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Short Communication

Vibration-induced stress priming during seed culture increases microalgal biomass in high shear field-cultivation

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ABSTRACT

Vibrational wave treatment has been used to increase proliferation of microalgae. When directly applied at large scale, however, turbulence can offset positive effects of vibration on microalgae proliferation. Moreover, severe hydrodynamic shear fields in the bioreactor decrease cell viability that detrimentally influence maximum yieldable biomass. In this study, vibration pretreatment (between 10–30 Hz and 0.15–0.45 G) was used to prime the cells for enhanced biomass. When exposed to 10 Hz at 0.15 G for 72 h and inoculated in baffled flasks of large shear fields (0.292 Pa for the average wall shear force (aveWSF) and 184 s^{-1} for the average shear strain rate (aveSSR)), microalgae showed 27% increase in biomass as well as 39% increase in corresponding amount of heterologous protein (i.e. GFP-3HA). Our results show that stress primed microalgae with vibrations can lead to improved proliferation that results in increased biomass production at industrial scale bioprocesses.

1. Introduction

Cellular stress response (CSR) consists of a wide range of molecular changes that cells undergo in response to environmental stress. Microbes are exposed to many environmental factors such as temperature changes, osmolality variations, and nutrient deprivation. These stressors cause the microbes to physiologically change through various signal transduction networks which can negatively affect their survival and reproduction. Afterwards, these cells reprogram their metabolism and adapt to their environment achieving proper cellular growth, proliferation, and development under the stressors. Priming is a special cellular phenomenon of CSR: cells that have previously experienced a temporary milder stress (priming) have an enhanced stress response to a second stress event (triggering) (Andrade-Linares et al., 2016a; Hilker et al., 2016). The primed state comes with a transient metabolic cost shifting the metabolism from growth to production of protective compounds, but primed microbes are more beneficial in terms of survival in a stress environment than non-primed ones. In plants, a caterpillar chewing-treatment made *Arabidopsis thaliana* respond more actively than the non-treated ones in a severe vibrational actuator treatment (Appel and Cocroft, 2014). In addition, fungi that already experienced milder heat also showed their priming effect on progeny colonial growth in extreme heat conditions (Andrade-Linares et al., 2016b). These results were supported by a stress response metabolite analysis, in which stress priming was found to activate the general stress response which helps the cells to prepare themselves.

Although this priming effect is helpful for living organisms' survival and their biomass, stress priming of *Chlamydomonas reinhardtii* (a model photosynthetic microalgae) and mechanical stimulation that the microalgae experience in bioprocesses have not been considered together. According to molecular biology for microalgal mechanotransduction, several mechanoreceptors were just found to be involved in its mechanotransductory pathway: *TRP11* encodes a flagellar mechanoreceptor protein, *CAV2* a flagellar voltage-dependent calcium channel, *ADF1*, which is a TRP family Ca^{2+} channel (Fujiu et al., 2009, 2011), and *MSC1* an intracellular mechanosensitive ion channel involved in the organization of the chloroplast membrane (Nakayama et al., 2007). Collisions deform the microalgal cell body. Thus, *C. reinhardtii* activates intracellular Ca^{2+} concentration-dependent collision-avoiding reactions which result in several behavioral responses (i.e., mechanoreception, flagella excision, phototaxis, and photophobic response). The mechanical stimulus during the collisions excites the cellular membrane potential to express mechanosensitive channels like the TRP family, especially TRP11 and TRP15, by which an influx of Ca^{2+} is triggered. When the membrane depolarization exceeds a threshold level, an action potential is generated at the flagella by the voltage-

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dependent calcium channels, CAV2. In order to increase microbial biomass, a realistic approach is necessary that uses the innate characteristics of the microbes, and this mechanotransduction pathway could be activated to prime cells to increase microalgal biomass when they are cultivated in industrial-scale bioreactors with harsh mechanical stresses.

Photosynthetic microalgae have recently been proposed as a resource to solve the problem that the world will face in supplying enough food and feed; they consist of abundant macronutrients and micronutrients (i.e., vitamins, iron, and necessary dietary elements), grow fast enough such that all countries could get more plant-based products but with less labour and costs, can be farmed with high productivity, and potentially use only small areas of arable land and fresh water, all of which are environmentally sustainable (Smetana et al., 2017). In order to increase the microalgal biomass, some research groups have studied the effect of ultrasound on microalgal growth rates (Han et al., 2016; Savchenko et al., 2017) because mechanical stimulus is known as one of the key factors to produce more biomass as well as metabolites which can be used as nutrients. Although the microalgae grew faster, the equipment necessary for ultrasound could not be achieved on an industrial scale. It has been pointed out that the costs for the microalgae process are too high, and the process has yet to be efficiently managed (Scharlemann and Laurance, 2008). In this regard, it is not cost-effective to set up a vibration generating system on a large industrial scale. In addition, its vibrational effect could be offset by the vigorous turbulence in the bioreactors. Turbulence in large-scale bioreactors is necessary to agitate all the required components in the media for cellular proliferation such as oxygen/carbon dioxide and nutrients to increase the biomass. Apparatus like stirring panels, agitators, or a baffled block in large-scale bioreactors also helps prevent microbes from clustering or settling down. However, the turbulence can give rise to hydrodynamic shear force fields leading to lower cellular viability which means that the use of vibration in industrial processes to increase the proliferation of microalgae is not applicable and that cells should be able to endure such harsh conditions (Gallardo-Rodríguez et al., 2016).

In this study, *Chlamydomonas reinhardtii* was pretreated with a lab-scale up/down vibration generating system and cultivated in baffled flasks which had a shear stress force similar to that of large-scale bioreactors, and the changes in RNA expression of mechanosensitive channels were analyzed. It was assumed that *C. reinhardtii* could (1) recognize a mild compressional/tensional mechanical stimulus generated from the vibration system and (2) become primed so that they could withstand a similar extent of shear stress from industrial bioreactors leading to an increased proliferation and biomass productivity.

2. Materials and methods

2.1. Microalgae cultivation

CC-124 (*mt- nit1 nit2* strain) from *Chlamydomonas Center* was cultivated in tris-acetate-phosphate (TAP) with NH_4^+ liquid medium under continuous low irradiance (50 μE) for 5–7 days until the cells just entered the stationary phase (Paik et al., 2017). A second inoculation of the suspension diluted the cells to 1/2500 in 5 ml of fresh medium to maintain the cells in a healthy state for further experiments. When the cellular state reached the late exponential phase again, the cells were diluted again with fresh TAP + N medium to 3×10^6 cells/ml. Then, 200 μl of the resuspended cells were spread onto 1.5% TAP + N agar plates (7.5 ml of a mixture of TAP + N and agar had been poured in Ø60 petri dishes and dried at room temperature for 5 days). The cells were treated for 72 h with up/down vibration from a vibration system (SONICWORLD/SONIX SW-R3.03) using different frequencies of 10, 30, and 100 Hz at 0.5 G, or different magnitudes of 0.15, 0.30, and 0.45 G at 10 Hz. Each resultant G-force was profiled with an accelerometer (Nagano, G-MEN DR20). After the vibration pretreatment, they were collected and inoculated in 30 ml of TAP + N medium in baffled flasks at a concentration of 5×10^4 cells/ml for about 10 days (Fig. 1).

Fig. 1. Schematic representation of the two steps for microalgal stress priming: the vibration generation system for stress priming (top right) and the baffled flask shaking incubator for high shear stress (middle). This steps were compared with the steps from a seed plate (top left) as a control.

2.2. Baffled flask

250 ml Erlenmeyer flasks (Duran, Cat. No.: 21 216 36) were symmetrically equipped with four side baffles positioned at 90° and four bottom baffles offset from the side baffles, and their heights were all 15 mm. These eight deep baffles were designed to enhance vigorous agitation providing almost the same hydrodynamic shear force field as in industrial bioreactors. The shaking frequency was set at 245 rpm (Fig. 3a).

2.3. RNA preparation and analysis

The microalgal cells were lysed with buffer containing 10 mM Tris-HCl (pH 8.0), 200 mM NaCl, 1% SDS, and 10 mM EDTA. Following the traditional acidic phenol-chloroform method, 1 μg of extracted mRNA was used to synthesize cDNA using reverse transcriptase (Thermo Scientific, Cat. No.: EP0441) with an oligo dT_{15} (IDT) primer and dNTPs. Then, 5 μl of cDNA (diluted 1:5) were amplified by SYBR Green I master mix (Roche Applied Science, Cat. No.: 04 707 516 001) using 5 pmol of each specific primer with 50 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s on a Lightcycler 480 II System (Roche Applied Science). Relative levels of specific mRNA to CBLP (*Chlamydomonas* β subunit-like polypeptide) were represented as normalized target/reference ratios.

2.4. CFD simulation for the shear force analysis

The CFX module of ANSYS student ver. 18.2, CFD (Computational Fluid Dynamics) software, was used to simulate and characterize the fluid flows in the baffled flasks. The simulations were performed as previously reported (Liua et al., 2016). Briefly, the gas-liquid interface was set as the free surface. The two-phase flow using the volume of fluid (VOF) model and the water turbulence using the RNG k-ε turbulence