Generation of lysolipin derivatives by genetic engineering

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Running title: Novel lysolipin derivatives

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Table S1: Media receipt for lysolipin production media NL800 and E1. All data refer to 1 L H_2O_{deion}.

Medium	Ingredients	pH
NL800	5 g glucose 10 g glycerin 10 g soluble starch 5 g soy flour full fat 2 g yeast extract 1 g NaCl 1 g $CaCO_3$	7.2
E1	20 g glucose 20 g soluble starch 5 g yeast extract 2.5 g pharmamedia 1 g $MgSO_4$ x $7H_2O$ 1.3 g KH_2PO_4 x $3H_2O$ 5 g NaCl 5 g $CaCO_3$	7.5

Table S2: Oligonucleotides used in this study. MunI restriction site is highlighted by bold letters.

Primer designation	Primer sequence (5'-3')
llpRI-CmR.MunI-F	TTGATTAGTCACGCCGCACCTAGTAGCCTGGCAACTATG**CAATTG**GACGTCTAAGAAACCATTAT
llpRI-CmR.MunI-R	GCCGGTTCCATCCCCGTCGGCTGGCCGGCGCGGGTCTCA**CAATTG**TTACGCCCCGCCCTGCCAC

Table S3: Bioactivity profile of lysolipin I and its derivatives as reported in patent WO/2007/079715. Improved bioactivities are highlighted in bold.

Lysolipin (derivative)	**MIC (µg/mL) (*E. coli* ATCC10536)**	**Cytotoxicity (IC50, [M]) (THP-1 (human lymphoblast))**
Lysolipin I (CBS42)	128	9.39E-09
CBS40	**3**	2.16E-08
CBS44	128	1.16E-07
CBS48	128	2.08E-07
CBS49	**30**	3.44E-07
CBS68	128	**1.58E-07**
CBS70	128	**2.99E-07**
CBS72	128	4.27E-08

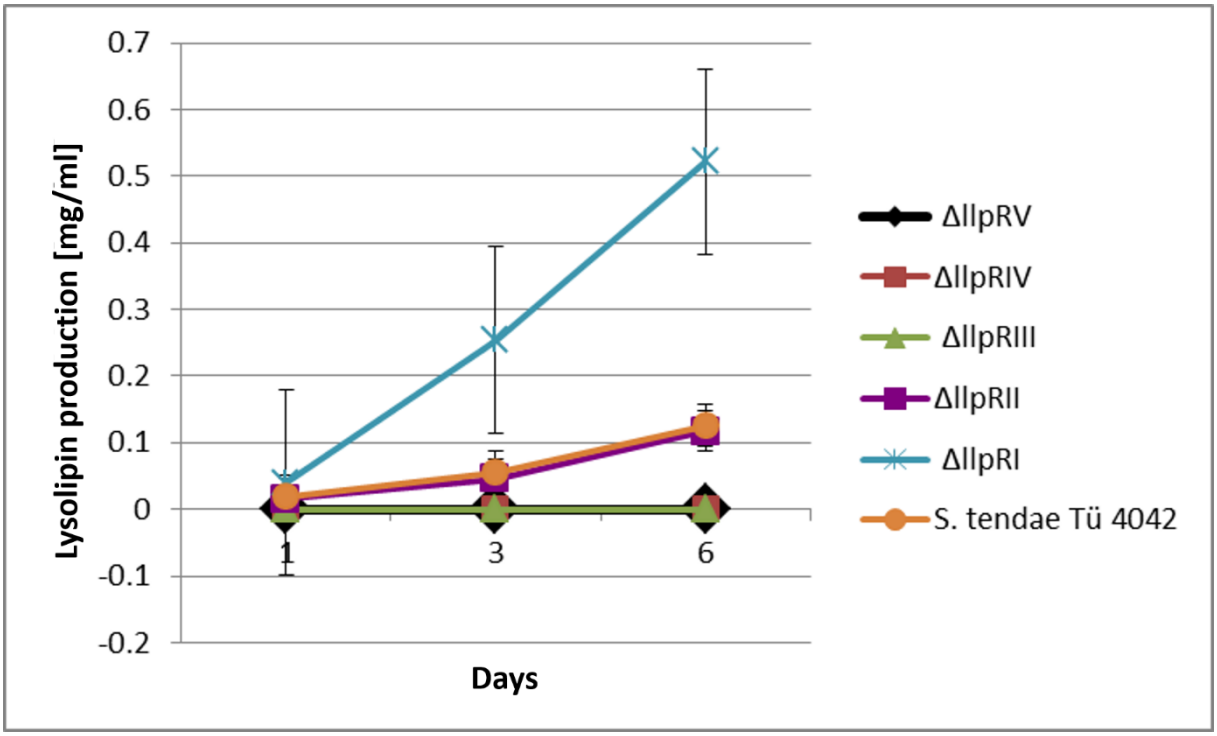

Figure S1: Lysolipin I production of *Streptomyces tendae* TÜ4042 and transcriptional regulatory mutants *S. tendae* Δ*llpRI*, Δ*llpRII,* Δ*llpRIII,* Δ*llpRIV,* and Δ*llpRV,* respectively, according to Rohrer, 2017. Cell cultures were harvested after 1, 2 and 6 days of cultivation in E1 medium. Lysolipin production was analysed by HPLC-MS by using a

lysolipin calibration curve. Results are shown from three independent biological replicates.

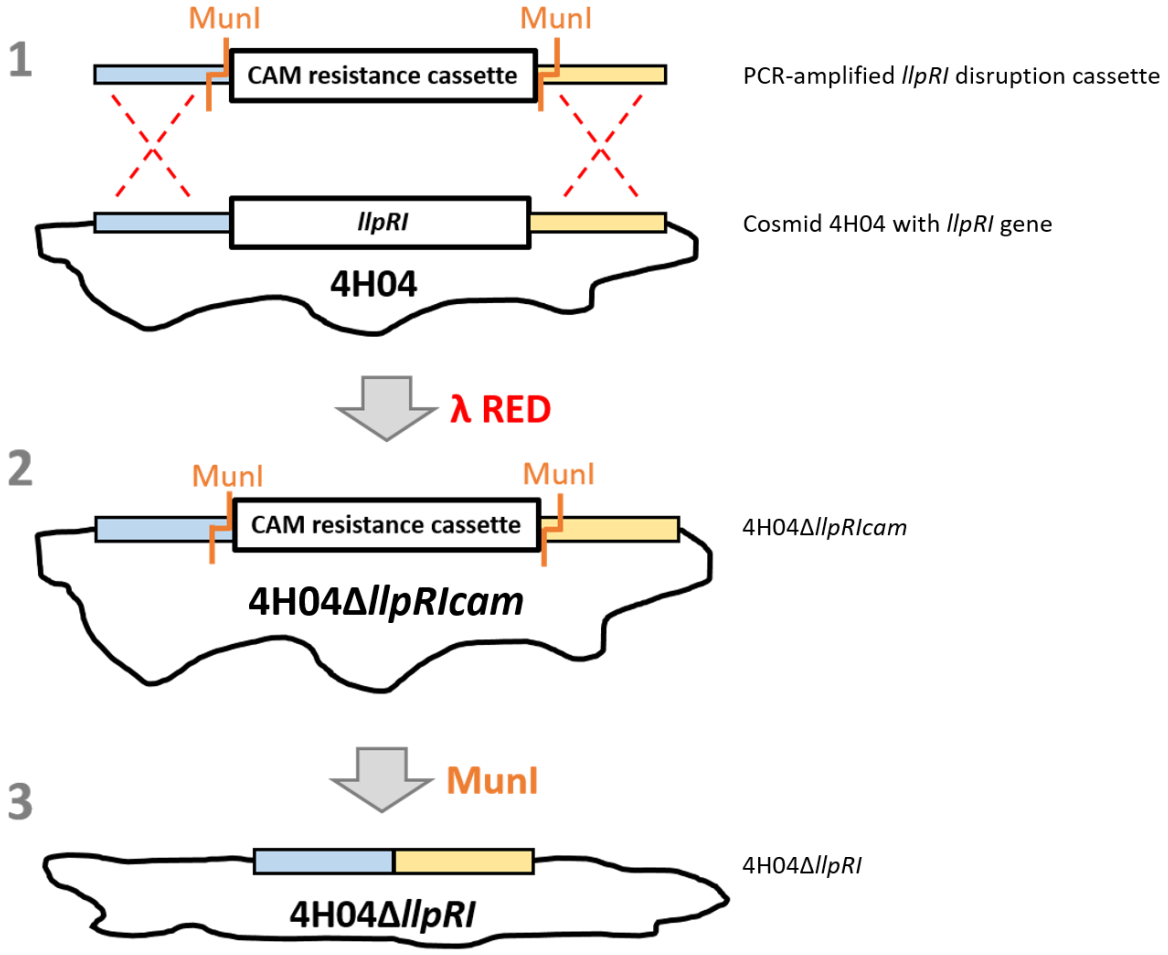

Figure S2: Schematic presentation of the cloning procedure to generate construct 4H04Δ*llpRI in E. coli*. Up- and downstream homologous regions of *llpRI* are indicated in blue and yellow, respectively. MunI restriction sites are shown in orange. Homologous recombination events are highlighted as red dashed crosses 1: λ-RED-driven recombination of homologous gene regions by a double crossover event to integrate the PCR-amplified *llpRI* disruption cassette into 4H04; 2: 4H04Δ*llpRIcam* with chloramphenicol resistance marker (CAM) and deleted *llpRI* gene; 3: 4H04Δ*llpRI* without CAM cassette after restriction with MunI, resulting in *llpRI* deletion.

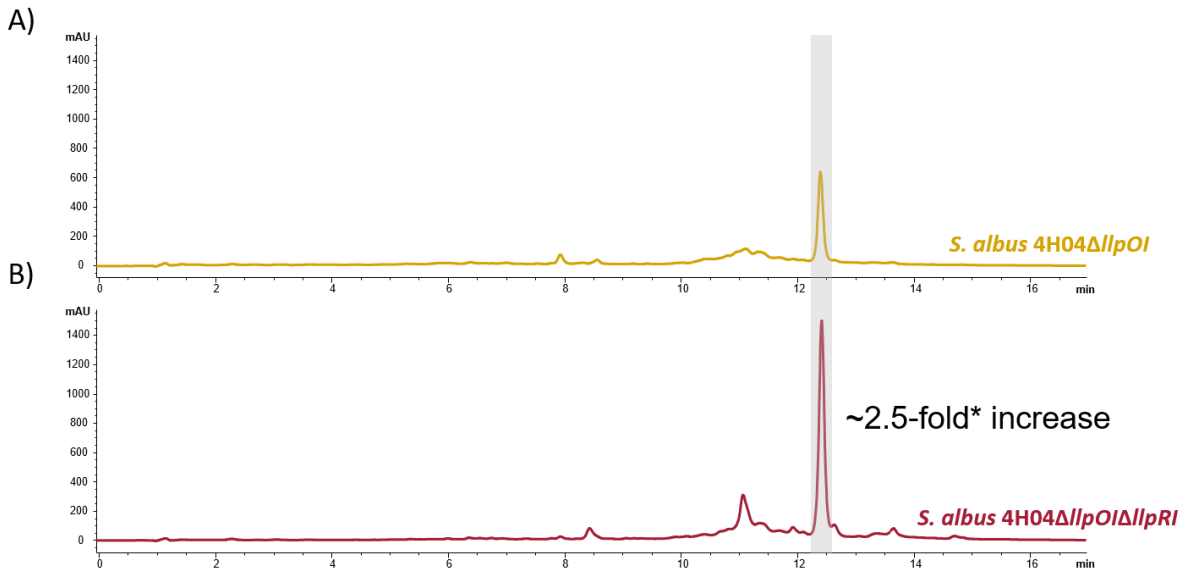

Figure S3: HPLC chromatograms of extract samples from A) *S. albus* 4H04Δ*llpOI* (yellow) and B) *S. albus* 4HO4Δ*llpOI*Δ*llpRI* (red). Peak at RT 12.4 correlates to lysolipin derivative (CBS40) production and is highlighted in grey. Samples were obtained from cultures grown for seven days at 28°C.

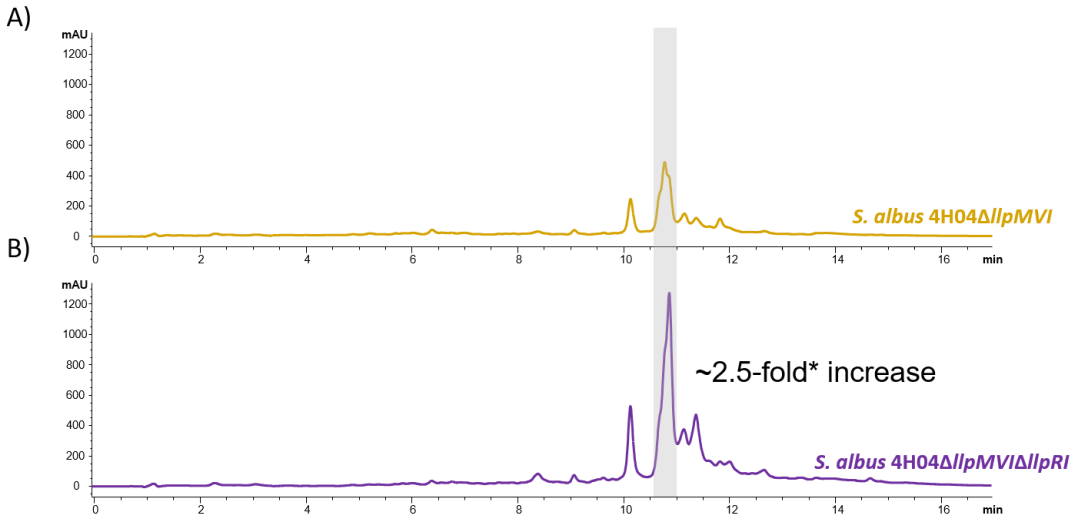

Figure S4: HPLC chromatograms of extract samples from A) *S. albus* 4H04Δ*llpMVI* (yellow) and B) *S. albus* 4H04Δ*llpMIV*Δ*llpRI* (purple). Peak at RT 10.6 correlates to lysolipin derivatives (CBS 70+72) production and is highlighted in grey. Samples were obtained from cultures grown for seven days at 28°C.

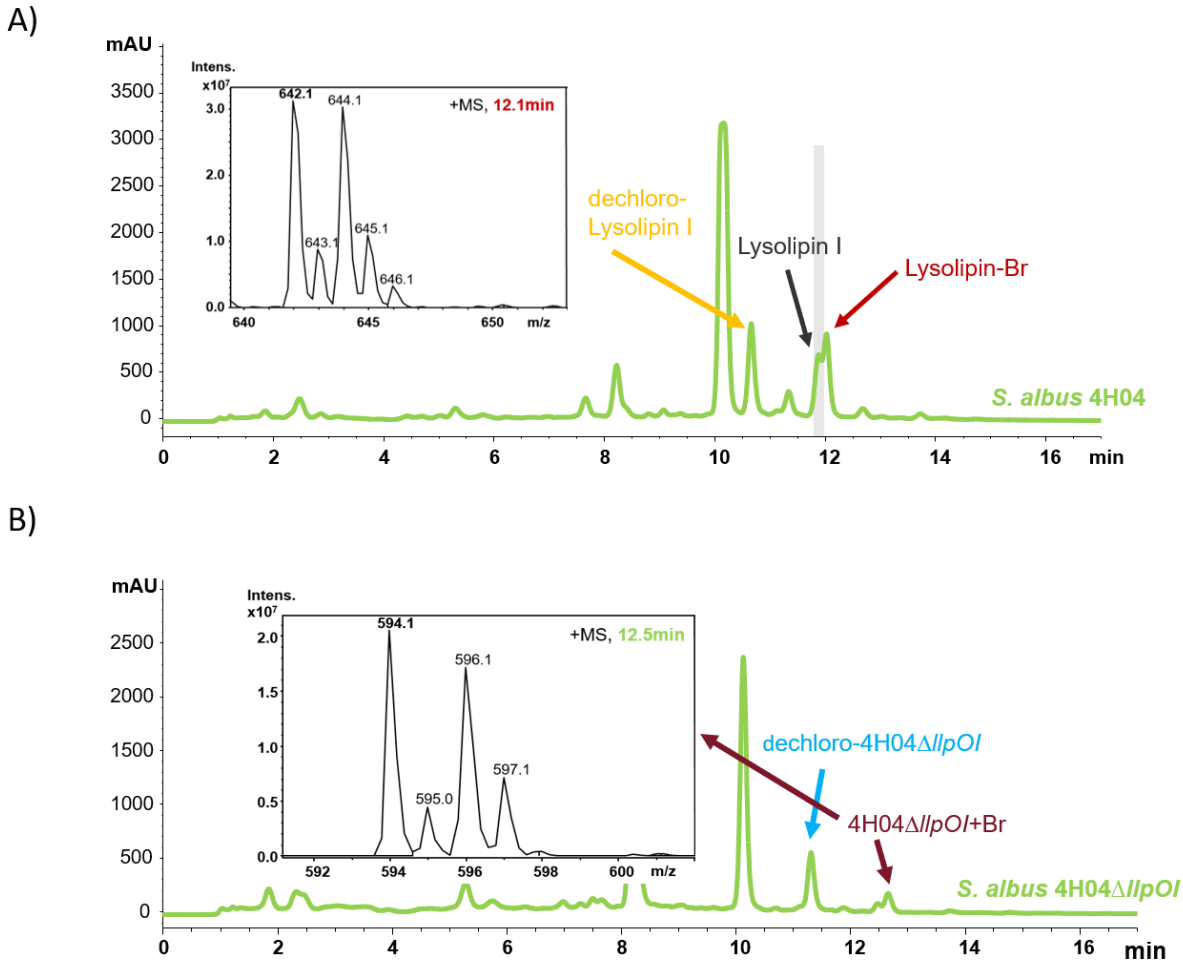

Figure S5: HPLC chromatograms of extract samples from A) *S. albus* 4H04 (green) and B) *S. albus* 4H04Δ*llpOI* (green). Peaks correlating to lysolipin I and lysolipin derivatives are highlighted by arrows. MS spectra are shown in black. Samples were obtained from cultures grown for seven days at 28°C.