Responses of the microalga *Chlorophyta* sp. to bacterial quorum sensing molecules (N-acylhomoserine lactones): aromatic protein-induced self-aggregation

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SUPPLEMENTARY INFORMATION

11 Pages including cover page

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Transcriptomic analysis.

Cells were centrifuged on the second day of *Chlorophyta sp.* cultivation and immediately frozen in liquid nitrogen. The total RNA used for the complementary DNA (cDNA) library construction was extracted using an RNeasy MinElute Cleanup Kit (Qiagen Inc., Germany) according to the manufacturer's instructions. Contaminating DNA was eliminated with RNase-Free DNase I (Takara Inc., Japan). The rRNA was eliminated from the total DNase I-treated RNA using a Ribo-Zero™ Magnetic Kit (Epicentre Inc., USA). To build the library, 100 ng of rRNA-depleted RNA, prepared with a NEB Next ® UltraTM Directional RNA Library Prep Kit for Illumina, was used (NEB, USA). After the library was built, the quality of the library was verified by high-sensitivity DNA chip detection using 10 ng of the library, cluster generation was performed using the TruSeq PE Cluster Kit (Illumina, USA) and the cBot system, and sequencing was performed on an Illumina HiseqTM2500 system. The no-reference-genome method of transcriptome data analysis was used. For quality control of the raw data and subsequent filtering, clean reads were assembled with the Trinity software program and redundant sequences were eliminated, producing non-redundant consensus reference sequences for subsequent GO, COG, pathway analysis, etc.

Molecular weight measurement

The molecular weight (MW) distribution of the organic matter in the EPS samples was determined using high gel filtration chromatography (GFC, Shimadzu) consisting of an HPLC (Shimadzu, LC-10A) equipped with a differential refractive index detector (Shimadzu, RID-10A). Glucan was used as the standard which can be used for a molecular weight range of 200-1000000 Daltons (Da). The column temperature was controlled at 40°C. Deionized doubly distilled water was used as the mobile phase with a flow rate of 0.6 mL/min, and the sample retention was 30 min.

EPS was extracted from the microalgal biomass before EEM analysis, according to the procedure described by Frolund (Frølund et al. 1996). The extracted EPS samples were filtered with 0.22 μm acetate filter before analysis.

Frølund B, Palmgren R, Keiding K, Nielsen PH (1996) Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Research 30(8):1749-1758

Excitation emission matrix (EEM) spectra

EPS was extracted from the microalgal biomass before EEM analysis according to the procedure described by Frolund (Frolund et al. 1996). The extracted EPS samples were then passed through a 0.22-μm acetate-filter, after which they were diluted with 50 mM phosphate buffer (pH 7.0 ± 0.1) to a suitable level (10 times in this work) within the measurement range of the EEM equipment.

EEM spectra were measured with a Hitachi F-7000 fluorescence spectrometer, scanning emissions from 220 to 600 nm after excitation ranging from 220 to 450 nm with 10 nm increments. The fluorescence data were processed using Panorama Fluorescence 3.1 software.

Frølund B, Palmgren R, Keiding K, Nielsen PH (1996) Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Research 30(8):1749-1758

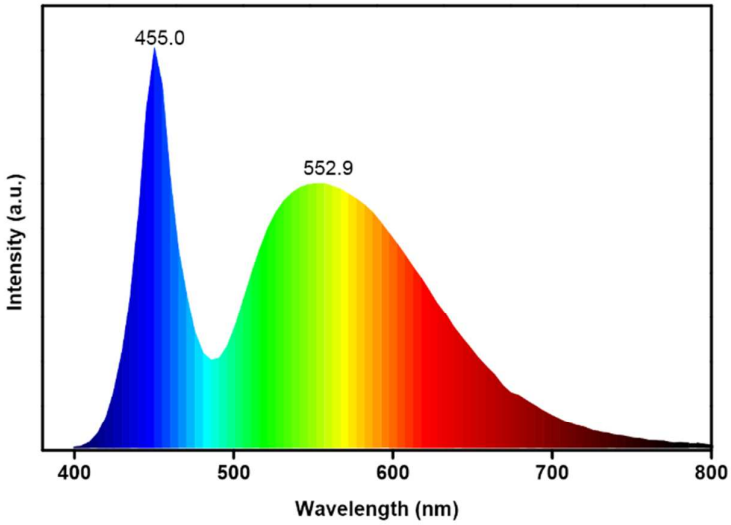

Figure S1. Light spectrum of the LED lamp.

Figure S2. Experimental procedures. (a) Extraction of signal molecules, AHLs, from activated sludge. (b) Microalga *Chlorophyta* sp. activation, heterotrophic cultivation and AHLs interaction.

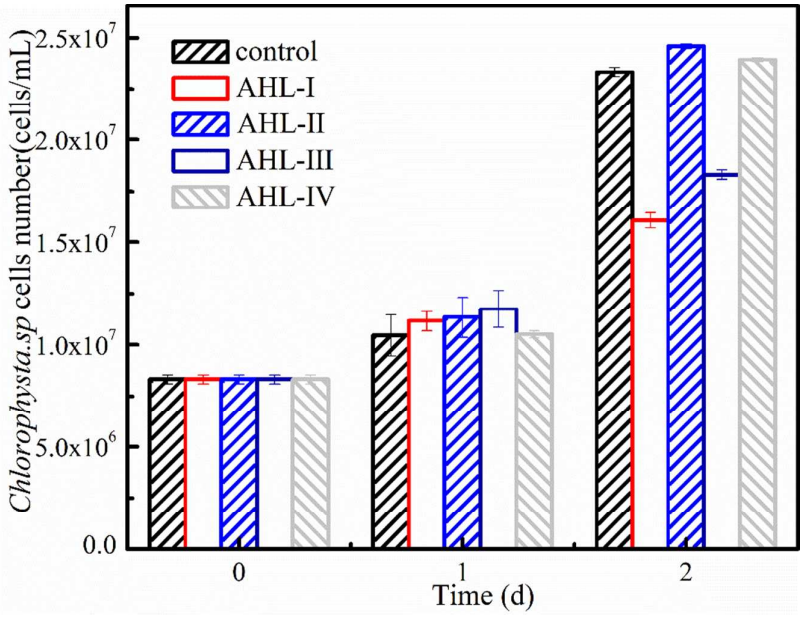

Figure S3. Growth of *Chlorophyta sp.* in response to AHLs.

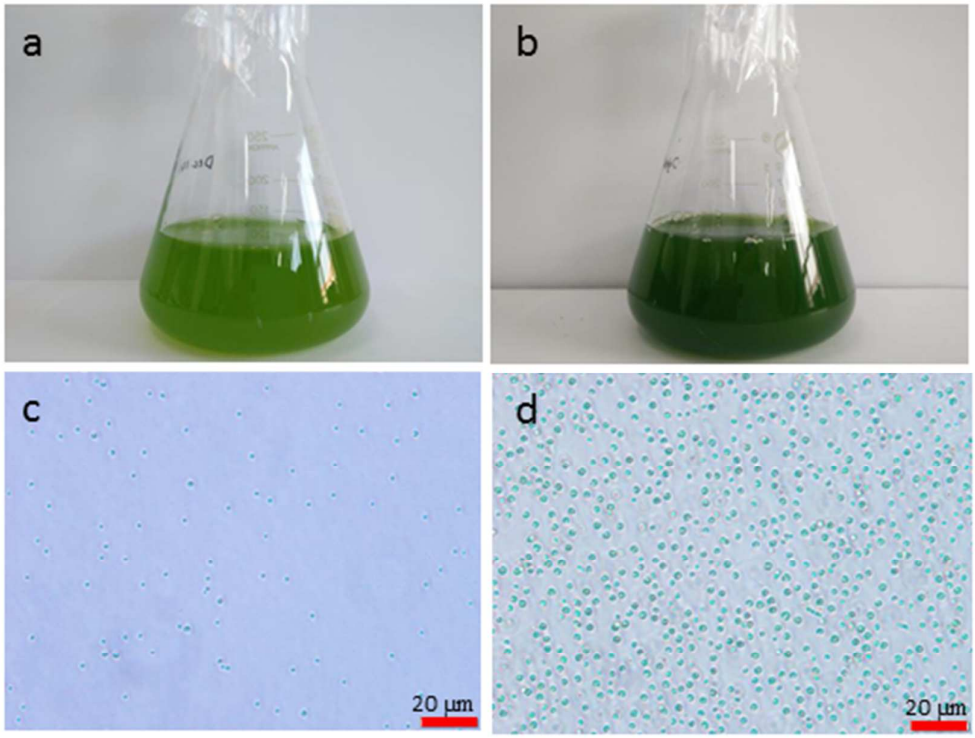

Figure S4. Typical macroscopic and microscopic observation of *Chlorophyta* sp. under heterotrophic conditions without AHLs supplementation. Day 1 (a, c), biomass=0.1589 g L^{-1}; Day 2 (b, d), biomass=0.5280 g L^{-1}.

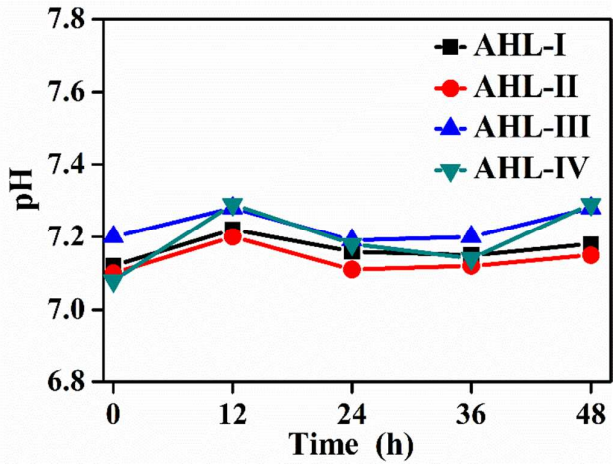

Figure S5. Change in pH of *Chlorophyta* sp. treated with AHLs.

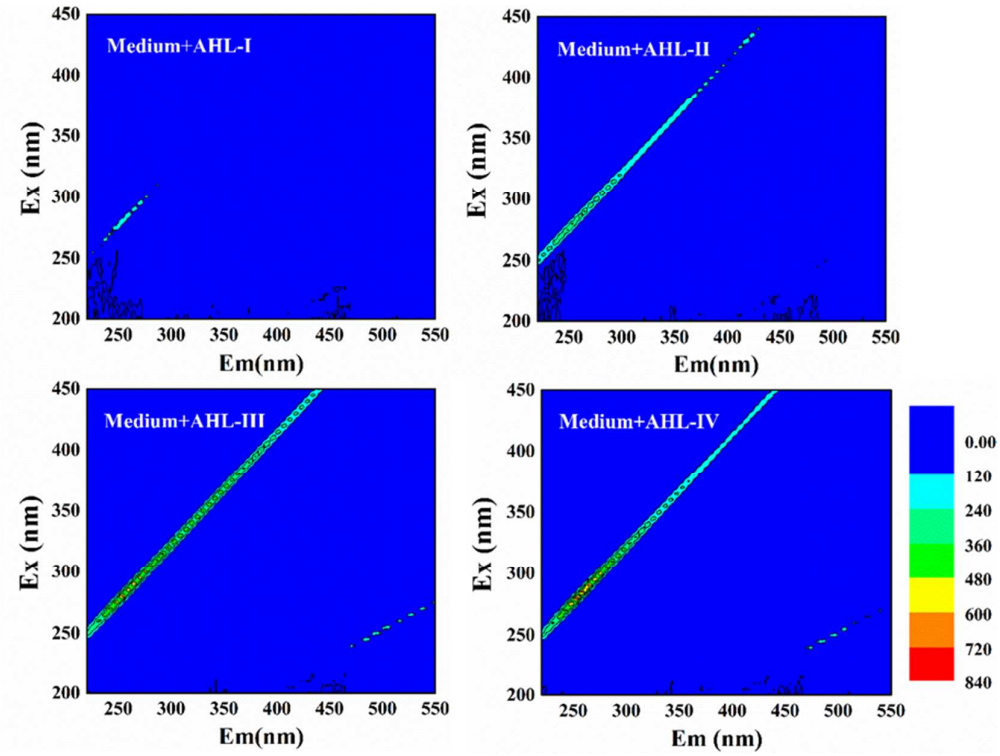

Figure S6. EEM spectrums of the synthetic medium plus the extracted AHLs (in absence of microalgae cultivation)