Biochemical Engineering Journal 101 (2015) 185–190

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

# Enhancing the oil extraction efficiency of *Chlorella vulgaris* with cell-disruptive pretreatment using active extracellular substances from *Bacillus thuringiensis* ITRI-G1

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ARTICLE INFO

*Article history:*
Received 5 March 2015
Received in revised form 29 April 2015
Accepted 31 May 2015
Available online 3 June 2015

*Keywords:*
Microalgae
Cell disruption
Liquid–liquid extraction
Downstream processing
Biological pretreatment

ABSTRACT

An isolated bacterium, identified as *Bacillus thuringiensis* ITRI-G1, was able to produce active extracellular substances (denoted as AES-Bt) that exhibited efficient cell disruptive ability for a lipid-rich microalga *Chlorella vulgaris* CNW-11. Pretreatment of the microalga with AES-Bt for 24 h led to an increase in the extracted oil content from 34.2% to 44.3% (dry weight basis), representing an enhanced oil extraction efficiency ($\eta_{oil}$) of 29.3%. The $\eta_{oil}$ value remained within the range of 26.3–33.2% when increasing the algae biomass loading from 5.0 g/l to 40.0 g/l (or decreasing AES-to-algae ratio from 200 to 25 ml/g), while the maximum $\eta_{oil}$ value was obtained when an AES-to-algae ratio of 50 ml/g was used. Finally, reuse of AES-Bt agent was also examined. The results show that the enhanced lipid extraction efficiency slightly decreased as the number of recycling times increased. However, after being recycled and reused for four times, the AES-Bt agents could still effectively disrupt the cell wall, leading to around 92% of the original oil extraction efficiency that was obtained when the AES-Bt agents were first used. This study demonstrates that biological cell-disruptive pretreatment using the AES-Bt agents could be an effective and environmentally friendly means of increasing oil extraction efficiency with microalgae.



## 1. Introduction

Biofuels are considered the most promising candidates for renewable energy, and are expected to play a crucial role in the global energy infrastructure in the future [1–4]. Biodiesel, one of the most commonly used biofuels, is recognized as an ideal recyclable energy carrier, and is thus also used as a possible primary energy source [5–7]. Commercial biodiesel is currently produced from animal fat, waste frying oil and vegetable oils [8]. However, using such feedstock for biodiesel production has raised the issues of competition with the food supply and with the use of arable land [9]. Therefore, microalgae, who can grow much more rapidly than terrestrial plants, and convert solar energy to chemical energy via $CO_2$ fixation more efficiently, are now being considered as a promising oil feedstock for making biodiesel [10].

The key technologies for the production of microalgae-based biodiesel include isolation of oil-rich microalgae, development of effective microalgae cultivation technology, harvesting of algal biomass, and extraction of oil from algal cells. The cell harvesting and oil extraction steps in particular account for most of energy consumption and production cost of microalgal biodiesel [11–13]. It is known that cell disruption is a key step to promote oil extraction from microalgae (such as *Chlorella* species), which usually have rigid cell walls that restrict the penetration of solvents. Conventional cell disruption methods for microalgae are mostly physical and chemical, such as sonication, milling, microwave-assisted extraction, homogenization, bead-beater treatment, and organic solvents treatment [14–18]. However, these may require high energy consumption or cause problems with waste treatment

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http://dx.doi.org/10.1016/j.bej.2015.05.020
1369-703X/

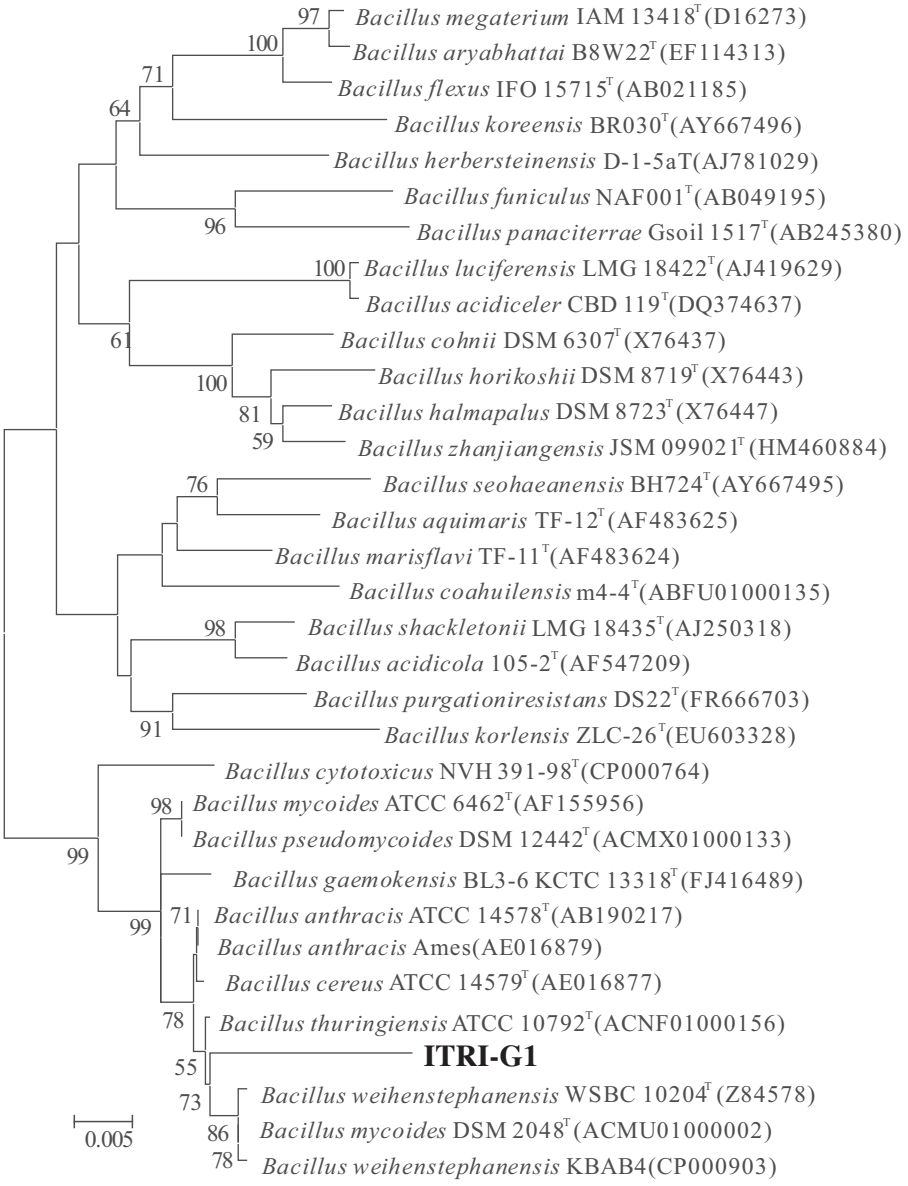


**Fig. 1.** Neighbor-joining showing phylogenetic positions of strain ITRI-G1 and related taxa based on 16S rDNA gene sequence comparisons. Bootstrap values are indicated at nodes. Only bootstrap values >50% are shown. Scale bar, 1% sequence dissimilarity (one substitution per 100 nt). Representative sequences in the dendrogram were obtained from GenBank (accession number in parentheses).

due to the use of toxic chemicals. Developing cost-effective and environmentally benign techniques for microalgae disruption to enhance microalgae oil/lipid extraction efficiency is, thus, critical step toward the success of commercial biodiesel production from microalgae.

It has been reported that algicidal bacteria, as an important fraction of biological species in hydrophytic ecosystems, play important roles in controlling algae blooms and limiting algal biomass production [19–22]. In our recent study, a cell-disruptive bacterial strain was also shown very effective in breaking the cell wall of *Chlorella* sp. [23]. Therefore, this study developed an innovative biological cell-disruption method using the active extracellular substances of an isolated strain *Bacillus thuringiensis* ITRI-G1 as cell-disruptive agents (denoted as AES-Bt agents) to promote oil extraction from a lipid-rich microalga *Chlorella vulgaris* CNW-11, which was used as feedstock for biodiesel production. The key operating factor of the AES-Bt pretreatment, namely the ratio of AES-Bt dose versus microalgal biomass loading (denoted as AES-to-algae ratio), was explored to identify the optimal ratio leading to the maximum enhancement of oil/lipid extraction efficiency. The reusability of the AES-Bt agents was also assessed by conducting repeated microalgal oil extraction with four consecutive recycles of the cell-disruptive agents. The aim of this work was to develop a cost-effective and environmentally benign method for cell disruption to facilitate oil extraction from microalgae.

## 2. Materials and methods

### 2.1. Microalgal strain and culture medium

The microalgal strain used in this work was isolated from freshwater in southern Taiwan and identified as *C. vulgaris* CNW-11. The microalga has a high oil content of over 40% per dry cell weight and was used as feedstock for biodiesel production. The medium used to cultivate the pure culture of *C. vulgaris* CNW-11 consisted of (g/l): $KNO_3$, 1.25; $KH_2PO_4$, 1.25; $MgSO_4\ 7H_2O$, 1; $CaCl_2$, 0.0835;