Variation of Extracellular Polymeric Substances (EPS) of *Chlamydomonas reinhardtii* under arsenic stress

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ABSTRACT: Extracellular Polymeric Substances (EPS) are an important component of microorganisms. In this paper, we first compared the effectiveness of EPS extraction methods for amodel green algae (*Chlamydomonas reinhardtii*), and then investigated variations of EPS under arsenic (As) stress. Components of the algal EPS with or without arsenite and arsenate treatments were analyzed. The results showed that the different EPS extraction methods ranked as follows: NaOH > heating > cation exchange resin > EDTA > centrifugation. However, NaOH extraction caused more cell disruption than the heating approach. The optimal extraction was heating at 50°C, under which the amount of EPS was $40.06 \pm 0.297 \text{ mg g}^{-1}$. Under $20 \mu\text{g L}^{-1}$ As(V) and $200 \mu\text{g L}^{-1}$ As(III), the amounts of EPS were 41.35 mg g^{-1} and 38.63 mg g^{-1} respectively, compared to control (46.4 mg g^{-1}), indicating that the EPS production of *C. reinhardtii* was not affected by As.

1 INTRODUCTION

Extracellular polymeric substances (EPS) are the products with a high molecular weight, and they usually secreted by microorganisms (such as algae). The EPS typically are composed of polysaccharides, proteins and nucleic acids. Additionally, as a crucial substance adhesion to cell surface, EPS has a variety of biological roles on algal cells due to its abundant functional groups (e.g., $-\text{COOH}$, $-\text{NH}$ etc.). EPS forms an important extracellular protective barrier on the algae cell surface, which can prevent toxicants. Moreover, those toxicants (e.g., Pb^{2+} , Cd^{2+}) also affect the production and composition of EPS. Research have shown that some microalgae has a strong ability to adsorb As and have the potential in removing the As pollution from water. However, the relation between microalgal EPS and As was not clear. Therefore, the objectives of this study were: (1) to establish a suitable method for the extraction and analysis of extracellular polymers substances (EPS) from *C. reinhardtii*, and (2) to quantify and analyze the algal EPS under various As treatments.

2 METHODS AND MATERIALS

2.1 Cultivation of algae and EPS extraction

The green alga *C. reinhardtii* in this study was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. EPS was extracted by the following methods: centrifugation, heating, NaOH, EDTA, CER treatments, respectively.

2.2 Effect of arsenic on EPS compositions and content

Upon exposure to different As(V) and As(III) concentrations, EPS from algae cultures was extracted using heating method. Proteins in EPS extract were determined by the modified Bradford method. The polysaccharides content in EPS was measured using the phenol-sulfuric method. Nucleic acids were determined by the diphenylamine colorimetric method.

3 RESULTS AND DISCUSSION

3.1 Comparison of EPS extraction methods

Table 1 showed that the amounts of EPS ranked as follows: NaOH > heating > cation exchange resin > EDTA > centrifugation. When NaOH (0.05 mol L^{-1}) was used, the amount of EPS reached 70.42 mg g^{-1} . However, this method caused higher cell disruption (DNA 0.94 mg g^{-1}) than others (Table 1). When the heating temperature was 50°C, the amount of EPS was $40.06 \pm 0.30 \text{ mg g}^{-1}$. Thus, heating method was chosen for EPS extraction from *C. reinhardtii*.

3.2 Variations of EPS contents and compositions under arsenic stress

The amount of EPS by the microalgae under the stress of different concentrations of As(V) in the 4-d cultivation are shown in Figure 1. When *C. reinhardtii* was inoculated in TAP media with 20, 100, 200, $500 \mu\text{g L}^{-1}$ of As(V), the EPS production at the 4-d

Table 1. Contents of EPS components measured by different extraction methods.

Extraction method	Yield of EPS (mg·g ⁻¹ DW)			
	polysaccharide	protein	DNA	DNA (%)
Centrifugation	0.53 ± 0.04d	ND	ND	ND
Heating	25.48 ± 0.21b	14.02 ± 0.4b	0.56 ± 0.03b	1.41 ± 0.071b
NaOH	28.71 ± 1.24a	40.77 ± 0.18a	0.94 ± 0.12a	1.36 ± 0.202b
EDTA	13.74 ± 1.38c	1.26 ± 0.09d	0.38 ± 0.04c	2.35 ± 0.353a
CER	27.88 ± 5.16a	7.21 ± 0.14c	ND	ND

Note: Data in table are means ± standard deviations. Different letters indicate significant difference between different extraction methods ($P < 0.05$). ND means not detected or below the detection limit. Data are means ± SD ($n = 3$).

Figure 1. Variations in EPS from *C. reinhardtii* after exposure to different concentrations of As(V). Data are means ± SD ($n = 3$).

decreased to ~43.7, 42.37, 41.54, 41.35 (mg g⁻¹ dry cell), respectively, compared to the cultures in the TAP media without arsenate (the control).

The total amount of EPS of *C. reinhardtii* decreased from 46.4 mg g⁻¹ (control) to 38.63 mg g⁻¹ with 200 µg L⁻¹ As (III) (Fig. 2). In this case, the protein of EPS significantly reduced by 45.72% (Fig. 2), which was much more than that with 20 µg L⁻¹ As(V).

The interaction of EPS with As has been suggested to decrease As toxicity to microorganisms. For example, *Rhizobium* strain VBCK1062 isolated from *Vigna radiata* plants showed an increase in EPS production when exposed to 5 mM As(V), however, there was a decline with concentration of As(V) higher than 5 mM (Deepika *et al.* 2016). On the contrary, the present study showed that EPS was decreased upon various As treatments, suggesting that the relationship between EPS and As is complicated and needs further investigations.

4 CONCLUSIONS

- 1) Heating method (50°C, 3 h) was considered as the suitable EPS extraction method with high extraction efficiency (40.06 mg g⁻¹), simple process and little cell disruption.
- 2) The amount of EPS production slightly decreased 5.82–11.53% when exposed to various arsenate

Figure 2. Variations of EPS from *C. reinhardtii* after exposure to arsenate (20 µg L⁻¹) and arsenite (200 µg L⁻¹). Data are means ± SD ($n = 3$).

and arsenite treatments, suggesting that EPS and As interactions are complex and more studies are needed.

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