## Supplementary Information for

## Towards biocontained cell factories: An Evolutionary Adapted Escherichia coli Strain Produces a New-to-nature Bioactive Lantibiotic Containing Thienopyrrole-Alanine

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**Supplementary figure S1: Genetic background for lichenicidin expression**. (a) Gene cluster coding for the biosynthesis of lichenicidin as isolated from *Bacillus licheniformis*. Genes required for expression of the single peptides Bli $\alpha$  and Bli $\beta$  in *E. coli*. (b) Genetic setup for high-level production of Bli $\beta$  in *E. coli* as published earlier.

leader peptide hexapeptide

M PA M M TA I M A G V M V A A S L A P M M K A Abu S R A

Supplementary figure S2: Amino acid sequence of the full length precursor peptide of Bliß.

Compound	Theoretical	nominal	Measured nominal mass	Mass error (ppm)
	mass			
Bliβ		3019.3601	3019.3617	0.5
Bli $\beta^{[3,2]Tpa}$		3025.3166	3025.3227	2.03

Supplementary table S1: Theoretical and measured nominal masses of Bliß and Bliß([3,2]Tpa<sup>9</sup>) detected with high-resolving ESI-Orbitrap-MS.



**Supplementary figure S4: Bliβ standard curve.** Liquid stocks of purified Bliβ peptide were prepared in 70% ACN. Concentrations applied for MS measurements were in the range 0.0001 mg mL<sup>-1</sup> to 0.5 mg mL<sup>-1</sup>. The concentration was plotted against the resulting peak area in MRM measurements as stated in the methods section. Line of best fit and coefficient of determination R<sup>2</sup> were calculated as indicated.