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Inducible microbial osmotic responses enable enhanced biosorption capability of cyanobacteria

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ABSTRACT

Microorganisms have evolved series of protective or adaptive responses to enhance survivability to fight against stresses in nature and engineered systems. Although the cellular responses and underlying mechanisms have been intensively studied, seldom research explored the possibilities of employing the microbial stress response via an inducible routine for applications of environmental benefits. In this study, we proposed and demonstrated that the biosorption of methylene blue by cyanobacteria could be enhanced via exerting higher osmotic stress, and the enhanced biosorption is attributed to the induced stress responses which elicited the combined effects of exopolysaccharides over-secretion and the up-regulated carbonic anhydrase activity. To our knowledge, this is the first study that employing the inducible physiological responses of microorganisms for enhanced environmental application, and we believe that this research will bring novel insights into the field of applied microbiology and environmental biotechnology.

1. Introduction

Microorganisms live in changing environments with various stresses. These stresses elicit protective or adaptive responses of exposed cells to enhance survivability [1]. Many researchers investigated the genetic and physiological variations in microbes when they were exposed to different environmental stimuli [2,3]. As reported, pH, temperature, antibiotics, nanoparticles and heavy metals could all induce the variation of surface properties including increased secretion of extracellular polymeric substances (EPS), as well as the change of transcriptional profiles of relevant genes [4–8]. Although the cellular stress responses and underlying mechanisms have been intensively studied in various microorganisms in both natural and engineered systems [9], there were no reports to our best knowledge that had taken advantage of the microbial stress response via an inducible routine for environmental benefits.

Environmental biotechnologies, especially biosorption, require sustainable biomaterials with promising performance to remove hazardous pollutants. Previous research demonstrated that the performance of biosorption relies on the cell surface properties to interact with target contaminants [10]. EPS are of grand importance to these specific features [11]. Given the inducing effects of external stress on EPS secretion, we proposed that exerting external stress on microorganisms could enhance its biosorption capacity via inducing microbial stress responses.

Ideal biomaterials for adsorption should possess adequate functional groups and originate from sustainable resources. In this sense, enriched microorganisms are promising candidates, and cyanobacteria have even further advantages. As autotrophs, they directly use CO_2 and solar energy to accumulate biomass. Thus, we chose *Synechococcus elongatus* PCC7942 (*S. elongatus*), a model cyanobacterium with well-characterized physiology and genetics, as an example to investigate whether external stress could induce cellular response and enhance biosorption capability. In addition, *Microcystis aeruginosa*, which is known to be one of the major genuses dominating the algal blooms and is desirable to be removed from aquatic ecosystems, was further tested to confirm the universality of our strategy. NaCl was selected to exert osmotic stress to the cell culture in order to enhance its biosorption capability, since, unlike antibiotics, nanoparticles, or other stressors, NaCl exhibits limited environmental impacts by itself.

In the present study, we demonstrated that it is feasible to exert external stress for enhancing biosorption via inducing the stress responses of cyanobacteria. The adsorption performance of induced cyanobacteria, surface properties as well as the transcriptional analysis of essential genes were investigated. We believe our

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strategy would develop a simple, cheap, and environmental sound method for enhanced biosorption with a novel angle via exploiting the stress responses of microorganisms.

2. Materials and methods

2.1. Strains, culture conditions and sample preparation

S. elongatus was obtained from ATCC (American Type Culture Collection), and the *M. aeruginosa* was obtained from Prof. Li Li's lab (Shandong University). Both of them were cultured in BG11 liquid medium (pH 7.1) at 30 °C with constant light (2000–3000 Lx). *S. elongatus* cells were harvested at stationary growth phase by centrifugation at 10,000*g* for 10 min at 4 °C and washed twice with phosphate buffer saline (PBS, pH 7.2). *M. aeruginosa* cells were harvested at stationary phase by centrifugation at 4,500*g* for 10 min at 4 °C and washed following the same protocol for *S. elongatus*. The pellets were resuspended in PBS and used for subsequent experiments. To distinguish the physical variations caused by osmotic stress from stress responses induced by stress shock for living cells, half of the harvested cells were treated by reacting with 2.5% glutaraldehyde for 15 min at 4 °C and considered as dead cells, to compare with the other half without glutaraldehyde treatment (living cells). Both living cells and dead cells were used for the adsorption experiment under the same conditions.

2.2. Osmotic shock and adsorption experiments

Living and dead cells were washed twice with deionized water and resuspended in different concentration of NaCl solution (0, 0.15, 0.25, 0.5 and 0.75 M); they were afterwards shaken for 24 h at 30 °C to enable the osmotic responses. Then, 500 μL of cyanobacterial suspensions were added to 50 mL centrifuge tubes, and 250 μL of 200 mg/L methylene blue (MB) were added as the adsorbates. Finally, the total volume of the solution in each tube was adjusted to 5 mL with deionized water. All tubes were shaken on an orbital shaker at 150 rpm in dark at 25 °C for 3 h to reach the adsorption equilibrium. After filtered by 0.45-μm cellulose acetate membrane, MB in the filtrate was analyzed by measuring the absorbance at 664 nm with a UV-2000 spectrophotometer (UNICO, USA). The performance of adsorption was evaluated by specific adsorption capacity, which is expressed as follow,

$$\text{Specific adsorption} = \frac{q}{q_c}$$

in which q is the adsorbed MB by osmotic stress induced cyanobacteria, mg/g dry weight; q_c is adsorbed MB by control, mg/g dry weight.

2.3. Extraction and characterization of EPS

Following the osmotic shock treatment for *S. elongates* and *M. aeruginosa*, EPS was extracted using a heating procedure (70 °C, 1 h) followed by centrifugation at 10,000 rpm for 15 min [12]. The supernatants were filtrated through a 0.45-μm cellulose acetate membrane and were considered as the EPS solution including exopolysaccharides (PS) and exoproteins (PN). The PS in EPS was measured by anthrone-sulphuric acid method [13], using glucose as the standard, and the PN was determined by the Bradford method [14], using bovine serum albumin as the standard.

2.4. Transcriptional analysis

Transcriptional variations of essential genes in the model cyanobacterium *S. elongatus* were further analyzed using quantitative reverse transcription polymerase chain reaction (qRT-PCR).

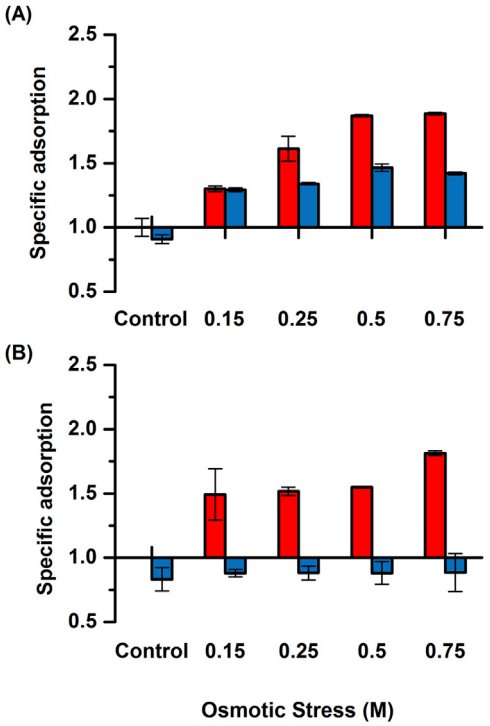

Fig. 1. Specific adsorption of living (red) and dead (blue) *S. elongatus* (A) and *M. aeruginosa* (B) cells with 0, 0.15, 0.25, 0.5 and 0.75 M NaCl shock. The adsorption capacity of living cells for both species exhibited significant increase compared with control, and the results were confirmed by statistical analysis ($p < 0.05$) as significant differences. Error bars represent ±1 SD from the means of at least triplicate measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

RNA was extracted with the RNAprep pure Cell/Bacteria Kit (Tiangen Biotech. Co. Ltd., Beijing, China). Reverse transcription was conducted with the Primescript™ RT reagent kit (Takara Biotech. Co. Ltd., Dalian, China). qRT-PCR was carried out using a Roche LC-480 real-time PCR system. All replicates of each sample were measured at least in triplicates. *rbcL*, *icfA*, *rpoD*, *KpsF* and *cpsB* were selected as targeted genes, and the 16 s rDNA sequence was used as the housekeeping gene. The qRT-PCR results were quantified using $2^{-\Delta\Delta Ct}$ method [15]. All experiments were followed by the protocols from manufacturers. Primer pairs designed for the target genes are listed in Table SI.

3. Results and discussion

3.1. Enhanced biosorption by inducing the osmotic responses of S. elongatus and M. aeruginosa

To study the effect of external salt stress, living and dead cells of two cyanobacterium species were resuspended in different concentrations of NaCl solutions, and MB was selected as a target adsorbate. After reaching the adsorption equilibrium, the specific adsorption of MB was calculated. For *S. elongates* living cells, the specific adsorption of MB increased by 1.30, 1.61, 1.87 and 1.89-fold with the cells that had been treated with 0.15, 0.25, 0.5, 0.75 M NaCl solution, respectively (Fig. 1A and Table SII). However, the increased adsorption of dead cells, which might resulted from the morphological variations, was not as significant as that of living cells. The more significant adsorption enhanced effect of osmotic stress on living cells than dead cells indicating that the increased adsorption was partly resulted from the induced stress responses of living cells, not only the physical change by osmotic stress. We further examinated the strategy on *M. aeruginosa*. Similar results were obtained