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BRIEF COMMUNICATION

Potential of arsenic bioremediation by a cyanobacterium isolated from the Salado River in the Atacama Desert

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Cyanobacteria and microalgae are recognized as excellent metal(loid)s-bioremediators of aquatic systems. We isolated a cyanobacterium from the Salado River in the Atacama Desert, northern Chile, which was identified as *Cyanobium* sp. Growth inhibition bioassays were conducted with arsenic and cadmium, and tolerance of *Cyanobium* to these metals was estimated. Removal of arsenic was assessed under different pH conditions and over time. We showed that the *Cyanobium* strain isolated from the Salado River has a greater tolerance to the arsenic and cadmium compounds than other species commonly used in metal(loid)s-bioremediation. Removal of up to 90% of arsenic was obtained in alkaline conditions, within the first 3 hours of exposure suggesting that *Cyanobium* sp. isolated from the Atacama Desert could be further studied with biotechnological purposes and to understand the evolutionary mechanisms of adaption to arid environments.

KEYWORDS: bioremediation; bioassays; Atacama Desert; *Cyanobium* sp.; metalloids; cyanobacteria

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INTRODUCTION

The extreme aridity of the Atacama Desert allows high concentrations of metals and metalloids in water bodies (Aránguiz-Acuña *et al.*, 2020). The Salado River (22°20′16″S 68°01′03″W–22°22′19″S 68°39′22″W) has average 1400 mg L^{-1} As concentrations in the whole basin (Romero *et al.*, 2003).

Arsenic transformation into various chemical species with different toxicity levels determinates that oxidation state of environmental As is important to estimate toxicity risk to aquatic biota (Franco *et al.*, 2015).

High tolerance to As observed in some microorganisms is mainly explained by mechanisms such as precipitation, chelation, compartmentalization, extraction, absorption, reduction, oxidation, methylation or production of arsenosugars (Slyemi and Bonnefoy, 2012). The pathway of extraction of As involves a prior reduction of As^{+5} to As^{+3} (Pandey *et al.*, 2013) and later a transfer of the methyl group of S-adenosylmethionine to As^{+3} (Shen *et al.*, 2013). Additionally, As^{+3} can be directly extracted through an integral membrane protein.

We tested the removal of As^{+5} from water by an isolated cyanobacterium in a pH range over time. We hypothesized that a phototroph isolated from a river located in the Atacama Desert will be more tolerant to metal(oid)s than foreign species and that these organisms may remove metals dissolved in water, potentially making them useful bioremediators of metal-concentrated waters.

MATERIALS AND METHODS

Water samples were collected from the Salado River (Fig. 1A, B, D) and filtered through 5-μm polycarbonate membrane filter to discard the zooplankton from the samples, then a second filtered was realized by 0.22-μm filters to concentrate microorganisms. This concentrated were cultured in 250-mL Erlenmeyer flasks containing F/2 medium solution at 1.5 g L^{-1} salinity (Guillard and Ryther, 1962) (continuous light, 55 μmol photon m^{-2} s^{-1} light intensity, 21 ± 1°C and gently agitation at 70 rpm). Inoculums were seeded after 2 weeks on F/2 agar culture medium in Petri dishes under conditions as before. The procedure was repeated for several months until isolate a single strain.

DNA extraction of isolated culture was performed follows Chelex® 100 protocols (Gómez *et al.*, 2002). 16S rRNA (ca. 661 bp) was amplified using the 27F and 1492R primers (Folmer *et al.*, 1994). Polymerase chain reaction was performed using the thermal profile following Gómez *et al.*, 2002 protocols. BLAST tool in the GenBank database was used to find the closest similarity to the sequence in the NCBI website. GenBank access number MK570611.

The tolerance of isolated cyanobacteria was tested for the inorganic arsenic, arsenite As^{+3} ($NaAsO_2$) and arsenate As^{+5} (Na_2HAsO_4); cadmium Cd^{+2} ($CdCl_2$) and a reference toxicant ($K_2Cr_2O_7$) (NCh, 2002; OECD, 2011). These metals were selected due to their high concentrations present in the site study (Romero *et al.*, 2003). Standard growth inhibition bioassays were conducted for 96 hours for a commonly used in toxicity bioassays microalgae, *Chlorella vulgaris* (OECD, 2011, without modification) and the cyanobacterium isolated in this study (replacing the freshwater culture medium by F/2 saltwater culture medium). Cell density was estimated by direct count in Neubauer camera under microscope. Growth rates $\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$ (Jiang *et al.*, 2011) and effective concentrations (ECx) were calculated for each compound and both species. μ and ECx were obtained by nonlinear regression on growth inhibition data and calculated in the drc package (Ritz *et al.*, 2015) in R 3.3.3 (R Core Team, 2017). Student *t*-test was used to compare EC50 values between species.

We exposed the isolated cyanobacterium to arsenate (As^{+5}) EC_{10} concentration for 96 hours at pH levels 7 and 9.5 to assess the removal dynamics. The pH value was adjusted daily (adding NaOH or HCl 1 M, when corresponded). The experiments were conducted in the same experimental conditions as before. The experimental samples were digested with 10 mL of concentrated nitric acid and 1 mL of 33% hydrogen peroxide in microwave and measures were done by Inductively coupled plasma optical emission spectrometry (ICP-OES). The metal removal efficiency (E, %) was calculated as $E = \frac{(C_0 - C_f) \times 100}{C_0}$ (Zhou *et al.*, 2012), where C_0 is initial and C_f final metal concentration of metals (mg L^{-1}) in the liquid solution.

RESULTS AND DISCUSSION

The isolated cyanobacterium was identified as *Cyanobium* sp. There is scarce record of the use of *Cyanobium* in toxicity assay (Yamagishi *et al.*, 2018). The *Cyanobium* isolated showed high tolerance to arsenic, the main metalloid present in the Atacama Desert waters, especially Salado River (Fig. 1C) and (Fig. 2A). EC_{50} values calculated for species and toxicants showed that *Cyanobium* sp. had higher tolerance than *C. vulgaris* for all the tested toxicants (Fig. 2A). Chlorella is commonly used in ecotoxicological studies due to its tolerance and detox ability (Landis and Yu, 2005).

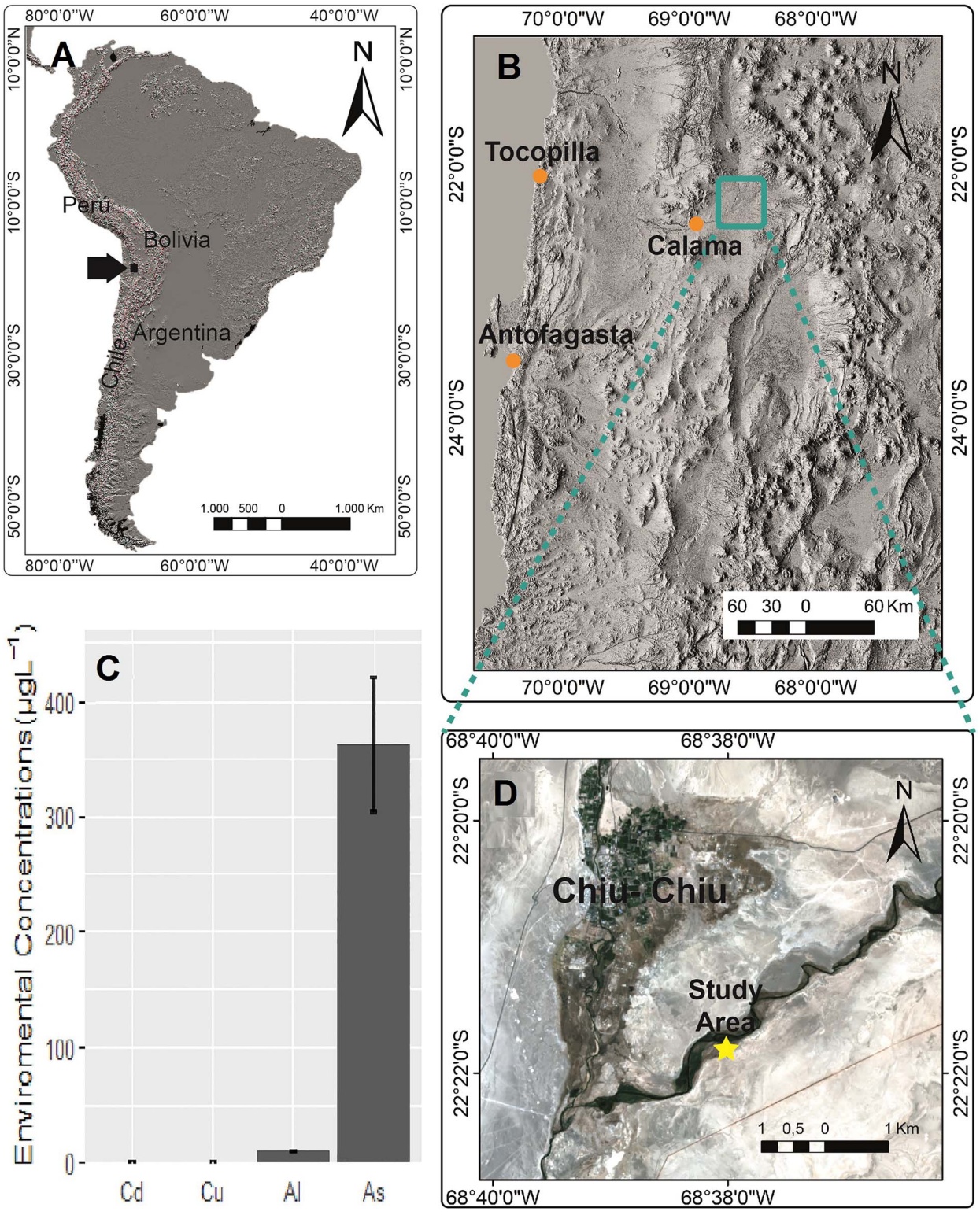

Fig. 1. (**A**), (**B**), (**D**) Maps of the study site. (A) Relative to its location within South America, (B) relative to its location within, Antofagasta region, North of Chile and (D) relative to Chiu Chiu village. (**C**) Metals quantified from water samples obtained from Salado River (Antofagasta Region, Chile). Cadmium, copper and aluminum were quantified by ICP-OES and arsenic by Atomic absorption spectroscopy (AAS).

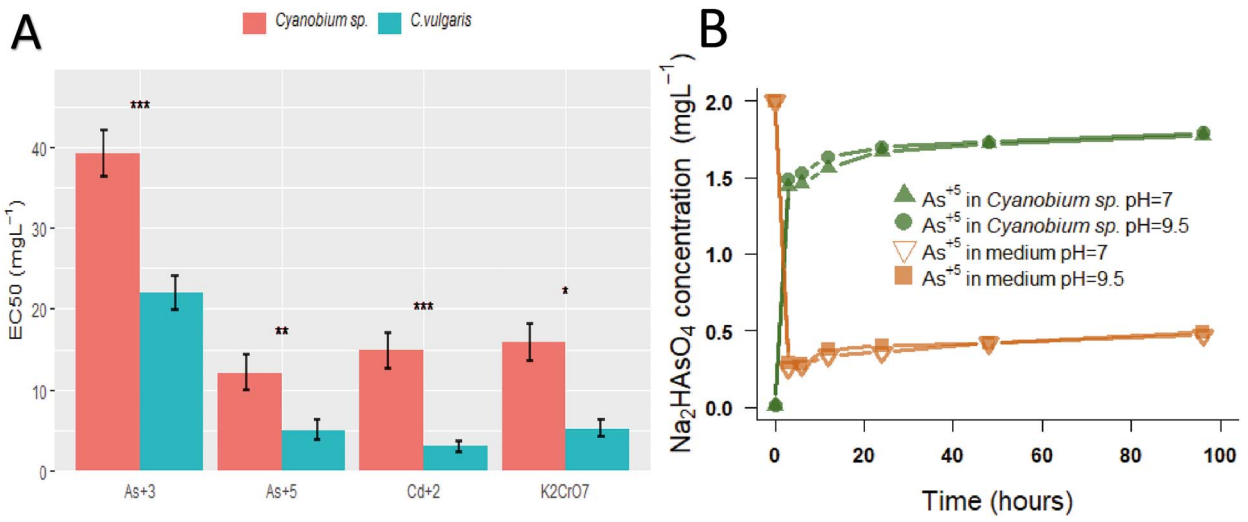

Fig. 2. (**A**) Removal of As^{+5} by *Cyanobium* sp. during 96-hour exposure. As^{+5} concentration measured in *Cyanobium* sp. biomass and medium of culture at two pH levels: 7.0 and 9.5. (**B**) Effective concentrations (EC50) obtained for *Cyanobium* sp. and *C. vulgaris* exposed to concentration ranges of As^{+3} ($NaAsO_3$), As^{+5} (Na_2HAsO_4), Cd^{+2} ($CdCl_2$) and the reference toxicant $K_2Cr_2O_7$. Asterisks show significant differences between species (Student *t*-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

The observed EC50 in *Cyanobium* sp. for As^{+3} was 3.36-fold higher than EC50 for As^{+5} (Fig. 2A), an uncommon result among prokaryotic species (Ferreira *et al.*, 2018; Franco *et al.*, 2015; Slyemi and Bonnefoy, 2012). This difference may be explained by relationship between As^{+5} uptake and phosphate group (Mkandawire *et al.*, 2004), due to these compound competes for the phosphate transporters. Therefore, at high concentration of PO_4 (as the culture medium used) organisms show a decreased As^{+5} uptake.

Removal efficiencies of As^{+5} by *Cyanobium* sp. after 96 hours of exposure were: $E = 40\%$ at pH = 5.5, $E = 88\%$ at pH = 7 and $E = 90\%$ at pH = 9.5 (Fig. 2B). Removal efficiency was higher in alkaline conditions. Biosorption of metals is greatly pH dependent: for some elements is decreased at very acid conditions (pH < 2.5) (Abbas *et al.*, 2014), whereas at high pH decreases the competition with H+, producing detoxification by another mechanism, the bioprecipitation (Blázquez *et al.*, 2005). Additionally, biosorption is a physicochemical process that reaches the equilibrium time in <2 hours (Ferreira *et al.*, 2018). As^{+5} removal by *Cyanobium* sp. reached 72% at pH 7 and 75% at pH 9.5 before 3 hours of exposure, with removal over 89% after 96-hour exposure (Fig. 2A), suggesting that biosorption may be used by *Cyanobium* sp. to remove As in the medium. However, specific mechanisms and biotransformation of arsenic forms displayed by *Cyanobium* from Salado River should be studied in the future to assess the potential scenarios of use in bioremediation.

Results obtained with *Cyanobium* from the Salado River are promising because the efficiency obtained was high compared with existing records. Broad phenotypic plasticity has been described previously for *Cyanobium* (Huber *et al.*, 2017), and responses to biotic and abiotic environmental drivers make it potentially useful for bioremediation, biotechnological purposes (Henrard *et al.*, 2011) or to the study of adaptive response to extreme environments.

CONCLUSION

Cyanobium sp., isolated from the Salado River located in the Atacama Desert, was more tolerant to tested metals and metalloid, compared with phytoplankton species used commonly in toxicity bioassay. Moreover, *Cyanobium* sp. removed As^{+5} from the medium culture within 3 hours of exposure. This could be explained due to the history of exposure of this strain to high natural concentrations of metal(oid)s and to the plasticity of *Cyanobium* to respond environmental conditions. Further research should be developed to identify specific mechanisms and their applications in biotechnological process.

AUTHOR CONTRIBUTIONS

Pablo Pérez-Portilla, Adriana Aránguiz-Acuña and Juan Araya collected and cultured the species. Adriana Aránguiz-Acuña and Juan Araya designed the experiments. Pablo Pérez-Portilla, Juan Araya and Karem Gallardo developed the experiments and the chemical analysis. Pablo

Pérez-Portilla, Adriana Aránguiz-Acuña and Karem Gallardo analyzed the data. All authors drafted, revised and approved the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The datasets used to support this study will be made available upon reasonable request.

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