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Quorum sensing molecules in activated sludge could trigger microalgae lipid synthesis

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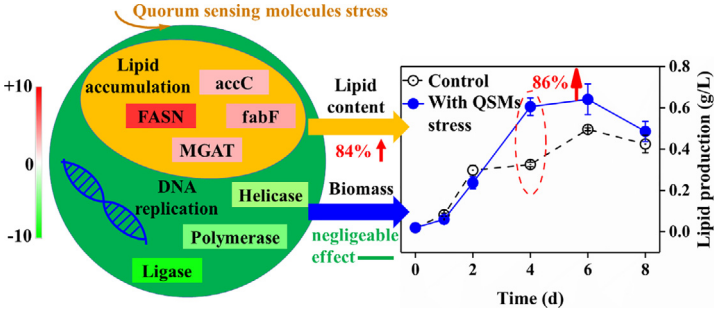
GRAPHICAL ABSTRACT



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ABSTRACT

Cultivating microalgae using wastewater is an economical strategy to produce biofuel; however, microbial contamination has to be controlled strictly. Microalgae lipid accumulation can be triggered by environmental pressures, and here, we studied whether microbial contamination is the pressure for microalgae. We hypothesized this pressure was forced via cell-to-cell communication with quorum sensing molecules (QSMs). In this work, we verified the impacts of QSMs produced by activated sludge (wastewater-born microbial consortiums) on both lipid content and biomass production of the microalgae *Chlorophyta* sp., since in combination, they determined lipid productivity. With QSMs stress, the lipid content of *Chlorophyta* sp. increased by ~84%, while biomass production decreased only slightly. Consistently, enzymes on the fatty acid synthesis pathways were generally up-regulated, while they were slightly down-regulated for DNA replication. In summary, the total lipid production improved by 86%. These results revealed the positive effects of microbial contamination on microalgae biofuel production.

1. Introduction

Microalgae are considered to be one of the most prospective biofuel feedstocks that can replace un-sustainable fossil fuels (Christenson and Sims, 2011; Colling-Klein et al., 2018). To reduce the considerable microalgae cultivation expense, coupling microalgae biofuel production with wastewater treatment has been suggested as a promising technology (Beuckels et al., 2015; Quiroz-Arita et al., 2015). An even more attractive strategy is to conduct heterotrophic cultivation in the coupling process, thus achieving a ~2 times higher growth rate of microalgal cells than under autotrophic cultivation (Kim et al., 2013). Recently, microalgae cultivation in various organic wastewaters has

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been investigated, e.g., municipal, agricultural, and industrial wastewaters (Chiu et al., 2015; Wang et al., 2015; Xiong et al., 2016).

To enhance microalgae biofuel production, microalgae biomass production is expected to be guaranteed. As a result, the axenic culture condition is typically controlled in laboratory studies, especially for heterotrophic cultivation, to avoid microbial contamination resulting in nutrient competition (Cho et al., 2015; He et al., 2013b; Zhou et al., 2012). However, microbial community evolution enriched with bacteria is always inevitable when using real wastewater (Fredrickson, 2015; He et al., 2013a; Higgins et al., 2016).

The interaction between biofuel-storing microalgae cells and wastewater-born microbes remains little known, however, it determines whether microbial contamination is always absolutely negative for microalgae lipid production. Here, we hypothesized that wastewater-born microbes, mainly composed by bacteria cells, could affect microalgae characteristics, according to several clues in the marine environment (Cho et al., 2017). It has been reported that bacterial cells could generate primary metabolites, e.g. CO_2 (Bai et al., 2015; Zhao et al., 2014), cofactors, and hormones, e.g. Vitamin B_{12} and indoleacetic acid (IAA) (Amin et al., 2015; Croft et al., 2005; Jusoh et al., 2015), to promote microalgae lipid productivity. Furthermore, bacterial cells were confirmed to communicate with marine algae (Tait et al., 2005), which affected algal cells' liberated spore settlement. Joint et al. indicated that bacteria and microalgae could interact via quorum sensing molecules (QSMs), which are cell-to-cell communication chemicals (Joint et al., 2002). Recently, our group reported that QSMs could stimulate microalgae self-aggregation by producing biomacromolecules (Zhou et al., 2017).

It is worth noting that this response of microalgae was a type of self-protective performance, which was driven by environmental pressure, i.e., bacterial competition. Interestingly, the microalgae lipid content could also be triggered under a number of environmental pressures, such as nitrogen starvation, phosphorus deficiency, and high salinity (Li et al., 2008, 2010; Takagi et al., 2006). However, it remains an open question whether microalgae lipid production could be triggered by QSMs pressure.

QSMs produced by wastewater born microbes may affect microalgae lipid synthesis by hypothesizing QSMs are a type of environmental stress for microalgae. However, little is known on this issue to date. The aim of this study was to verify the impact of wastewater-born microbes' QSMs on microalgae lipid production. Both microalgae biomass production and lipid content were of particular interest, as in combination, they determine biofuel productivity. We extracted QSMs from fresh activated sludge, since it is the most popular form of wastewater-born microbes (Feng et al., 2014). Co-culture of microalgae and bacterial cells was not employed to avoid bacterial competition caused substrates consumption and followed an un-objective evaluation on QSMs impacts (Chiu et al., 2015). To the best of our knowledge, this is the first study to report the effect of wastewater-born microbes on microalgae lipid production from the view of cell-to-cell communication.

2. Materials and methods

2.1. Microalgae species and cultivation

Chlorophyta sp. (FACHB-729) was selected as model microalgae, due to its wide distribution and abundant lipid production (He et al., 2013b). The strain was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology in Wuhan, China. The preservation and rejuvenation of *Chlorophyta* sp. was conducted according to our previous report (Zhou et al., 2017). To adapt the experimental environment, *Chlorophyta* sp. was cultivated under heterotrophic conditions for two generations prior to QSMs treatment. Heterotrophic cultivation (inoculated by 10% v/v) was conducted in BG11 medium (Zhou et al., 2017), supplemented with 5.0 g/L glucose, at 25 ± 1 °C with agitation at 150 rpm (ZWY-240, Zhicheng Shanghai, China).

2.2. Experimental protocols

Fresh activated sludge, with typical wastewater-born microbes consortiums, was obtained from the aeration tank of a sewage treatment plant in Changchun, China. Activated sludge QSMs were extracted in 2 h of sampling before the microbes decayed, via acidified ethyl acetate (0.1% acetic acid) using a rotary evaporator (OSB-2100, EYELA, Japan) (Feng et al., 2014; Ravn et al., 2001). Each liter of bacterial suspension was sonicated at 4 °C for 5 min, and then centrifuged at 10,000 rpm (17 700*g*) for 10 min. After that, the supernatant was mixed with an equal volume of acidified ethyl acetate (0.1% acetic acid). Then, the mixture was mixed thoroughly for 1.5 h and subsequently settled for 20 min. The upper layer was separated and dried in a rotary evaporator (OSB-2100, EYELA, Japan) at 35 °C. The extraction process was repeated three times using the same procedure.

To verify the effects of the QSMs amount on microalgal lipid production, QSMs were extracted from different amounts of activated sludge, 0 g, 0.5 g, 1.0 g, and 2.5 g in dry weight, respectively. QSMs were extracted according to above procedures from each sludge samples, so that the dry residuals which contained QSMs at four different amounts were obtained. They were dissolved in 5 mL methanol respectively, and the solutions were added to 150 mL *Chlorophyta* sp. culture at a dosage of 0.5% (v/v). At last, *Chlorophyta* sp. cultivation with different QSMs dosages were denoted as control, Q(+), Q(++), and Q(+++), respectively. Moreover, the typical QSMs in activated sludge, C_6-HSL (N-hexanoyl-L-Homoserine lactone, sigma), was employed to investigate the impacts of pure QSMs on *Chlorophyta* sp.. All protocols were conducted in triplicate.

2.3. Biomass, growth, and lipid accumulation

Microalgae biomass was determined via dry weight (Fu et al., 2017). The maximum specific growth rate (μ_{max}) was evaluated according to the modified method of Hanes-Woolfe (Dowd and Riggs, 1965; Herndon and Cochlan, 2007). The utilization of glucose was measured using anthrone-sulfuric method (Dubois et al., 1956).

The lipid content was evaluated via the gravity ratio (%) of lipid and biomass (Yoo et al., 2010), in which the lipids were extracted via the modified chloroform–methanol (2:1, v/v) method (Bligh and Dyer, 1959). The chloroform layer contained the lipids in the bottom and the methanolic layer contained the non-lipids components. To obtain the isolated chloroform layer, we controlled the final ration of methanol/chloroform /water was 1/1/0.9. Lipid production and lipid productivity were calculated via Eqs. (1) and (2), respectively.

$$\text{Lipid production (g/L)} = \text{Biomass (g/L)} \times \text{Lipid content (\%)} \quad (1)$$

$$\text{Lipid productivity (g/L·d)} = \frac{[\text{Biomass (g/L)} \times \text{Lipid content (\%)}]_t - [\text{Biomass (g/L)} \times \text{Lipid content (\%)}]_{initial}}{\text{cultivation time (d)}} \quad (2)$$

2.4. Transcriptomic analysis

Cells were centrifuged on the second day of *Chlorophyta* sp. cultivation, for control, Q(+), Q(++) and Q(+++), respectively, 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