Effect of symbiotic bacteria on the accumulation and transformation of arsenite by *Chlorella salina*

Y.X. Wang, Q.N. Yu & Y. Ge

Jiangsu Key laboratory of Marine Biology, College of Resources and Environmental Sciences,
Nanjing Agricultural University, Nanjing, China

ABSTRACT: Microalgae and bacteria are usually symbiotic in the environment. Algae-bacteria consortia may be potentially applied in wastewater treatment and environmental remediation. Previous research has shown that the algae-bacteria consortia have a strong ability of removing arsenic (As) from solution. However, little is known about the effects of symbiotic bacteria on uptake and metabolism of arsenite (As(III)) by microalgae. In this study, we investigated the effects of symbiotic bacteria on As(III) accumulation and transformation by *Chlorella salina*.

1 INTRODUCTION

Arsenic (As) is a ubiquitously distributed and a non-threshold carcinogen, ranking at the top of the priority list of hazardous substances. Microalgae are widely found in the environment. They are single celled organisms with large cell surface area, strong absorption and adsorption capacity for As, and great potential for the treatment of As contaminated wastewater. Bacteria and microalgae often coexist during the wastewater treatment processes. Although the bacteria may compete with the microalgae for nutrients (Bai *et al.*, 2015), they could also facilitate microalgal harvesting by forming algal-bacterial aggregates. Studies have shown that microalgal metabolism of As(III) includes extracellular oxidation and intracellular methylation, complexation with glutathione (GSH), PCs and stored in the vacuole for detoxification (Jiang *et al.*, 2011). However, little is known about the effects of symbiotic bacteria on As(III) metabolism in microalgae.

2 METHODS/EXPERIMENTAL

Chlorella salina was purchased from Marine Germplasm Bank of China, Chinese Academy of Sciences, Qingdao. *Agrobacterium tumefaciens* and *Erythrobacter citreus* were isolated and identified as the symbiotic bacteria from *C. salina*.

In this study, 0~750 $\mu\text{g L}^{-1}$ arsenite [As(III)] was set up, and the adsorption, absorption and morphological transformation of As(III) by axenic and non-axenic *C. salina* were measured seven days after treatment.

Determination of total As contents in sterile and non-axenic *C. salina* cells was done through digestion at 120°C. The As species of algal cells were extracted with 1.75% HNO_3 . HPLC-HG-AFS was used for the As speciation analyses.

3 RESULTS AND DISCUSSION

Chlorella salina was exposed to a series of concentrations of As(III), however As(V) was the main species in the culture medium and non-axenic algae cells (Figs. 1, 2); but for the axenic *C. salina*, As(III) was the main form in the culture medium (Fig. 3).

Figure 1. Speciation of As in the culture medium of non-axenic *C. salina*.

Figure 2. Speciation of As in the non-axenic *C. salina* cells.

Figure 3. Speciation of As in the culture of axenic *C. salina*.

Figure 4. Speciation of As in the axenic *C. salina* cells.

Figure 5. Speciation of As in the culture of *A. tumefaciens*.

As(V) was the main species of intracellular As and a small amount of As(III), MMA and DMA were also detected in these cells. These As species accounted for 2.14%–4.32% of total As in the *C. salina* cells.

There were only As(III) and As(V) forms in the cells of axenic *C. salina*, the ratios of As(V) and As(III) were 44.66%–58.30% and 41.70%–55.34%, respectively (Fig. 4).

As(III) was the main form in the culture medium when the symbiotic bacterium, *A. tumefaciens*, was cultured alone (Fig. 5). The proportion of As(V) was only 7.73%–13.56%, indicating that the bacteria might be an arsenate reduction bacterium.

Results of this study showed that the presence of symbiotic bacteria increased the accumulation and transformation of As by algae cells. After 7 d of treatment with different concentrations of As(III), the removal rates of arsenic in the solution from *C. salina* were 18.92%–55.12%, higher than that of axenic *C. salina* (12.82%–27.43%) and symbiotic bacteria (1.86%–16.19%).

This study showed that bacteria significantly increased the accumulation of arsenic in *C. salina* ($P < 0.05$), and improved the removal of As(III) from water. Symbiotic bacteria increased the amount of EPS on the surface of *C. salina* and increased the binding sites for As, resulting in significantly higher As adsorption by the non-axenic *C. salina*.

In the presence of symbiotic bacteria, As(V) is the major As species in the *C. salina* cells and culture medium, indicating that symbiotic bacteria may increase the expression of the arsenite oxidase gene and lead to the higher oxidation of As(III). Further experiments are being conducted to reveal its mechanisms.

4 CONCLUSIONS

Symbiotic bacteria significantly affected the accumulation of As in the *C. salina* cells. Consortia of the bacteria and *C. salina* could affect the oxidation and methylation of As(III) in the algal cells.

ACKNOWLEDGEMENTS

Financial support from Natural Science Foundation of China (31400450, 31770548) are greatly appreciated.

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