Rearranged Biosynthetic Gene Cluster and Synthesis of Hassallidin E in *Planktothrix serta* PCC 8927

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Supplementary methods

Details of the preparation of the extract for LC-MS. Methanol extract was analyzed first with low resolution LC-ESI-ITMS (Agilent 1100 Series LC/MSD Ion Trap XCT Plus, Agilent Technologies, Palo Alto, CA, USA). Five µL sample was injected to Luna C18 column (2.1 x 100 mm, 5 µm, Phenomenex, Torrance, CA, USA) which was eluted from 30 % to 70 % of acetonitrile in 0.1 % HCOOH in 49 min at 40°C with a flow rate of 0.15 mL.min^{-1}. Mass spectral data was accumulated in ultra scan positive electrospray ionization mode (26,000 *m/z* s^{-1}) at scan range of *m/z* 300 – 2200 and by averaging three spectra. High-resolution UPLC-QTOF analyses were performed with Acquity I-Class UPLC - Synapt G2-Si HDMS (Waters Corp., Milford, MA, USA) system. From 0.1 to 1 µL sample was injected to Cortecst UPLC® C18+ column (2.1 x 50 mm, 1.6 µm, Waters). The sample was eluted at 40°C with a flow rate of 0.3 mL.min^{-1}, from 10 % to 70 % acetonitrile in 0.1 % HCOOH in 5 min, then to 95 % in 0.01 min, kept there 1.99 min, then back to 10 % in 0.5 min and finally kept there 2.5 min before next run. QTOF was calibrated with sodium formate giving a calibrated mass range from *m/z* 91.036 - 472.726 to 1178.651-2121.195 depending of the run. Leucine Enkephalin was used at 10 s intervals as a lock mass reference compound. Mass spectral data was accumulated in positive electrospray ionization resolution mode at scan range from *m/z* 50-200 to *m/z* 1500-2200 depending of the run.

Supplementary figures

Figure S1. Chromatograms and mass spectra from methanol extract of *Planktothrix serta* PCC 8927 obtained by UPLC-QTOFMS. A: Total ion current chromatogram (TICC) and extracted ion *m/z* 1248.7 chromatogram (EIC) of the protonated aglycon fragment show the elution of hassallidin E. B: Mass spectrum at 4.07 min. C: Product ion spectra of protonated hassallidin E (*m/z* 1410.7) at 4.07 min. D: Product ion spectra of protonated aglycon (*m/z* 1248.7) at 4.07 min. Immonium ions of amino acids Dhb, threonine, methyl threonine, glutamine and tyrosine can be seen in the low mass part of the spectrum magnified in the insert. E: Mass spectrum at 4.1 min show minor amounts of the linear methyl ester of hassallidin E together with the native hassallidin E in aged sample. F: Product ion spectra (calibrated up to *m/z* 1178.7) of the Na^+ adduct of methylated linear hassallidin E shows the amino acid sequence and the localization of the hexose in the compound. The presence of this hexose unit in hassallidin E was shown by the readily lost 162 Da neutral typical fragment from the protonated molecule immediately in the ion source (MS) and secondly in the collision cell (MS^2) (this figure and figure S2). Also linear forms of Hassallidin are visible. The depsipeptides, which contain lactone bond closing the macrolicyclic ring, often form lianer peptide in methanol. Here in an aged hassallidin E methanol extract, an open chain of Hassallidin E methyl ester (m/z 1442

[M+H]⁺, *m/z* 1464 [M+Na]⁺) was found due to the methanolysis of the lactone bond. The *m/z* values of the different ions are detailed in Table 2. Hex = hexose residue (162 Da), Hexose (180 Da).

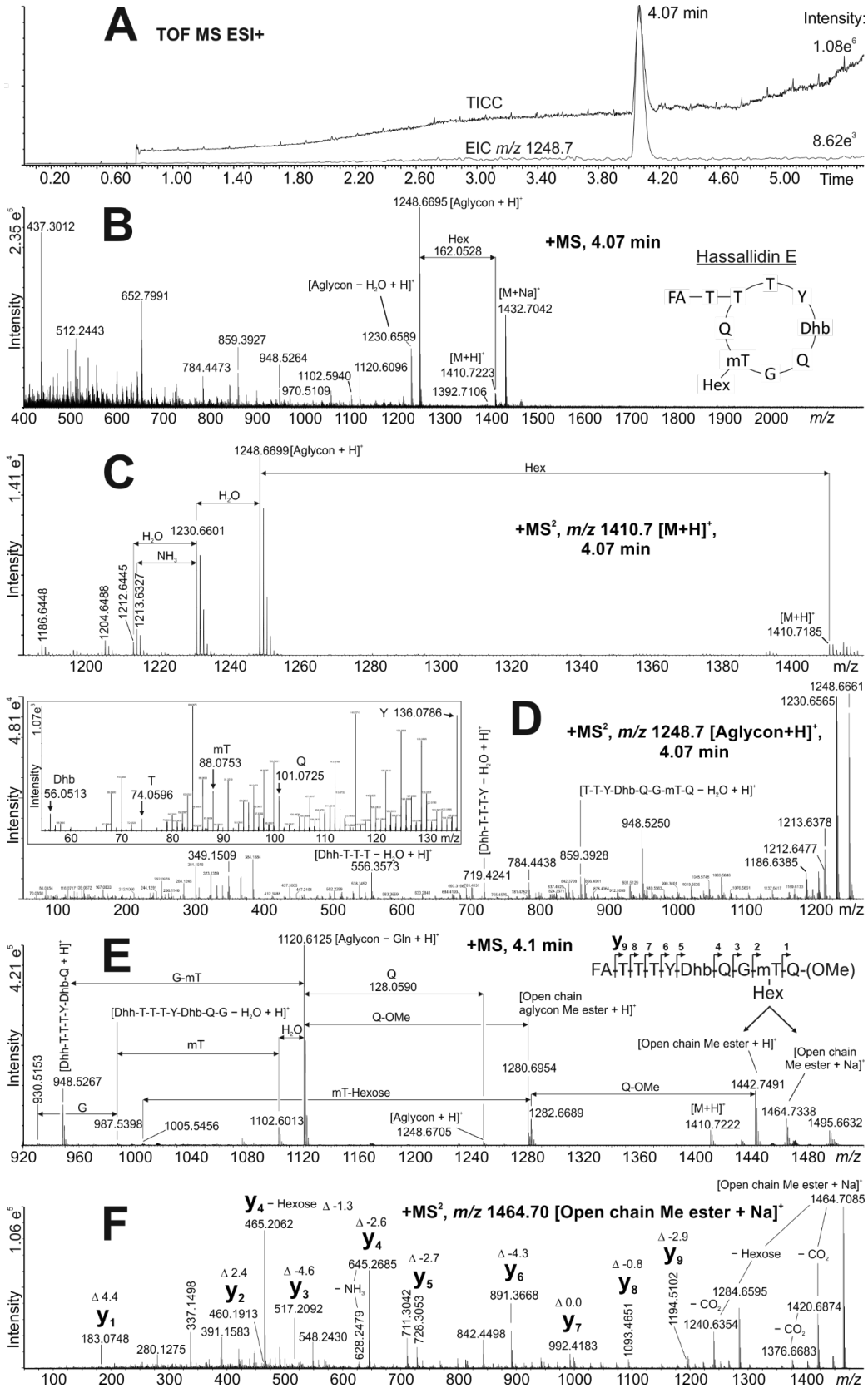

Figure S2. Chromatograms and product ion spectra from methanol extract of *Planktothrix serta* PCC 8927 obtained by LC-ITMS. In the upper panel total ion current chromatogram (TICC), UV (280 nm) and extracted ion *m/z* 1410 ([M+H]$^+$) chromatograms (EIC) and in the lower panel product ion spectrum of protonated hassallidin E and its aglycon. Hex = hexose residue (162 Da), FA = 2,3-dihydroxyhexadecanoic.

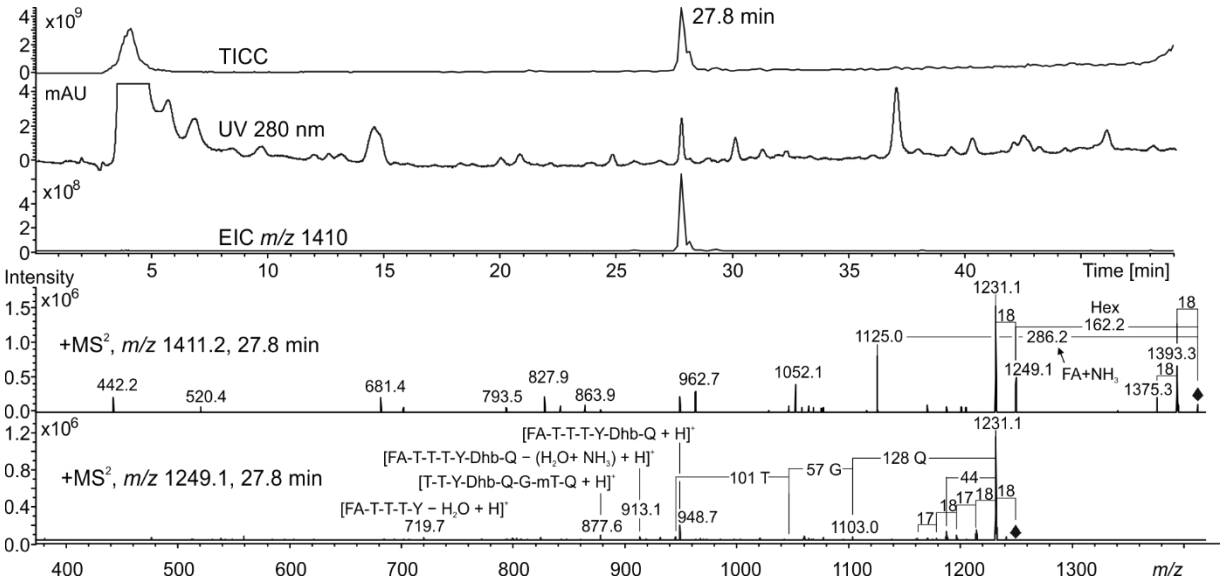

Figure S3. UV spectrum of hassallidin E obtained from the peak apex of 27.8 min in the chromatogram presented in Figure S2.

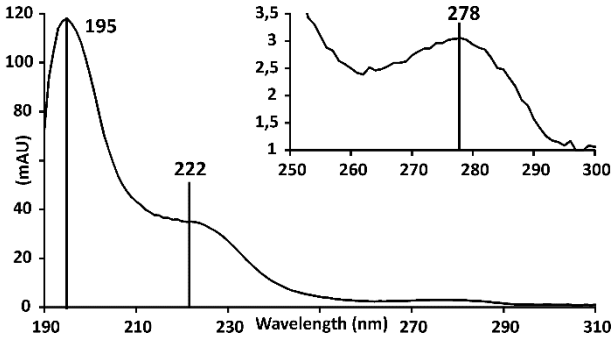

Figure S4. Effect of growth medium (BG11 and Z8) and temperature (18 and 25 °C) on the hassallidin E production per *Planktothrix serta* PCC 8927 biomass dry weight. Amounts of hassallidin E were compared based on the corresponding peak areas of the total ion current chromatogram (TICC).

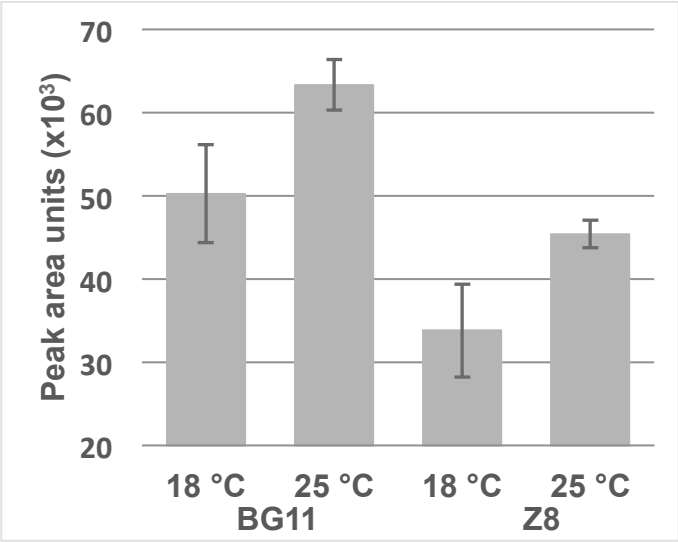

Figure S5. Disk diffusion assays. Compared antifungal activity against *Candida albicans* HAMBI 485 of *Planktothrix serta* PCC 8927 crude extract with crude extract of *Anabaena* sp. 258 (A) and with the antifungal compound Nystatin (B). The disk containing only extraction solvent (MeOH) serves as negative control.

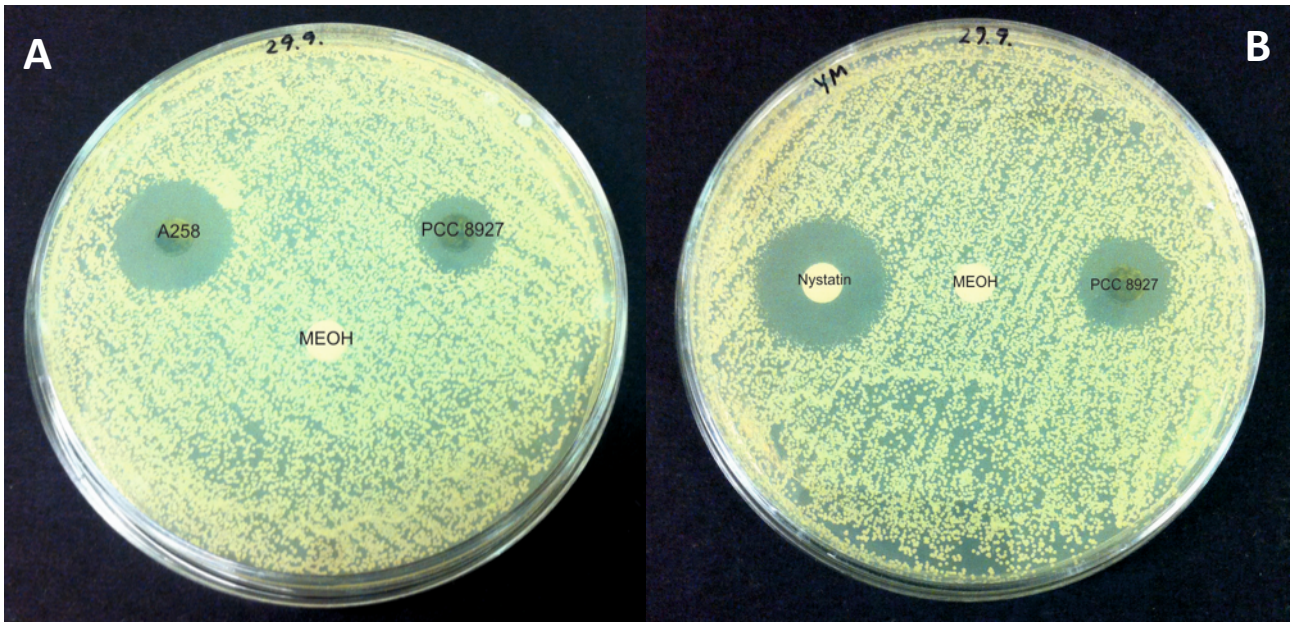

Figure S6. Mass spectrum of the purified hassallidin E used for antifungal assays. The spectrum was obtained with positive ionization. Analysis (deconvoluted spectrum) showed that the hassallidin is exclusively in the cyclic peptide form.