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Metal removal of cyanobacterial exopolysaccharides by uronic acid content and monosaccharide composition

Sahlan Ozturk [a,*], Belma Aslim [b], Zekiye Suludere [b], Sema Tan [c]

[a] Nevsehir University, Faculty of Science and Arts, Department of Molecular Biology and Genetics, Nevsehir, Turkey
[b] Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey
[c] Kirikkale University, Faculty of Science and Arts, Department of Biology, Kirikkale, Turkey

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ABSTRACT

In the present study, chromium, cadmium and metal mixed (chromium + cadmium) removal and its association with exopolysaccharides and uronic acids production in *Synechocystis* sp. BASO671 were investigated. It was investigated that BASO671 showed different removal ability when exposed to each metal solely and mixed metal. EPS production by BASO671 was increased following exposure to 15 and 35 ppm Cr(VI), Cd(II) and Cr(VI) + Cd(II). Monomer composition of EPS was changed after metal treatment. Uronic acid contents of metal treated cells were higher than control cells of each isolate. Also, glucuronic acid content and galactronic acid content of EPS correlated with uronic acid contents of cells. Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis confirmed that a considerable amount of metals had precipitated on the cell surface. Fourier transform infrared spectrum analysis of EPSs indicated the presence of C—H and C—O group, which may serve as binding sites for divalent cations.

1. Introduction

Toxic metallic trace elements that contaminate the environment are of increasing economic, public health and environmental significance and concern.

Methods of treating metallic trace element contaminated effluents currently consist of chemical precipitation, solvent extraction, dialysis, electrolytic extraction, reverse osmosis, evaporative methods, treatment with ion-exchange resins, carbon adsorption and dilution. In recent years, there has been a significant effort to search for new methods of metallic trace element removal from contaminated sites. Biological methods to remove metals from liquid effluents present many potential advantages. Metallic trace element accumulation processes by biological cells are grouped together under the general term "biosorption" (Salehizadeh & Shojaosadati, 2003).

Many microorganisms are capable of secreting high molecular mass polymers, which can either be released into the surrounding environment (extracellular polysaccharides, exopolysaccharides or extracellular polymeric substances (EPSs)) or remain attached to the cell surface (capsular polysaccharides). ESPs are mainly composed of polysaccharides, proteins, humic substances, nucleic acids, and lipids, containing ionizable functional groups such as carboxylic, phosphoric, amino and hydroxylic groups (Liu & Fang, 2002). These polysaccharides are believed to protect bacterial cells from desiccation, metallic trace elements or other environmental stresses, including host immune responses, and to produce biofilms, thus enhancing the cell's chances of colonizing special ecological niches. In metallic trace element pollution, bacterial exopolymers have become an alternative of interest as metal binding agents in the detoxification of contaminated waters (McEldowney, 2000).

Cyanobacteria, or blue-green algae, are ubiquitous microorganisms that occur naturally and serve as one of the biomaterials with a high capacity for removing metallic trace elements from contaminated waters. They have been known since long as a potential EPS producer. The presence of proteins, uronic acid, pyruvic acid, and O-methyl, O-acetyl and sulfate groups emphasizes the complex nature of cyanobacterial EPS (Bender & Phillips, 2004). The cell surface of cyanobacteria consists of polysaccharides, proteins and lipids, which act as a basic binding site for metallic trace elements. Therefore, it is the most important organism for environment in terms of removing wastes from the water.

In our study, the cyanobacterial strain, *Synechocystis* sp. BASO671, produced exopolysaccharides during the normal growth process. The aim of this study is to determine the metal removal behaviour of *Synechocystis* sp. BASO671 in terms of the relation between metal removals, EPS production. It also aims to determine the effect of Cr(VI), Cd(II) and Cr(VI) + Cd(II) on EPS production, EPS

* Corresponding author. Tel.: +90 0384 228 10 00; fax: +90 312 228 10 43.
E-mail address: sahlan.ozturk@nevsehir.edu.tr (S. Ozturk).

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monomer composition, uronic acid content of EPS and functional groups of EPS using this cyanobacterial isolate.

2. Materials and methods

2.1. Culture conditions and microorganisms

BASO671 was isolated from Uncali Stream (Antalya), Turkey. Isolation and purification of the isolate were performed by dilution and plating of water samples. Isolate were grown in BG-11 medium: [$NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4{\cdot}7H_2O$, 0.075; $CaCl_2{\cdot}2H_2O$, 0.036; citric acid, 0.006; iron(III) ammonium citrate, 0.006; Na_2-EDTA, 0.001; Na_2CO_3, 0.02 g L^{-1}, 1 mL]; trace elements solution (H_3BO_3, 61; $MnSO_4{\cdot}H_2O$, 169; $ZnSO_4{\cdot}7H_2O$, 287; $CuSO_4{\cdot}5H_2O$, 2.5; $(NH_4)6Mo7O_{24}{\cdot}4H_2O$, 12.5 mg L^{-1}) pH 6.8 at 25 °C with light/dark cycle of 12/12 h by using an incubator shaker (MINITRON), for 20 days. The agitation of the incubator shaker was 100 rpm during incubation. The intensity of light during the light period was 3000 lux.

2.2. 16S rRNA-based identification of cyanobacterial isolate

Genomic DNA was extracted using the DNeasy® Blood and Tissue Kit (Cat. No.: 69504, QIAGEN, UK). Cyanobacterial 16S rRNA gene sequences were amplified using cyanobacteria-specific primers as previously described (Nubel, Garcia-Pichel, & Muyzer, 1997): CYA106F (5′-CGGACGGGTGAGTAACGCGTGA-3′) and CYA781R (5′-GACTACAGGGGTATCTAATCCCTTT-3′). Also BACF (5′-GCCAGGGGAGCGAAAGGGATTAGA-3′) and BACR (5′-CATGGTGTGACGGGCGGTGTG-3′) primers which were designed by one of the authors (B. Aslim) were used for amplification. PCR amplifications were performed with a Hybaid thermocycler (ThermoHybaid, UK) and conditions were evaluated as described (Nubel et al., 1997). Sizes of the amplified fragment for CYA106F–CYA781R and BACF–BACR primers were 640 and 629 bp, respectively. The sequencing process was performed via the REFGEN process (Ankara, Turkey), and the sequences obtained were searched against the GenBank DNA database using the blast function.

2.3. Cr(VI) and Cd(II) toxicity

Solutions of different metal concentrations were prepared by dissolving $CdCl_2$ and $K_2Cr_2O_7$ (Merck) in distilled water to reach metal concentrations of 15 and 35 ppm. Cr(VI) and Cd(II) solutions were sterilized by filtering them with a 0.2 μm pore size filter. Experiments were carried out using 100 mL of BG11 in 150 mL glass Erlenmeyer flask Cr(VI) and Cd(II) resistance of cyanobacterial cultures were investigated by determining chlorophyll-a (Hirschberg & Chamovitz, 1994) every 48 h, for a period of 12 days. Also, flasks containing medium lacking Cr(VI) and Cd(II) were inoculated in the same manner to serve as controls. Cr(VI) and Cd(II) resistance were evaluated by comparison with the controls. The main values and the standard deviation were calculated from the data obtained with triplicate trials.

The EC_{50} determined by probit analysis (Finney, 1971) was defined as the Cr(VI) and Cd(II) concentration required to cause 50% mortality within 6 days.

2.4. Cr(VI) and Cd(II) removal

The removal of Cr(VI) and Cd(II) by *Synechocystis* sp. BASO671 was evaluated using a modified method described by Matsunaga, Takeyama, Nakao, and Yamazawa (1999). Isolates (OD_{664}, 2.5) were exposed to 10 ppm Cr(VI), Cd(II) and Cr(VI) + Cd(II) for 7 days in BG11 medium at 25 °C with a light/dark cycle of 12/12 h using an incubator shaker. The intensity of light employed during the light period was 3000 lux. Isolates of 1 mL which were exposed to metals were assayed for 0–7 daytime intervals. Metal removal was determined as metal in the medium, metal adsorbed on the surfaces of the cells, and metal accumulated in the cells. The concentration of Cr(VI) and Cd(II) was measured by an atomic absorption spectrophotometer (AA-6600, Shimadzu). The cromium or cadmium removal rate (%) was calculated as follows: (amount of removed Cr or Cd)/(amount of initial Cr or Cd) × 100. Samples were centrifuged (10,000 rpm) and residual Cr(VI) and Cd(II) in the medium was determined in the supernatant. The pellet was further washed with 1 mL of 10 mM EDTA solution for desorption of Cr(VI) and Cd(II) from the cell surfaces and centrifuged (10,000 rpm) once again. Cr(VI) and Cd(II) adsorbed onto the cell surfaces were separated from this supernatant. The amount of intracellular accumulation of Cr(VI) and Cd(II) were determined by measuring the Cr(VI) and Cd(II) content in the pellet, and resuspended and sonicated (Vibra Cell) at 50 MHz on ice in 1 mL of 1 N HNO_3 using an atomic absorption spectrophotometer.

2.5. Isolation, purification and characterization of exopolysaccharides (EPSs)

EPS was extracted by the modified procedure of Cérantola, Bounéry, Segonds, Marty, and Montrozier (2000). After 20 days of cultivation, cells were harvested at room temperature by centrifugation at 10,000 rpm for 10 min. The supernatant was removed. After the pellet was dissolved in 1 mL deionized distilled water, the solution was boiled for 15 min at 100 °C. It was then kept at room temperature for 10 min and added to 3 μl of 85% TCA. The resultant mixture was centrifuged at 10,000 rpm for 30 min. The supernatant which contained EPS was pooled and equal volume of ethanol was added. The mixture was kept at 4 °C overnight and was centrifuged at 10,000 rpm for 30 min again. Precipitate was then washed two times with 96% ethanol and centrifuged at 10,000 rpm for 30 min. Final precipitate was dissolved in 1 mL deionized distilled water and stored at −20 °C. Total carbohydrate contents of the EPS samples were determined by the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956) using glucose as a reference standard (Torino, Taranto, Sesma, & de Valdez, 2001). The main values were calculated from the data obtained with triplicate trials.

The monosaccharide composition of freeze-dried extracellular polysaccharide samples was determined with HPLC (VARIAN ProStar) by using Metacarb 87H column (300 mm × 7.8 mm, Cat. No.: 5210). The organic acids were determined with PDA detector (VARIAN 330) (210 nm), while the extracellular polysaccharides were determined with RI detector (VARIAN 350), connected after the PDA detector. The analyze conditions are mobile phase 0.008 N H_2SO_4, flow rate 0.4 mL min^{-1} at 35 °C. Monomer analyze of EPS was carried out at the Central Laboratory, Molecular Biology and Biotechnology R&D Centre, Middle East Technical University.

2.6. Effect of Cr(VI) and Cd(II) on EPS production

Equal biomasses of the isolates were inoculated into 500 mL flasks containing 300 mL of BG-11 with 15 and 35 ppm Cr(VI), Cd(II) and Cr(VI) + Cd(II) concentrations. Isolates were incubated at 25 °C with light/dark cycle of 12/12 h by using an incubator shaker (MINITRON), for 7 days. The intensity of light during the light period was 3000 lux. Initial and final biomass concentrations of the isolates were investigated and equalled by determining chlorophyll-a. Cells were collected by centrifugation at 10,000 rpm for 10 min at room temperature. EPS was isolated as described by Cérantola et al. (2000) and total EPS (mg L^{-1}) was estimated by the phenol-sulfuric method (Cérantola et al., 2000). The main values and the standard deviation were calculated from the data obtained with triplicate trials.