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Increasing the extraction efficiency of algal lipid for biodiesel production: Novel application of algal viruses

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Various studies have been conducted recently using microalgal system for the production of algal lipid for biodiesel production. This study aimed at increasing the extraction efficiency of algal lipid from *Chlorella* sp. by the application of *Chlorella* viruses. The calorific value of lipid from *Chlorella* sp. has been reported to be higher than that of fresh water microalgae, making it a potential candidate to be used as biofuel. This is the first report on novel application of microalgal viruses for improving the extraction efficiency of algal lipid for biodiesel production through viral lysis of *Chlorella vulgaris*. The algal lipid extraction efficiency, increased by 11.68% in the case of *Chlorella* virus treated *C. vulgaris* when compared with *Chlorella* virus untreated *C. vulgaris*. The application of *Chlorella* virus and the viral encoded lytic enzymes for increasing the extraction efficiency of algal lipid will be helpful in optimizing algal biofuel industry in the future.

Key words: Micro-algal system, biodiesel, algal lipid, *Chlorella* viruses, viral lysis.

INTRODUCTION

Microalgae are a promising candidate for biodiesel production. Microalgae have comparatively high oil content, biomass productivity and are more sustainable and environmentally friendly than petroleum-derived fuels (Hu et al., 2008). Some microalgae are known to produce high amounts of lipids and can be applied in bioprocess to produce alternative oils for biodiesel manufacture (Ratledge, 2005). Nevertheless, the microalgal biodiesel has not been widely commercialized mainly due to its high costs. The microalgal biodiesel production can be optimized for cost effectiveness and increasing the algal lipid extraction efficiency (Li et al., 2008; Pittman et al., 2011).

Chlorella viruses have large icosahedral capsids with an internal membrane enclosing their ds DNA genome that infect certain strains of the unicellular green alga *Chlorella* (chlorovirus or *Chlorella* virus; Phycodnaviridae) which are ubiquitous in natural environments (Yamada et al., 1991; Zhang et al., 1988; Wulfmeyer et al., 2012). In a normal lytic cycle, virus particles attach to the surface of host *Chlorella* cells and degrade the cell wall at the point of attachment; the viral core is then released into

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the host cytoplasm, leaving an empty capsid on the cell wall. Within 6 to 8 h, post infection, mature viral progenies exit the cells after cell lysis. Both the initial and final stages of the viral replication cycle obviously require cell wall-degrading activities, but little is known about the nature and origin of the enzymes that degrade the cell wall (Van Etten et al., 1991).

A common characteristic of virus-sensitive *Chlorella* strains are a rigid cell wall containing uronic acids and glucosamine in addition to other polysaccharides such as glucose, rhamnose, galactose, xylose, arabinose, mannose and glycoprotein matrix providing the cells with a formidable defense against its environment (Kapaun et al., 1992; Kapaun and Reisser, 1995; Gerken et al., 2013). Based on different microalgae and culture conditions such as temperature, nutrient and light intensity; microalgal lipid content and composition are varied (Converti et al., 2009; Solovchenco et al., 2008). The oil content of some microalgae such as *Scenedesmus* sp., *Chlorella* sp., *Neochloris oleoabundans* can be from 20 to 50% of total cell dry weight (Gouveia and Oliveira, 2009), revealing the significant potential of biodiesel production. Fatty acid methyl esters (FAME) originating from vegetable oils and animal fats are known as biodiesel, which have also been characterized in microalgae and are non-toxic. The exploitation of microalgae for commercial biodiesel production is not yet a commercial reality and still warrants further research and development (Benemann, 2008).

Algae for biodiesel have been studied for many years for production of hydrogen, methane, vegetable oils (triglycerides, for biodiesel), hydrocarbons and ethanol. The high calorific value lipids generated by the microalgae and cyanobacteria are used for biodiesel (Illman et al., 2000). A mechanism that could concept-tually scale-up the yield of biodiesel precursors has not yet been demonstrated effectively (Benemann, 2008). Beside the selection of candidate microalgal strains for high production of biodiesel precursors, the present research challenge is also on increasing the extraction efficiency of biodiesel precursors (Sierra et al., 2008) from algae *Chlorella vulgaris* by using a novel approach like *Chlorella* virus mediated lysis.

MATERIALS AND METHODS

Isolation and identification of microalgae

Recently, a report demonstrated that the calorific value of *C. vulgaris* was found to be 28 kJ/g, which was highest among the freshwater microalgae making it a promising candidate to be exploited for alternate fuel (Scragg et al., 2002). Fifteen (15) algal strains were isolated from different aquatic ecosystems in and around Nagpur; out of which one was identified as *C. vulgaris*. The microalgae was identified by light microscopy analysis (Leica DM2500 Wien, Austria) and was used for further experimental studies.

Chlorella vulgaris genome sequencing and sequence analysis

The microalgal species was confirmed by partial sequence of 18S rRNA of microalgae using outsourcing the sequencing services to Macrogen Inc. Seoul, South Korea. The sequencing was performed using the primers and conditions as prescribed by Hoshina and Fujiwara (2012). The partial nucleotide sequence was subjected to Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990).

Propogation of *C. vulgaris* microalgae

The isolated *C. vulgaris* microalgae was propagated in nitrate depleted Bold Basal Media (Rippka, 1988) at 28 ± 2°C and light intensity of 350 µmol m^{-2} s^{-1} for a week using a light meter and subsequently sub-cultured (Scragg et al., 2002). The sub-cultured microalgae strain of isolated *C. vulgaris* in exponential phase was subjected to further study as depicted in the workflow (Figure 1).

Isolation of *C. vulgaris* specific virus by plaque assay method

The surface water sample collected from Ambazari Lake, Nagpur, Maharashtra, India was used for isolation of *C. vulgaris* specific virus. The water sample was filtered through 0.2 µm polycarbonate membranes (Millipore, India.); 100 µl of the filtrate was inoculated into 900-µl exponentially growing culture of isolated *C. vulgaris*. The cultures were incubated for a week. The virus was isolated from the lysed culture using the plaque assay procedure using Bold Basal Media with 1% agar (Figure 2). In brief, a 100 µl lysed culture was mixed with 200 µl of the host cells at a concentration of 1×10^8 to 2×10^8 cells per ml. The mixture was poured with 5.5 ml of 1% Bold Basal Media and incubated in the light at 28 ± 2°C (Van Etten, 1982).

Purification and enrichment of *C. vulgaris* specific virus

The single plaque was selected for enrichment in exponentially growing *C. vulgaris* culture in Bold Basal Media and was incubated at 28 ± 2°C and light intensity of 350 µmol m^{-2} s^{-1} for one week (Rippka, 1988).

Extraction of lipid from micro-algal biomass

Extraction and estimation of lipid content from micro-algal biomass with and without algal virus treatment was carried out by following the standard protocol of Bligh and Dyer (1959). Each experiment was done in triplicate. The mean and standard deviation of the experimental results was calculated using MS-Excel.

RESULTS AND DISCUSSION

Chlorella vulgaris genome sequencing and sequence analysis

The microalgae partial sequence accession was subjected to NCBI-BLAST that showed 100 and 99% identity with other *C. vulgaris* nucleotide sequences submitted to genbank with accession numbers AB699112.1 and JX185298.1 respectively.

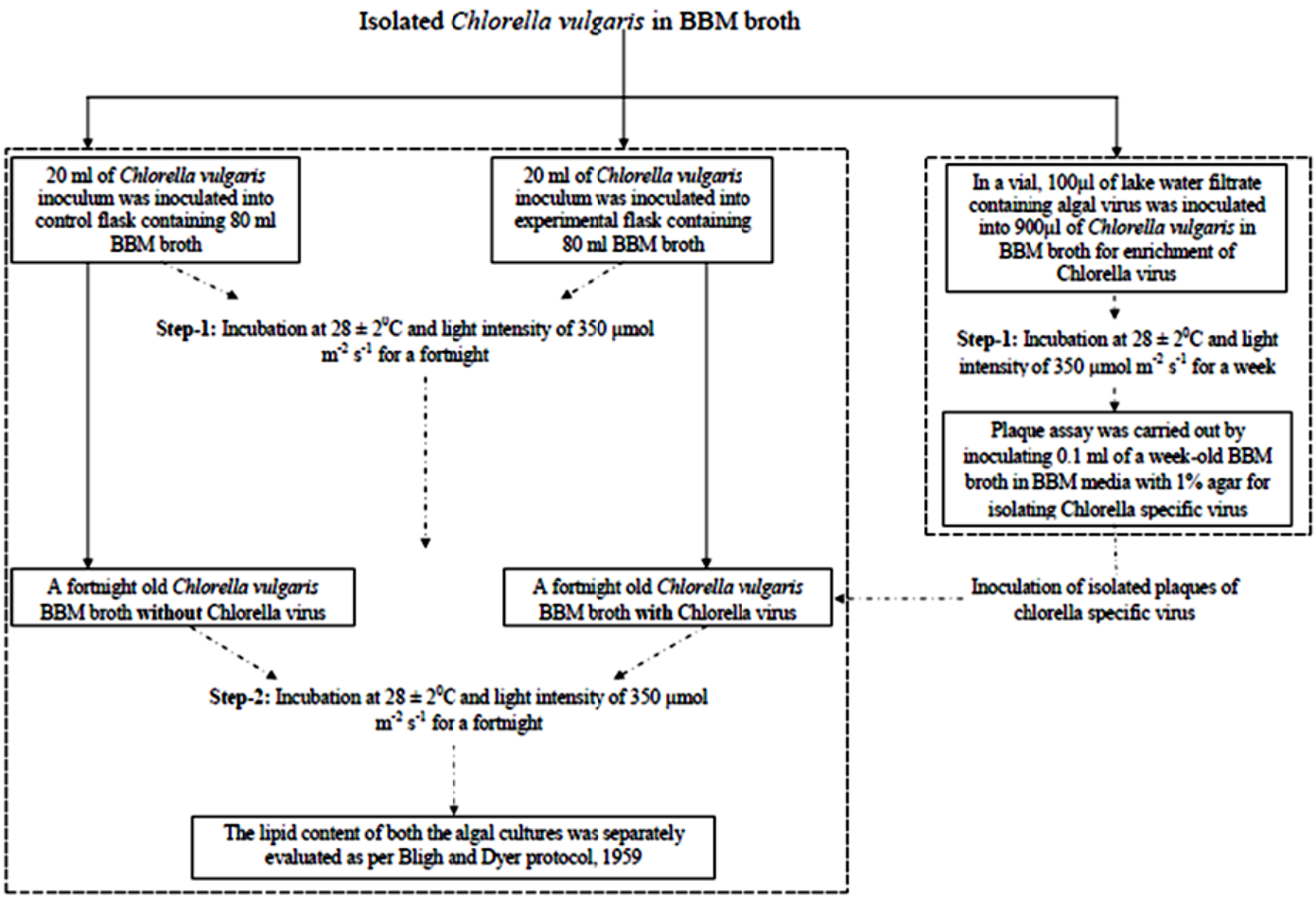

Figure 1. Workflow for increasing the extraction efficiency of bio-diesel precursor from algae through application of algal viruses.

Figure 2. *Chlorella vulgaris* specific algal virus isolated on bold basal media with 1% agar.

Table 1. Lipid content of *C. vulgaris*.

Experimental set	Lipid content (g g^{-1})	Percentage lipid content (%)
C. vulgaris treated with Chlorella virus (Experimental)	0.291 ± 0.017	29.1
C. vulgaris not treated with Chlorella virus (Control)	0.257 ± 0.02	25.7

*Data indicates the mean ± SD which was measured from five replicates.

Extraction of lipid from algal virus treated and untreated microalgal culture

C. vulgaris showed promising lipid extraction efficiency when treated with *Chlorella* virus. The lipid yield of *C. vulgaris* treated with *Chlorella* virus was found to be 0.291 ± 0.017 g/g and the lipid yield of *C. vulgaris* not treated with *Chlorella* virus was found to be 0.257 ± 0.02 g/g of biomass. Hence, algal lysis due to virus yielded an increased percentage of lipid content that is 29.1%, which was significantly higher than the untreated *C. vulgaris* that is 25.7%. The result implied that algal lipid extraction efficiency, increased by 11.68% in the case of *Chlorella* virus treated *C. vulgaris* when compared with *Chlorella* virus untreated *C. vulgaris* as shown in Table 1.

Productions of precursors for biodiesel have already been well established (Chiu et al., 2008). This study reports for the first time the application of *Chlorella* virus for increasing algal lipid extraction efficiency from *C. vulgaris*. We found from our results that *Chlorella* virus could be helpful in increasing the extraction efficiency of the algal lipid through viral mediated lysis of *C. vulgaris* under optimum conditions. We observed that the application of *Chlorella* viruses could effectively increase the extraction efficiency of algal lipid from *C. vulgaris*; similarly, viruses can be isolated against other microalgae for enhanced algal lipid recovery.

However, this method has some limitations as far as efficiency is concerned, such as development of resistance after frequent use of viruses (*Chlorella* viruses, in this case); lysogenic conversion of the viruses within their specific hosts; and the unpredictability of viral lysis event in the culture. The limitations can be minimized; as resistant algal strain can be screened for lytic viruses from environmental samples, which is simple and affordable, lysogen formation may be minimized by maintaining optimum conditions for algal growth. The lysis events can be monitored by microscopic observation of culture after viral addition and incubation for desired period depending on culture volume. Nevertheless, we considered that there is need to explore fluid dynamics between two fluids for improving the extraction efficiency of algal lipid for ultimately potentiating the biodiesel production. Further algal virus encoded products in the form of formulations can also be employed for efficient lysis of biodiesel producing algae commercially.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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