt=6.94 min.

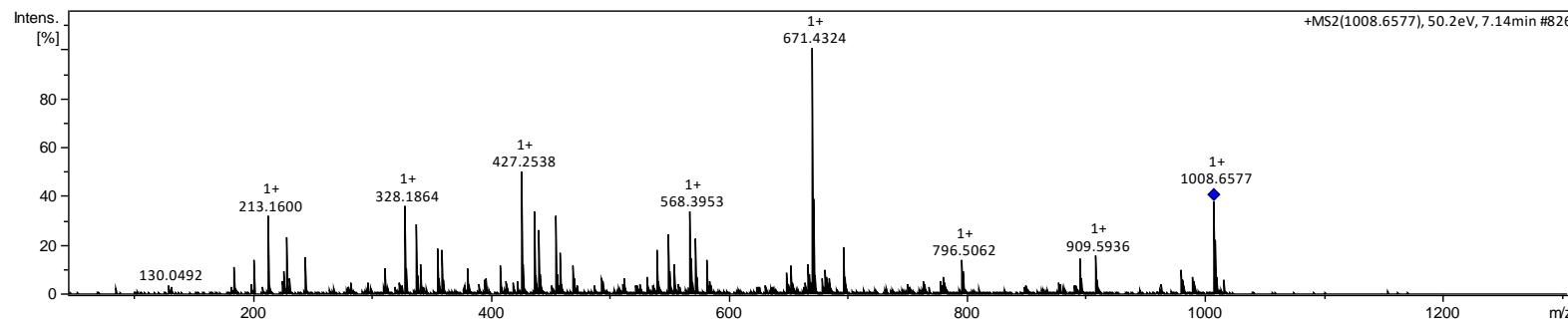
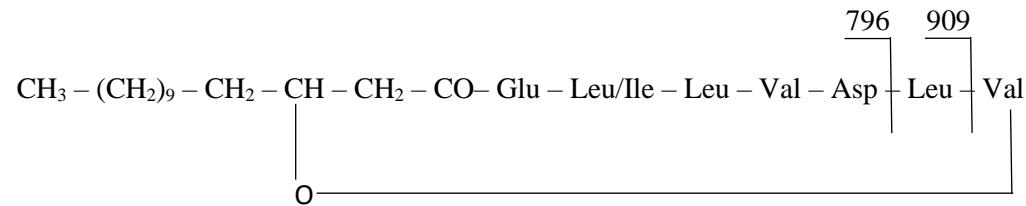
C13 surfactin A:





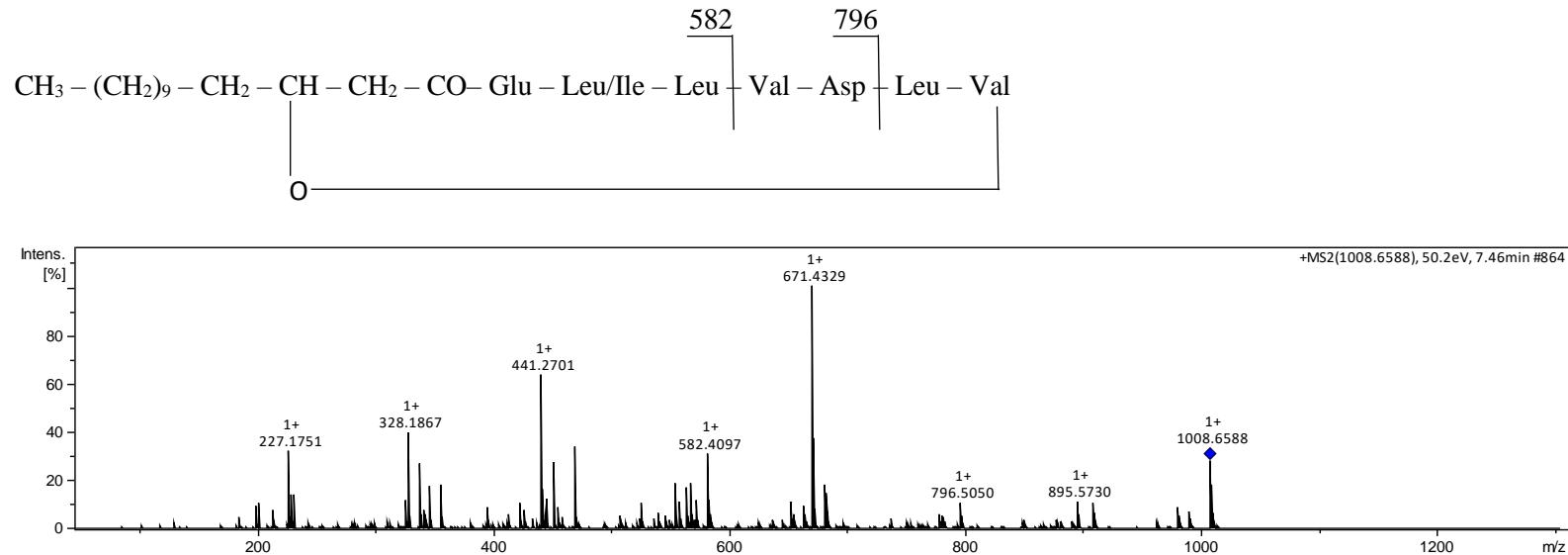
MS/MS spectrum of the  $[M+H]^+$  1008.66 m/z ion at Rt=7.20 min.

C14 surfactin B:



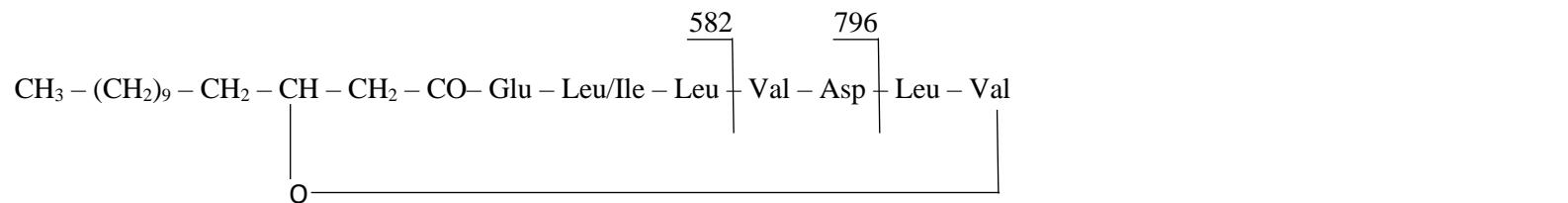
MS/MS spectrum of the  $[M+H]^+$  1008.66 m/z ion at Rt=7.50 min.

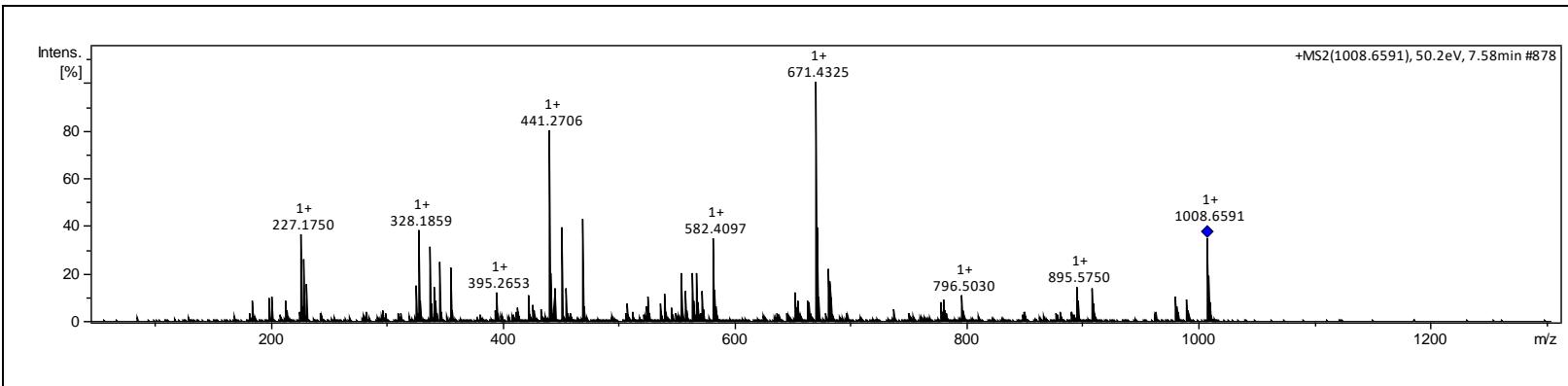
C14 surfactin B:



MS/MS spectrum of the  $[M+H]^+$  1008.66 m/z ion at Rt=7.62 min.

C14 surfactin B:

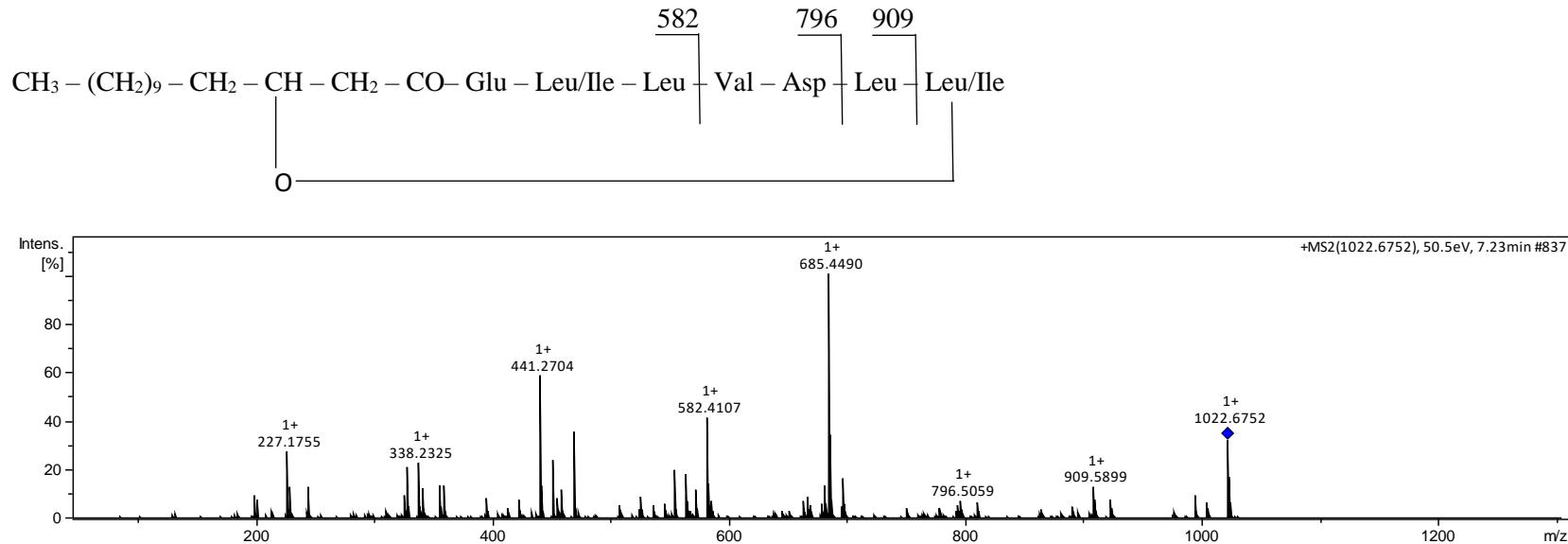




**Figure S3.** MS/MS spectra of  $[M+H]^+$  1008.66 m/z ions detected in culture supernatants of *B. subtilis* #309.

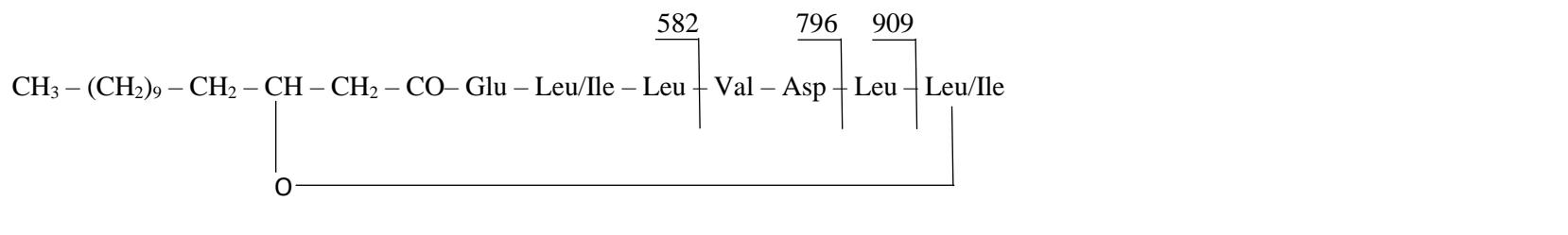
MS/MS spectrum of the  $[M+H]^+$  1022.68 m/z ion at Rt=7.31 min.

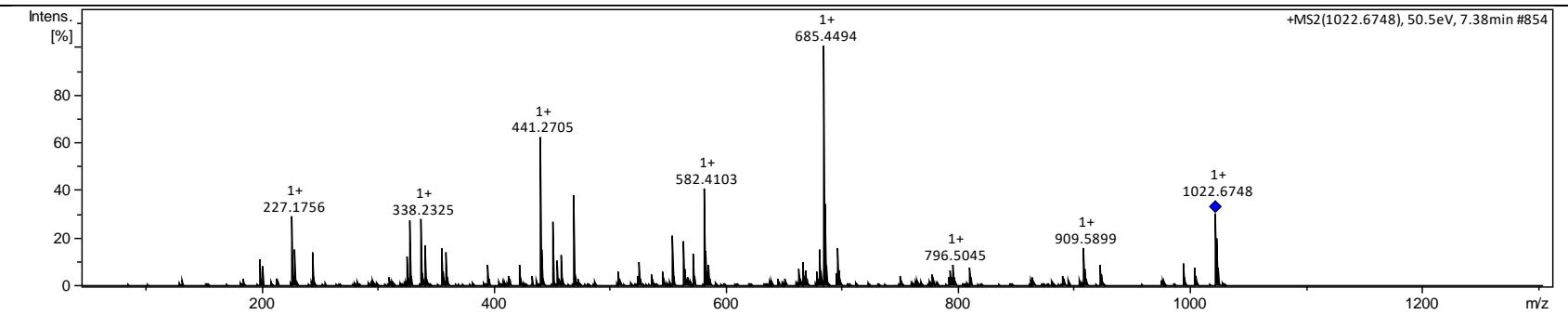
C14 surfactin A:



MS/MS spectrum of the  $[M+H]^+$  1022.68 m/z ion at Rt=7.38 min.

C14 surfactin A:



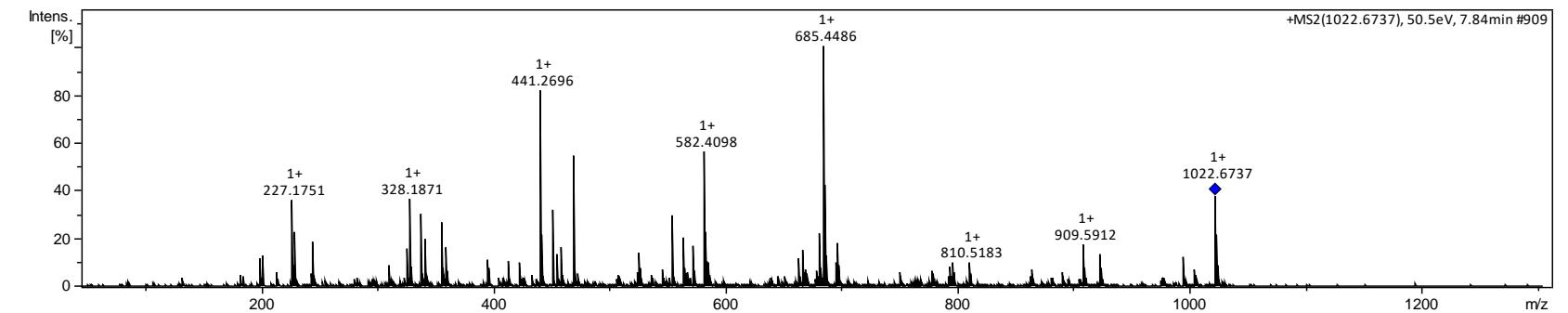
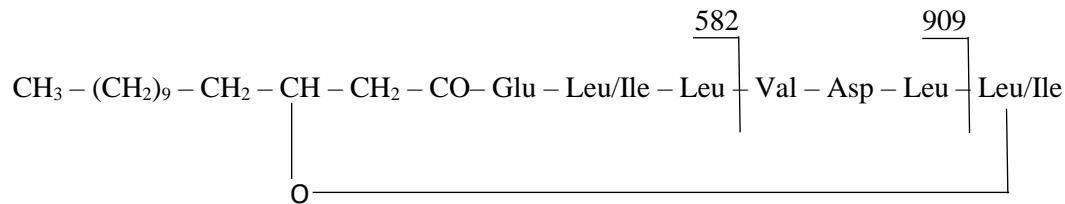


MS/MS spectrum of the  $[M+H]^+$  1022.68 m/z ion at Rt=7.55 min.

Good quality spectrum could not be collected due to the high intensity of coeluting ions  $[M+H]^+$  1008.66 m/z and 1036.69 m/z

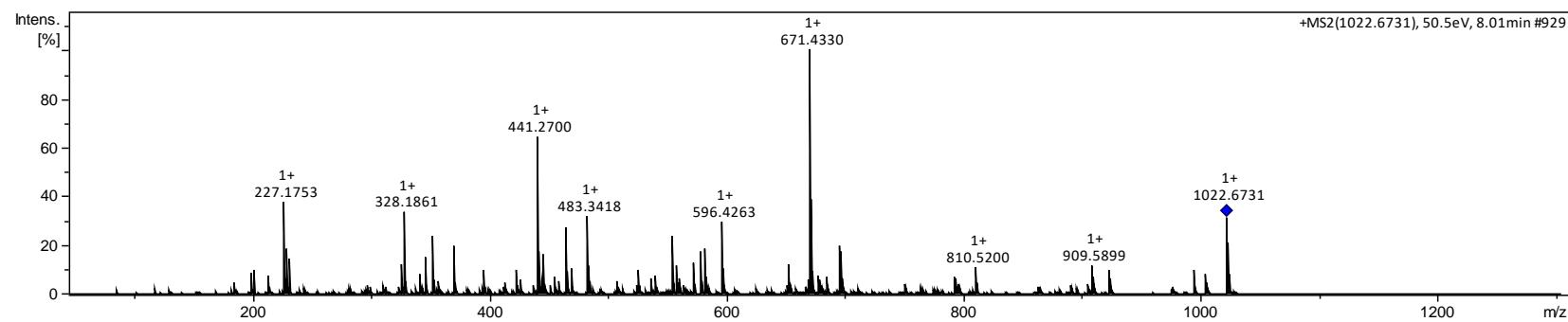
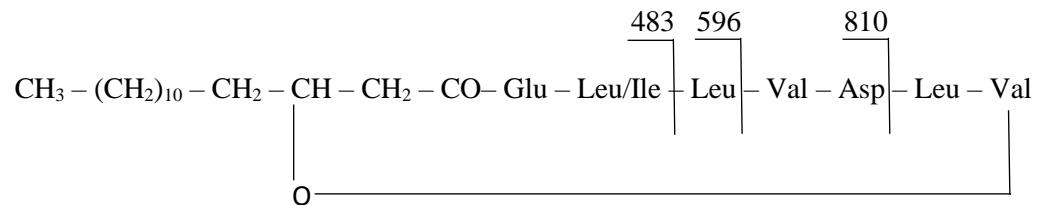
MS/MS spectrum of the  $[M+H]^+$  1022.68 m/z ion at Rt=7.88 min.

### C14 surfactin A:



MS/MS spectrum of the  $[M+H]^+$  1022.68 m/z ion at Rt=8.07 min.

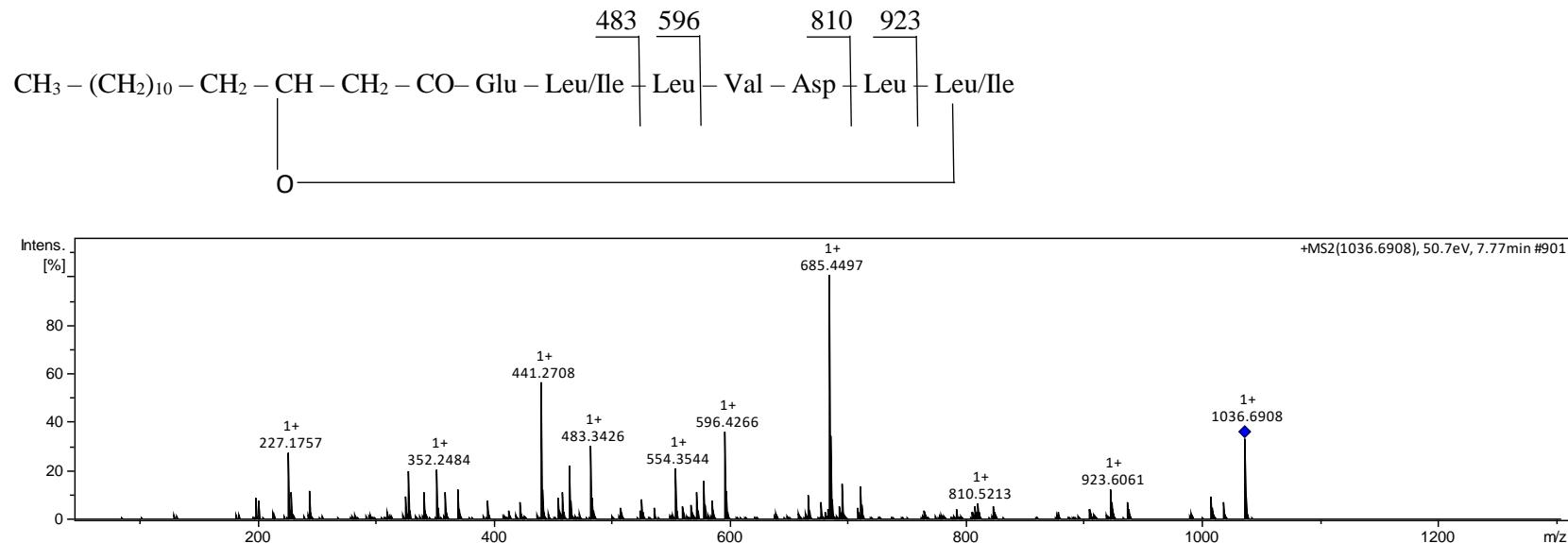
C15 surfactin B:



**Figure S4.** MS/MS spectra of  $[M+H]^+$  1022.68 m/z ions detected in culture supernatants of *B. subtilis* #309.

MS/MS spectrum of the  $[M+H]^+$  1036.69 m/z ion at Rt=7.83 min.

C15 surfactin A:

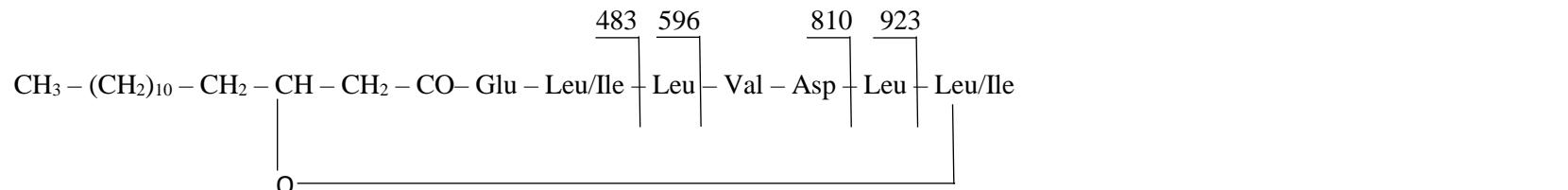


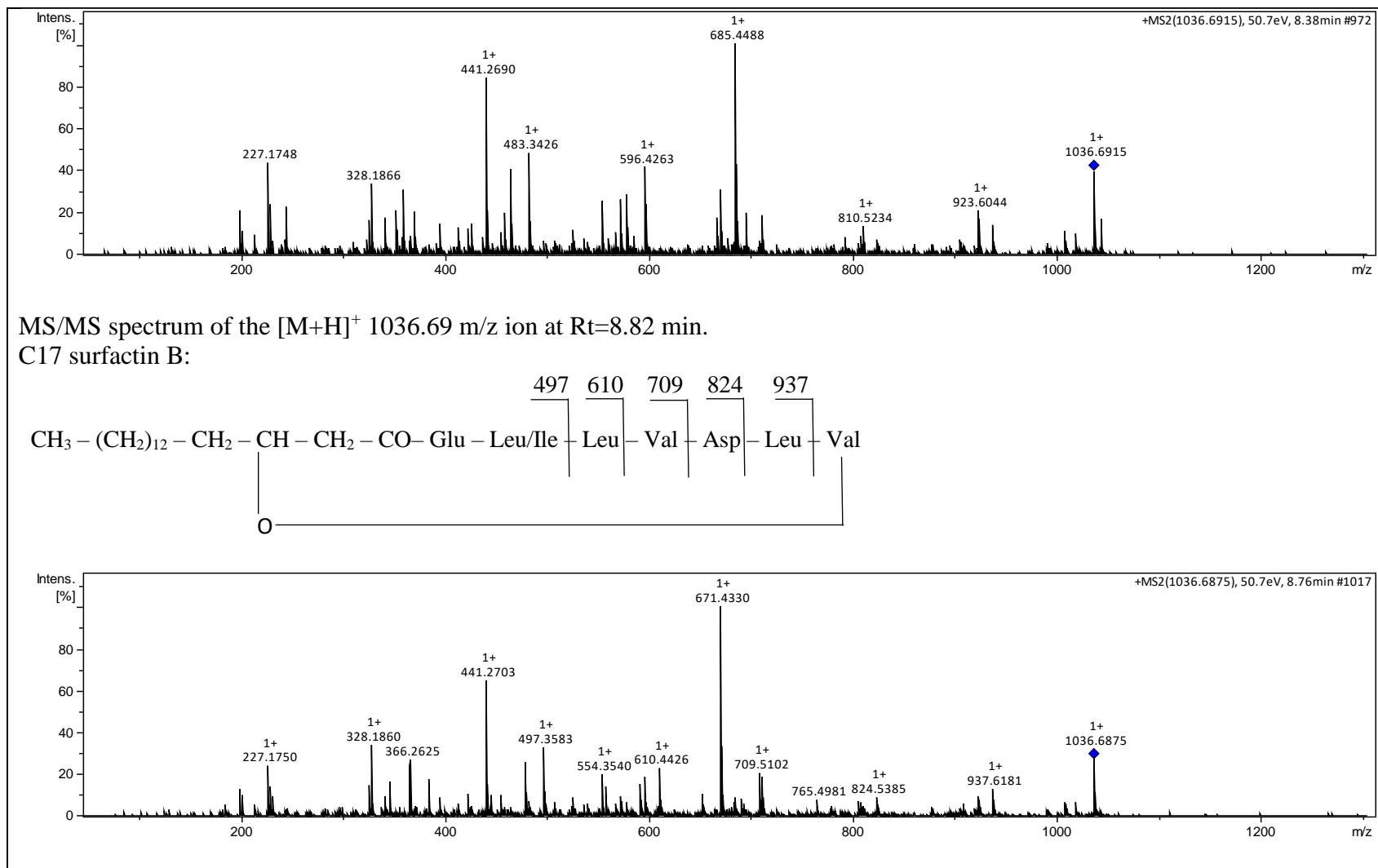
MS/MS spectrum of the  $[M+H]^+$  1036.69 m/z ion at Rt=8.12 min.

Good quality spectrum could not be collected due to the high intensity of coeluting ions  $[M+H]^+$  1022.68 m/z

MS/MS spectrum of the  $[M+H]^+$  1036.69 m/z ion at Rt=8.51 min.

C15 surfactin A:

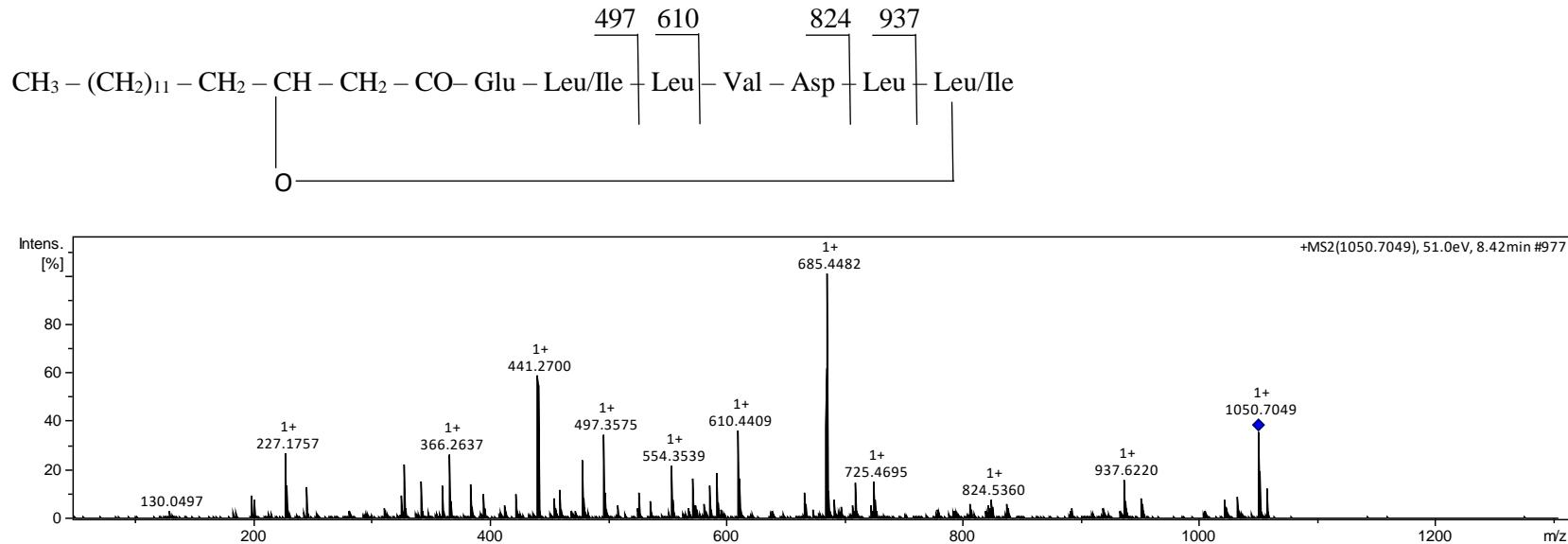




**Figure S5.** MS/MS spectra of [M+H]<sup>+</sup> 1036.69 m/z ions detected in culture supernatants of *B. subtilis* #309.

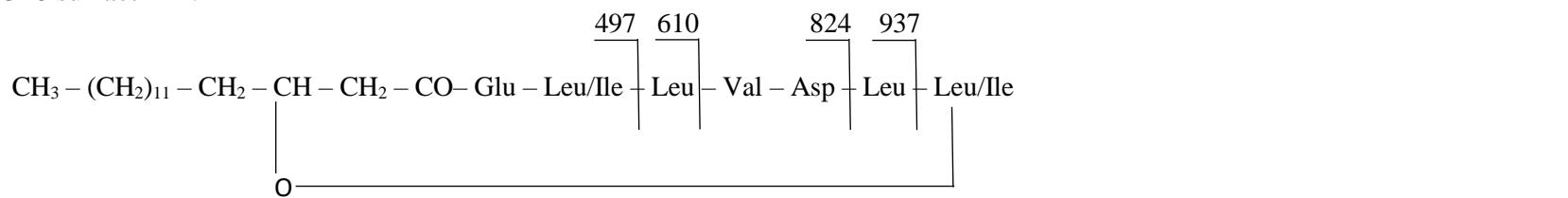
MS/MS spectrum of the  $[M+H]^+$  1050.71 m/z ion at Rt=8.50 min.

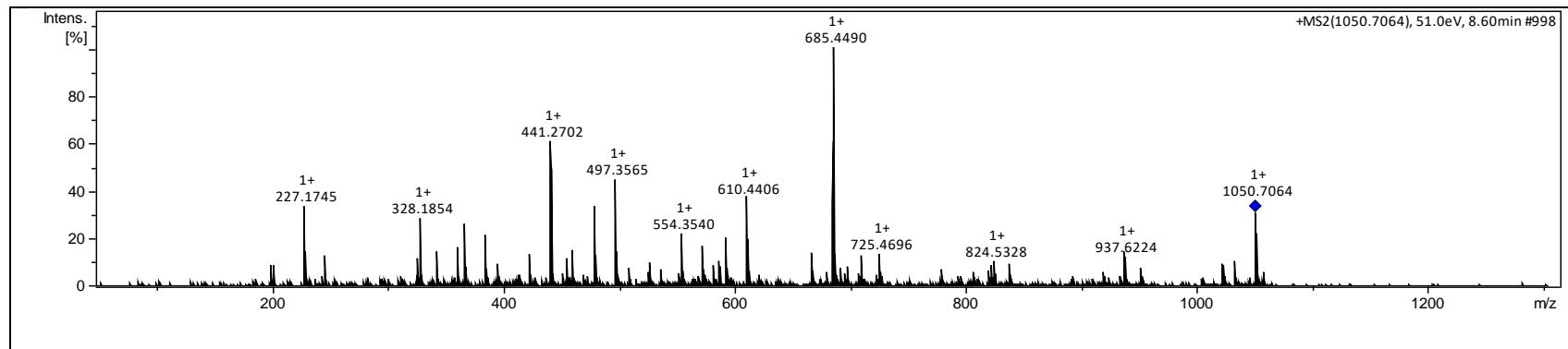
C16 surfactin A:



MS/MS spectrum of the  $[M+H]^+$  1050.71 m/z ion at Rt=8.65 min.

C16 surfactin A:





**Figure S6.** MS/MS spectra of  $[M+H]^+$  1050.71 m/z ions detected in culture supernatants of *B. subtilis* #309.



**Figure S7.** Thin-layer chromatography (TLC) analysis of lipopeptide produced by *B. subtilis* #309. Developed with chloroform/methanol/water (65:25:4 vol/vol/vol); detected with bromothymol blue.

**Table S1.** Composition of crude glycerol from different sources [1].

Symbol	G1	G2	G3	G4	G5
Waste product derived from [%]:	Biodiesel production	Biodiesel production	Stearin production	Soap production	Pure glycerol
Glycerol content	80	50	42	80	100
Nitrogen content	0.014	0.078	0.136	0.041	0
NaCl	5.47	3.04	1.23	7.59	0
Ash	6.34	3.62	1.35	8.76	0
Water	8.16	43.42	55.3	3.6	0

**Table S2.** Elemental composition of crude glycerol from different sources [1].

Crude Glycerol	The content of elements (mg/kg)							
	Cu	Mg	Fe	Zn	K	Na	Cl	Ca
<b>G1</b>	0.39 ± 0.04	22.55 ± 1.02	5.91 ± 0.20	1.41 ± 0.13	65.89 ± 4.88	23120.03 ± 764.10	33200 ± 425.56	132.21 ± 5.45
<b>G2</b>	0.12 ± 0.08	15.26 ± 0.45	2.31 ± 0.68	1.13 ± 0.34	72.74 ± 8.98	13102.66 ± 519.93	18500 ± 30.00	97.06 ± 9.44
<b>G3</b>	0.03 ± 0.01	5.305 ± 1.06	458.38 ± 45.84	1.251 ± 0.25	63.137 ± 6.31	5224.84 ± 261.24	7400 ± 59.26	461.95 ± 46.19
<b>G4</b>	0.66 ± 0.08	5.454 ± 0.44	26.42 ± 1.20	1.30 ± 0.40	231.32 ± 4.77	31632.89 ± 521.66	46000 ± 21.20	52.36 ± 10.93

**Table S3.** Relative abundance (%) of surfactin structural analogues present in standard surfactin (Merck) and surfactin extracts obtained from cultures of *Bacillus subtilis* #309 grown in mineral salts medium (MSM) supplemented with glycerol from different sources. The results represent the mean  $\pm$  standard deviation of three independent experiments.

Surfactin analogue	Surfactin standard	G1 (Biodiesel)	G2 (Biodiesel)	G3 (Stearin)	G4 (Soap)	G5 (Pure glycerol)
C12 Surfactin	2.9 $\pm$ 0.1	1.1 $\pm$ 0.0	1.1 $\pm$ 0.0	2.0 $\pm$ 0.1	1.5 $\pm$ 0.0	0.9 $\pm$ 0.0
C13 Surfactin	14.4 $\pm$ 0.5	10.6 $\pm$ 0.1	10.8 $\pm$ 0.1	12.5 $\pm$ 0.1	15.8 $\pm$ 0.1	8.9 $\pm$ 0.1
C14 Surfactin	35.9 $\pm$ 0.2	54.8 $\pm$ 0.1	53.1 $\pm$ 0.1	54.3 $\pm$ 0.3	42.0 $\pm$ 0.3	51.6 $\pm$ 0.2
C15 Surfactin	41.8 $\pm$ 0.4	26.9 $\pm$ 0.1	28.6 $\pm$ 0.2	25.2 $\pm$ 0.2	34.7 $\pm$ 0.2	31.5 $\pm$ 0.2
C16 Surfactin	3.0 $\pm$ 0.0	3.4 $\pm$ 0.1	3.6 $\pm$ 0.0	2.8 $\pm$ 0.1	2.8 $\pm$ 0.0	4.4 $\pm$ 0.1
C17 Surfactin	0.1 $\pm$ 0.0	1.2 $\pm$ 0.0	1.0 $\pm$ 0.1	0.8 $\pm$ 0.0	0.6 $\pm$ 0.0	1.1 $\pm$ 0.1

## References

1. Dobrowolski, A.; Mituła, P.; Rymowicz, W.; Mirończuk, A.M. Efficient conversion of crude glycerol from various industrial wastes into single cell oil by yeast *Yarrowia lipolytica*. *Bioresour. Technol.* **2016**, *207*, 237–243, doi:10.1016/j.biortech.2016.02.039.