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Short Communication

Biosorption of heavy metals by obligate halophilic fungi

Amna Bano [b, c], Javaid Hussain [a], Ali Akbar [d], Khalid Mehmood [c], Muhammad Anwar [c], Muhammad Sharif Hasni [c], Sami Ullah [b], Sumbal Sajid [c], Imran Ali [a, c, e, *]

[a] Southwest University of Science and Technology, Mianyang, Sichuan 621010, China
[b] Department of Chemistry, University of Balochistan, Quetta 87300, Pakistan
[c] Institute of Biochemistry, University of Balochistan, Quetta 87300, Pakistan
[d] Department of Microbiology, University of Balochistan, Quetta 87300, Pakistan
[e] Plant Biomass Utilization Research Unit, Botany Department, Chulalongkorn University, Bangkok 10330, Thailand

HIGHLIGHTS

- First report of using obligate halophilic fungi for biosorption of heavy metals.
- Cadmium, copper, ferrous, manganese, lead and zinc were effectively removed from their medium by obligate halophilic fungi.
- *A. flavus* and *S. halophilus* showed best performance for the biosorption of heavy metals.
- Over all, Fe and Zn were most removed by obligate halophilic fungi.
- This study provides a cost effective solution of removing heavy metals.

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ABSTRACT

The presence of heavy metals in the environment poses a serious threat to human health. Remediation of this problem using microorganisms has been widely researched to find a sustainable solution. Obligate halophilic fungi comprising *Aspergillus flavus*, *Aspergillus gracilis*, *Aspergillus penicillioides* (sp. 1), *Aspergillus penicillioides* (sp. 2), *Aspergillus restrictus* and *Sterigmatomyces halophilus* were used for the biosorption of cadmium, copper, ferrous, manganese, lead and zinc. The metals were supplemented as salts in potato dextrose broth for the growth of obligate halophilic fungi and incubated for 14 days. The supernatant and biomass were obtained by the acid digestion method. The biosorption was screened by atomic absorption spectroscopy. All tested fungi showed moderate to high adsorption of heavy metals, amongst which *A. flavus* and *S. halophilus* showed the best average adsorption of all heavy metals studied, with an average of 86 and 83%, respectively. On average, Fe and Zn are best removed from the liquid media of obligate halophilic fungi, with an average of 85 and 84%, respectively. This pioneering study of biosorption by obligate halophilic fungi using inexpensive media in stagnant conditions provides a cost-effective environmental solution for the removal of heavy metals.

1. Introduction

Microorganisms are the most utilised organisms in biotechnology (Akbar et al., 2014, 2016). Global warming and climate change are creating extreme changes in the environment and scientists must be ready to counteract any upcoming problems (Ali, 2014; Ali et al., 2014a). Extremophilic microbes are tailor-made to live in extreme conditions (Ali et al., 2013b). Extremophiles are receiving attention from researchers due to the variety of extreme metabolites they harbour (Ali et al., 2013a). Microorganisms are frequently being isolated from hypersaline habitats and can grow *in vitro* in high salt concentrations and termed as halophilic microorganisms (Ali et al., 2016a). Generally, the salt-in strategy is exhibited in halophilic microorganisms, whereby the salt is accumulated in the cytoplasm to allow greater diffusion of water from the external environment (Ali et al., 2016a).

Halophilic microorganisms are presently screened in excess for their primary and secondary metabolites (Ali et al., 2014a).

* Corresponding author. Institute of Biochemistry, University of Balochistan, Quetta 87300, Pakistan.
E-mail addresses: imranalisheik@gmail.com, imran.ali@um.uob.edu.pk (I. Ali).
URL: http://www.rubem.com.pk

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Important biotechnological metabolites are frequently isolated from halophilic microorganisms, since they have been found to secrete poly-extremozymes which can work in various extreme conditions (Ali et al., 2015). Compatible solutes can work as stress protectants (Sepcic et al., 2010), biosurfactants, saline wastewater remediants (Ali et al., 2014b) bioplastics and antioxidants (Ali et al., 2014c). The basic advantage of having extremolytes from halophilic microorganisms is that they have the ability to work better in low water activity, which can be an unavoidable condition in several industrial operations or in living organisms such as humans, where different ions and metabolites are present in various conditions (Ali et al., 2016b).

It was pre-assumed that halophilic fungi could carry better biosorption in low water activity; therefore, obligate halophilic fungi were tested for their removal potential of heavy metals in the presence of salt concentration. To the best of our knowledge, this is the first ever report of using halophilic, especially obligate halophilic fungi, for the biosorption of heavy metals.

2. Materials and methods

2.1. Growth and heavy metal supplementation

The obligate halophilic fungi in this study were isolated from a man-made solar saltern in Phetchaburi, Thailand (Ali et al., 2013a). The fungi were deposited at the Thailand Institute of Scientific and Technological Research (TISTR) for public access. These fungi included *Aspergillus flavus* (TISTR 3637), *Aspergillus gracilis* (TISTR 3638), *Aspergillus penicillioides* (sp. 1) (TISTR 3639), *Aspergillus penicillioides* (sp. 2) (TISTR 3640), *Aspergillus restrictus* (3641) and *Sterigmatomyces halophilus* (TISTR 5926). With the exception of *A. flavus*, which was grown in at least 10% of NaCl, all fungi required a 5% minimum concentration of NaCl. Potato Dextrose Broth (PDB) (liquid medium) and Potato Dextrose Agar (PDA) (solid medium) were used for the growth of fungi (Ali et al., 2014a, 2014b; Hu et al., 2016a, b; Huang et al., 2017).

The fungi were inoculated into a series of 100 mL Erlenmeyer's flasks containing 50 mL of PDB supplemented with 1000 ppm concentrations of $CdCl_2.H_2O$, $CuCl_2.2H_2O$, $Fe(NH_4)_2SO_4.6H_2O$, $MnCl_2$, $Pb(NO_3)_2$ and $Zn(NO_3)_2$. The flasks were incubated for 14 days at laboratory temperatures along with control mediums having no fungal presence.

The fungal mats were obtained from the growth medium by filtering through Whatman filter paper and filtrate was collected and dried in an oven at 80 °C for 12 h and the dry weight was estimated (Jaeckel et al., 2005).

2.2. Acid digestion method

The protocol available from the United States Environmental Protection Agency (EPA, 1996) was used with the required modifications. Approximately 0.15 mg of dried fungal samples were placed in a 100 mL Erlenmeyer flask. Twenty mL of 65% HNO_3 and 3 mL of deionised (DI) water was added to each flask, which was then covered with a watch glass. After 24 h, the flasks were heated on a hot plate at 95–105 °C. Further heating was performed by adding 10 mL of 35% HCl until no reaction with the analyte was noticed. The samples were digested by reflux. After cooling, 3 mL of 30% H_2O_2 was added to each sample and the temperature was increased to 105 °C until the mixtures became colourless. After cooling, the solutions were filtered through ashless filter paper by repeated washing to remove the insoluble particles and then brought to a final volume of 50 mL with DI water. The same method was chosen for liquid filtrate.

2.3. Analysis of heavy metal remediation

The removal of heavy metals in the fungal medium was determined by using atomic absorption spectroscopy (AAS) (PerkinElmer 800, USA) by following the methodology explained by Srivastava and Thakur (2006). Biosorption from obligate halophilic fungi was calculated by the methodology explained by Ahmad et al. (2006).

2.4. Statistical analysis

Each test was performed in triplicate. The results are shown in averages with standard deviations. Microsoft Excel was used for statistical analysis.

3. Results

3.1. Cadmium removal

Cadmium (Cd) is considered toxic to life and has an unknown function in biogeochemical cycles. Fig. 1 shows that *A. flavus* and *S. halophilus* have been found to remove a notable concentration of Cd. The remaining fungi in this study absorbed Cd moderately. Cd has been found to suppress the growth of microorganisms (Xu et al., 2017).

3.2. Copper removal

Copper (Cu) has been found to activate various enzymes such as oxidases in fungi (Guillén and Machuca, 2008). *A. flavus*, *A. restrictus*, and *S. halophilus* were found to absorb the Cu considerably, with a good amount of growth as well (Fig. 2). Cu has been reported earlier to inhibit fungal growth (Nasim et al., 2008). In one study, it was found that Cu increased the acidity of fungal medium (Guillén and Machuca, 2008), which does not suit these obligate halophiles as they have been found to grow better in a neutral to alkaline medium (Ali et al., 2013a).

3.3. Ferrous removal

Ferrous (Fe) is considered essential for any living organisms because of its regulatory effect on the cell cycle (Weiss, 2005). In this study, all fungi have been found to show high absorbance of Fe from their media (Fig. 3). *A. restrictus* has shown the least absorbance by approximately 64% of value. High concentrations of Fe can cause cellular injury in living organisms (Britton et al., 2002).

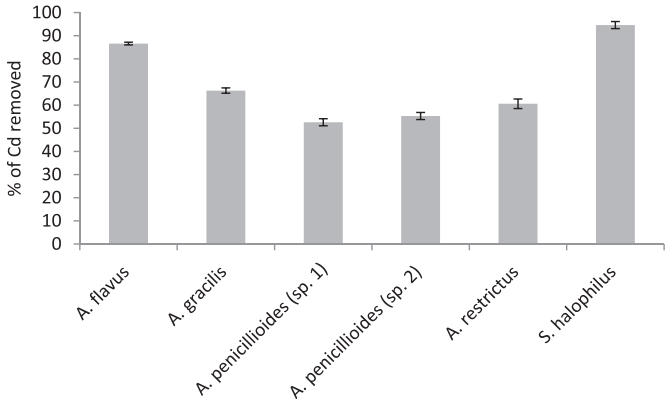

Fig. 1. Bar graph showing the percentage of cadmium removed by each obligate halophilic fungi, along with standard deviations.