Removal of arsenic by fungal strains of *Fusarium*

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ABSTRACT: Bioremediation of groundwater contaminates with arsenic using microorganisms has long been a research topic, has the advantage of being environmentally friendly and cost effective. Many fungi studied are resistant to arsenic such as *Trichoderma*, *Penicillium* and *Fusarium*. The genus *Fusarium* is very common contaminant in our region. Different strains of *Fusarium* were exposed to Arsenic. The strains were cultured for 2, large mycelia growth was observed. The level of arsenic decreased markedly. The reduction of the arsenic content in the water was very good, so it is feasible to carry out removal processes.

1 INTRODUCTION

In Argentina, the arsenic road begins in Jujuy and Salta, moves through Tucumán, La Rioja, Catamarca, San Juan, Chaco and Santiago del Estero; other provinces, until reaching the Atlantic coast, on that route, there is the dissolution of arsenical compounds, contaminating the aquifers that move through much of the territory of Argentina (Bhattacharya *et al.*, 2006; Bundschuh *et al.*, 2008, 2012; Nicolli *et al.*, 2012). The use of bacteria in ex situ groundwater bioreactor action processes, using equipment specially designed to carry out the process and reaching a complete biological treatment is possible (Pellizzari *et al.*, 2015).

The objective of our study is to observe the different mechanisms that can be performed by strains of the genus *Fusarium*, to develop bioassay systems of arsenic and other trace elements.

2 METHODS/EXPERIMENTAL

2.1 Materials

The experiments were carried out using *Fusarium* isolated in the province of Chaco. The colonies were obtained from the collection of microbiology, from cotton harvested in the region and from contaminated soil.

Fusarium culture was performed on potato dextrose agar (PDA) using 5, 10, 15, 20, 30 and 50 mgAs(V) L⁻¹ stock solutions by dissolving sodium arsenate (Na₂HAsO₄·7H₂O) in deionizing water. The serial dilution and counting methods were used in extended plates. *Fusarium* populations were counted as colony forming units (cfu) in triplicate in medium plates and colonies were counted after 7 days of incubation at 30°C.

The strains were purified on Bengal pink agar, which was incubated at 30°C for 7 days, mycelia growth was measured radials 8 hours.

2.2 Method

To isolate metal resistant fungal strains, the cultivation medium PDA with As(V). This experiment was carried out in triplicate with completely randomized design from 20 day at 30°C old pure cultures of each fungal isolate were inoculated individually onto Petri plates containing different concentrations of arsenate. Colony diameter for radial growth was measured during the incubation period. The degree of tolerance was compared to a control without As(V).

The cultures were identified at the specie level on the basis of macroscopic and microscopic characteristics. Colony diameter for radial growth (cm) was measured during the incubation period. Slide extension with cotton blue lactophenol for the observation of hyphae, conidiophores and conidia, examined under a Leica-DM2500 microscope in a 100× magnification. Pure cultures of common *Fusarium* were identified by comparing the phenotypic characteristic features of each fungi described in the Fungi and Food Spoilage (Pitt & Hocking, 2009).

Pure fungal isolates were maintained on agar Rose Bengal, under laboratory conditions. Arsenic tolerance was detected by growth in the presence of arsenic. Comparisons of growth were made, considering high tolerance to the strains that presented the highest growth rate.

All *Fusarium* strains were purified on Bengal Rose agar, which was incubated at 30°C for 7 days, mycelia growth was measured radials every 8 hours.

Phenotypic changes of *Fusarium* in the presence of arsenic were determined by sowing the strains in Bengal Rose Agar, formulated with stock solution of

Figure 1. System designed with natural air circulation.

arsenic at 1, 5, 10, 20, 40, 50, 60 and 80 mg As(V) L⁻¹, respectively. Fungal growth was controlled by radial measurement of mycelium every 8 hours for 7 days. Seeds were grown in arsenic-free medium as a control.

For the study of the removal of arsenic, the *Fusarium* inoculate were prepared from a pure fungal culture of 15 days and inoculated in a system designed with natural air circulation (Fig. 1), with a depth of 3 cm broth formulated with 0.5% of NaCl, 1% glucose, and 10 mg As(V) L⁻¹, using sodium arsenate salt.

3 RESULTS AND DISCUSSION

3.1 Results

The purpose of this investigation was to obtain soil fungi from arsenic-contaminated agricultural soils for their possible exploitation in arsenic bioremediation studies. A total of 5 *Fusarium* strains were enumerated. The most frequently encountered isolates from the soil samples of all the sites were *Fusarium oryzae*.

It was observed that the growth rate of all strains was similar below 30 mg As(V) L⁻¹. At higher concentrations, the species that presented considerable growth was *Fusarium oryzae*, is significantly high and can accumulate important amounts of arsenic, which reached a tolerance of 60 mg As(V) L⁻¹, above this value, no strain had positive growth at pH 7.

Fusarium oryzae grew very well in the culture trays with 1 mg As(V) L⁻¹. The mycelium covered the surface of the tray in 24 h. The flotation of the mycelium allowed draining the liquid of removal easily. The percentage of removal of arsenic was 90% in 15 days. We can say that *Fusarium* would be very good organism to work with toxic metals.

In the growth of *Fusarium* with As(V), phenotypic differences of the fungus were observed in comparison with the control culture. Sexual sporulation is observed, produced by cellular stress, which generates the presence of arsenic in the culture medium.

The pH of medium can affect metal-fungal responses by its effects on metal speciation and cell physiology and metabolism, indirectly and the capacity of bio-volatilization of arsenic by *Trichoderma* sp., *Neocosmospora* sp., *Rhizopus* sp. and *Penicillium* sp., were considered as good strains for the removal of arsenic (Srivastava *et al.*, 2011). *Fusarium oryzae*, was the only one that reached 60 mg As(V) L⁻¹, being the most tolerant (Monzon & Pellizzari, 2016).

4 CONCLUSIONS

The objective of this work was to study the biological removal of arsenic using strains of *Fusarium*, which in previous studies demonstrated high tolerance. The behavior of different species of *Fusarium* with highly aggressive metabolism against arsenic has been observed. The results obtained allow us to deduce that *Fusarium* has a high capacity to remove arsenic. Our fundamental purpose is to improve the quality of water in the area in order to reduce or reduce exposure to arsenic of the resident population through the application of low cost methodologies to improve water quality in general.

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REFERENCES

- Bhattacharya, P., Claesson, M., Bundschuh, J., Sracek, O., Fagerberg, J., Jacks, G., Martin, R.A. & Storniolo, A.R. 2006. Distribution and mobility of arsenic in the Rio Dulce alluvial aquifers in Santiago del Estero Province, Argentina. *Sci. Total Environ.* 358(1–3):97–120.
- Bundschuh, J., Litter, M.I. & Bhattacharya, P. 2012. Arsenic in Latin America, an unrevealed continent: occurrence, health effects and mitigation. *Sci. Total Environ.* 429: 1.
- Bundschuh, J., Nicolli, H.B., Blanco, M.C., Blarasin, M., Fariás S., Cumbal, L., Cornejo, L., Acarapi, J., Lienqueo, H., Arenas, M., Guèrèquiz, R., Bhattacharya, P., García, M.E., Quintanilla, J., Deschamps, E., Viola, Z., Castro de Esparza, M.L., Rodríguez, J., Pérez-Carrera, A. & Fernández Cirelli, A. 2008. Distribución de arsénico en la región Sudamericana. In: J. Bundschuh, A. Perez Carrera, M.I. Litter M.I. (eds) *Distribución del Arsénico en las Regiones Ibérica e Iberoamericana*. CYTED, IBEROARSEN, pp. 137–185.
- Monzón, E.H. & Pellizzari, E.E. 2016. Tolerancia al arsénico por *Fusarium oryzae*. IV Simposio Argentino de Procesos Biotecnológicos. Universidad Nacional de Quilmes.
- Nicolli, H.B., Bundschuh, J., Blanco, M.C., Ofelia C. Tujchneider, O.C., Panarello, H.O., Dapeña, C. & Rusansky, J.E. 2012. Arsenic and associated trace-elements in ground water from the Chaco-Pampean plain, Argentina: results from 100 years of research. *Sci. Total Environ.* 429: 36–56.
- Pellizzari E.E., Marinich L.G., Flores Cabrera, S.A. & Gimenez, M.C. 2015. Application of processes for the removal of arsenic by bacteria from groundwaters. *International Conference on Food and Biosystems Engineering*, 28–31 May 2015, Mykonos Island, FaBE 2015-044.
- Pitt, J.I. & Hocking, A.D. 2009. The ecology of fungal food spoilage. In: *Fungi and Food Spoilage*. Springer US.
- Srivastava, P.K., Vaish, A., Dwivedi, S., Chakrabarty, D., Singh, N. & Tripathi, R.D. 2011. Biological removal of arsenic pollution by soil fungi. *Sci. Total Environ.* 409(12): 2430–2442.