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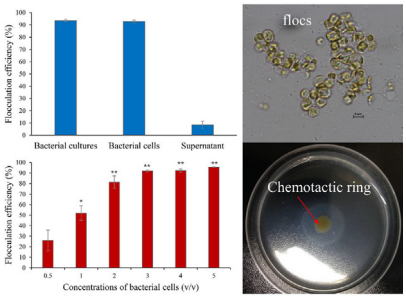
Effective harvesting of the marine microalga *Thalassiosira pseudonana* by *Marinobacter* sp. FL06

Xueqian Lei, Wei Zheng, Hongyan Ding, Xiaoying Zhu, Qiuliang Chen, Hong Xu, Tianling Zheng, Yun Tian*

State Key Laboratory of Marine Environmental Science and Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, School of Life Sciences, Xiamen University, Xiamen, China

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, *Marinobacter* sp. FL06 was used to effectively harvest the energy-producing microalga *Thalassiosira pseudonana* through direct flocculation. Strain FL06 showed 92.7% flocculating efficiency against *T. pseudonana*, and no metal ion was added for the flocculation process, resulting in a more environmentally friendly process. The flocculation efficiency of FL06 was stable over a wide range of pH values and temperatures, indicating that the application of this bacteria has potential advantages under various conditions. Strain FL06 also exhibited flocculation activity against different microalgae, indicating that the strain can be used to harvest multiple types of microalgae. Strain FL06 showed high chemotactic ability toward algal cells, suggesting that chemotaxis is important for flocculation. This study provides the first demonstration that the *Marinobacter* genus could be used to harvest *T. pseudonana* biomass. In summary, the results showed that FL06 has the potential for effective harvesting of microalgal biomass.

1. Introduction

The use of microalgae for the production of renewable fuels has been extensively studied, and microalgae have been identified as competent feedstocks for the extraction of valuable products, including triacylglycerol, pigments and polyunsaturated fatty acids (Ummalyma et al., 2017; Wan et al., 2015). The use of microalgae for biofuel production has attracted increasing attention, but the currently available technologies for harvesting microalgal biomass are not well developed (Milledge and Heaven, 2013). Various technologies, including centrifugation, flocculation, filtration and screening, flotation, electrophoretic techniques and gravity sedimentation, have been used to

* Corresponding author at: School of Life Sciences, Xiamen University, Xiamen 361102, China.
E-mail address: tianyun@xmu.edu.cn (Y. Tian).

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harvest microalgal cells (Abinandan et al., 2018; Barros et al., 2015; Pragya et al., 2013). Although the use of centrifugation for microalgae harvesting yields high-value products, the use of this technique for harvest microalgae to obtain low-value products is far more expensive and energy-intensive due to the high investment and operating costs required (Uduman et al., 2010). From an engineering standpoint, the productivity of filtration for the separation of algal cells from bulk culture is too low, and the membranes need to be cleaned regularly (Wan et al., 2015). Electrophoresis-based techniques offer numerous advantages, but the equipment costs and energy requirements remain too high for sustainable large-scale applications (Barros et al., 2015).

Flocculation is commonly used in wastewater treatment, mining, and drinking water production and has attracted attention for microalgae harvesting (Kim et al., 2011). The harvesting of microalgae by flocculation primarily occurs via physical flocculation, chemical flocculation and bioflocculation (Vandamme et al., 2013). In general, physical flocculation can be induced by electro-flocculation, ultrasound, and magnetic separation (Bosma et al., 2003; Ho et al., 2017; Shi et al., 2017). Chemical flocculation can be performed with inorganic polymers, organic polymers and inorganic flocculants (Gerchman et al., 2017; Kim et al., 2017; Ummalyma et al., 2016). Physical flocculation is cleaner process, but this process requires special electrical devices, and is difficult to scale up. Chemical flocculation is highly efficient but might cause secondary pollution in the environment. Compared with these methods, bioflocculation using microbes and bioflocculants shows promise for microalgae harvesting because it is safe and environmentally friendly (Lam and Lee, 2012; Wan et al., 2015).

Bioflocculation has been successfully applied for wastewater treatment and the use of this technique use for microalgae harvesting has attracted attention (Kim et al., 2011). The published studies on bioflocculation have focused on three types: microorganism-associated bioflocculation (Gärdes et al., 2011; Powell and Hill, 2013; Wang et al., 2012), microalgal cell self-flocculation (Alam et al., 2014; Salim et al., 2014) and microbial bioflocculant-associated bioflocculation. The bioflocculants produced by *Cobetia marina* L03 (Lei et al., 2015), *Solibacillus silvestris* W01 (Wan et al., 2013) and *Bacillus licheniformis* CGMCC 2876 (Ndikubwimana et al., 2014) have been used to harvest various types of microalgae. Most of the identified flocculating bacteria show flocculation activity against algal cells due to the excretion of extracellular bioflocculants, which indicate that these bacteria exhibit indirect flocculation activity. Few studies have focused on the direct flocculation activity of flocculating bacteria against algal cells. Algal cells are difficult to separate from massive quantities of culture medium because of their small size and their colloidal stability in suspension (Vandamme et al., 2013). In addition, it is more difficult to harvest marine microalgal biomass than freshwater microalgal biomass due to the high ionic strength of seawater (Wan et al., 2013). Microalgal species of Bacillariophyta, particularly the model diatom *Thalassiosira pseudonana*, have been extensively studied for biofuel production and metabolism (Armbrust et al., 2004; Levitan et al., 2014; Zendejas et al., 2012). Therefore, the potential use of flocculating bacteria to harvest biomass of the marine microalga *T. pseudonana* through direct flocculation should be investigated.

In this study, the flocculating bacterium FL06 was isolated and identified, which was found to exhibit flocculation activity against *T. pseudonana* biomass. The flocculation efficiency and flocculation mechanism of strain FL06 against *T. pseudonana* were investigated. The effects of different algal pH values and temperatures on the flocculation efficiency of strain FL06 were evaluated. The flocculation activity of strain FL06 against different candidate biofuel-producing algae was confirmed. This study provides the first demonstration of the use of a strain of the *Marinobacter* genus for harvesting *T. pseudonana* biomass. The purpose of this study was to explore the flocculation process and mechanism of strain FL06 against microalgal biomass.

2. Materials and methods

2.1. Algal culture and bacterial culture

The alga *Thalassiosira pseudonana* CCMP1335 was purchased from the National Center for Marine Algae and Microbiota. The diatom was cultivated in f/2 medium prepared with seawater (Guillard, 1975) at 20 ± 1 °C under a 12-h light:12-h dark cycle with a light intensity of 50 μmol photons $m^{-2} s^{-1}$. The algae *Nitzschia closterium* f. minutissima, *Chaetoceros muelleri*, *Phaeodactylum tricornutum*, *Thalassiosira weissflogii*, *Chlorella* sp. and *Dunaliella salina* were supplied by the Center for Collections of Marine Bacteria and Phytoplankton of the State Key Laboratory of Marine Environmental Science (Xiamen University) and grown in f/2 medium as described above.

Marinobacter sp. FL06 was isolated from a mangrove sediment sample collected from the Yunxiao Mangrove National Nature Reserve, Fujian Province, China. Ten-fold serial dilutions of the samples were prepared using sterile seawater, and 0.1-mL aliquots of each dilution were spread on 2216 E agar and incubated for 5 days at 28 °C. Individual colonies with distinct morphologies were purified three times and inoculated in 2216E broth to determine their flocculation activity, and strain FL06, which showed high flocculation efficiency against *T. pseudonana*, was isolated.

2.2. Identification of the flocculating bacterium

The genomic DNA of FL06 was extracted, and its 16S rRNA gene sequence was amplified by PCR using primers 27F and 1492R (DeLong, 1992). The purified PCR product was produced using a TIANquick Midi Purification Kit. Sequences of closely related taxa were obtained from the EzTaxon-e and GenBank databases, and a phylogenetic analysis was performed using the program mega 5 (Tamura et al., 2011). Evolutionary distance analyses and clustering were performed using the neighbor-joining method (Saitou and Nei, 1987). Bootstrap values were evaluated based on 1000 replications.

2.3. Analysis of flocculation mode and activity

Strain FL06 was inoculated in 2216E broth and incubated on a shaker at 150 rpm and 28 °C for 24 h. The cells were collected by centrifugation at 6000 × *g* for 10 min, and the supernatant was filtered by 0.22-μm Millipore membrane. The remaining pellets were washed twice with f/2 medium, and the cells were then resuspended in the same volume of f/2 medium. The washed bacterial cells, cell-free supernatant and bacterial culture obtained were added to the algal cultures to obtain a final concentration of 3% (v/v) in triplicate. The control bacterial culture and supernatant samples consisted of algae that were grown normally with an added equal volume of sterile 2216E broth to avoid any influence from the medium. The control samples of washed bacterial cells were algae that were grown normally with an equal volume of f/2 medium. After 3 h of algal cell flocculation, an aliquot of the culture was pipetted from a height of two-thirds from the bottom to evaluate the flocculation effect, and measurements were obtained using a spectrometer at a wavelength of 680 nm. The flocculation efficiency was calculated according to the equation (Li et al., 2017):

$$\text{Flocculation efficiency } (\%) = (A-B)/A \times 100 \quad (1)$$

where A is the absorbency (OD) at 680 nm of the control culture and B is the OD_{680} of the culture after flocculation.

The effects of different concentrations on flocculation efficiency were investigated further. Washed bacterial cells were centrifuged at 6000 × *g* for 10 min, washed twice with sterile f/2 medium and resuspended to concentrations of 0.5, 1, 2, 3, 4 and 5% in sterile f/2 medium. The control was algae grown normally with the addition of an equal volume of f/2 medium.