



■ Author(s)

Bourzama G¹  <https://orcid.org/0000-0001-6728-4907>
Ouled-Haddar H²  <https://orcid.org/0000-0002-3880-7550>
Marrouche M³  <https://orcid.org/0000-0001-9202-2554>
Aliouat A¹  <https://orcid.org/0000-0003-4177-9017>

¹ Microbiology Laboratory, Department of Biochemistry, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria

² University of Mohamed Seddik Benyahia, Jijel, Laboratory of Molecular Toxicology, 18000, Jijel, Algeria

³ Department of Microbiology and Food Science, Faculty of Sciences, Seddike Benyahia University, Jijel, Algeria.

■ Mail Address

Corresponding author e-mail address
Ghania Bourzama
Department of Biochemistry, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria.
Phone: 213784303342
Email: gbourzama@yahoo.fr

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Iron Uptake by Fungi Isolated from Arcelor Mittal -Annaba- in the Northeast of Algeria

ABSTRACT

The Metal pollution is one of the major risks in the world nowadays. Iron is an essential metal for growth and proliferation of a vast majority of organisms, but it can be toxic to human health and other living beings in the environment at high concentrations due to its increased industrial activity. Fungi have a remarkable capacity to uptake and detoxify iron metal using different mechanisms such as bioaccumulation. Thus, the aim of this work is to study the ability of iron uptake by the fungal strains isolated from Arcelor Mittal -Annaba- in Algeria. Three strains were screened at high concentration of iron (1 g/l) and their capacity to uptake iron has been studied on Czapek Yeast Agar medium. The amounts of uptaken iron ions were estimated in the same liquid medium using Atomic Absorption Spectroscopy (AAS). The results of the iron uptake by these screened strains showed that *Cladosporium cladosporioides* uptakes the highest concentration of iron (347.7 ppm), *Aspergillus niger* was able to accumulate up to 170 ppm of this metal while, the lowest uptake of this metal was shown by *Penicillium citrinum* with 106.43 ppm. It was found that the spore germination of three fungal strains was low when the medium is supplemented with high concentrations of iron. This indicated the potential of these fungal strains as biological agents for removal of iron from the industrial effluents containing high concentrations of it.

INTRODUCTION

Iron is an essential metal for growth and proliferation of a vast majority of microorganisms. This essentiality derives from the importance of iron in its biochemically accessible valence status that plays a role in a wide variety of electron transfer processes and enzymatic activities (Kosman, 2003). However, it could be toxic to human health and other living beings in the environment at high concentrations.

The presence of this metal in waste stream ground and drinking water is a very serious concern since the ions of this metal are toxic to various life forms (Wasiu *et al.*, 2016). However, the heavy metals and other constituents leach into the soil and damage the flora and fauna on Earth (Gayatri *et al.*, 2017). The use of microorganisms for decontaminating the environment encumbered with heavy metal pollutants through biosorption is considered as a good option for bioremediation.

Recent developments in the field of environmental biotechnology include the search for microorganisms as potent sorbents for heavy metals. Fungi can tolerate and detoxify metals in various ways. Many mechanisms are used by fungal strains to eliminate metals, in instance adsorption, precipitation, enzymatic conversion, active intracellular metal uptake, and mainly biosorption (Ramírez Calderón *et al.*, 2020). Biosorption is a process of metal uptake conducted by either living or



dead biomass through the binding of metal ions on the cell wall and extracellular materials (Bishnoi & Garima, 2005). The natural screening of fungal strains may provide a fast and natural source for metal removal. Fe is known as the most environmental pollutant that is frequently produced from human and industrial activities. Thus, in this study, we aim to search for fungal strains that are able to eliminate the iron metal under laboratorial conditions.

MATERIAL AND METHODS

Isolation and identification of the fungal strains

Isolation and purification of the fungal strains from soil of Arcelor Mittal, in Annaba / Algeria (with 7.8 mg.Kg⁻¹ of iron) were performed on PDA (Potatoes Dextrose Agar) at 25 °C for 7 days (Pochon & Tardieux, 1962).

The identification was carried out based on the macroscopic and microscopic characters (Harrigan & McCance, 1976; Oteng-Gyang, 1984; Botton *et al.*, 1999). To obtain pure cultures, the isolates were subcultured on PDA at 25°C for 7 days and preserved at 4°C. These pure cultures served as a source of inoculums to be used in the assays of screening, uptake and fungal sporulation.

Screening of iron resistant fungal strains

This experiment identifies the most effective strains that have the ability to grow on PDA medium containing high concentrations of iron (FeSO₄, 7H₂O). Petri dishes were inoculated and the cultures were incubated at 25°C for one week. The diameters of the radial growth of these fungi were measured and compared with the control plates (without Fe) (Anahid *et al.*, 2011; Levinskaitė *et al.*, 2009).

Effect of iron concentrations on fungal sporulation

To counting the spores of the screened strains, a series of dilutions (10⁻¹ - 10⁻⁵) was performed from the spore solution of the screened strains. The amount of spores in the dilution 10⁻⁵ was counted on Malassez cell under optical microscopy (objective x100) (Hopwood *et al.*, 1985). The relationship used for calculation is as follows (Solis-Pereira *et al.*, 1993):

$$N = n \cdot 10^5 \cdot F$$

Where N is the number of spores per ml initial suspension, n is the medium number of spores in the counting cell and F is the dilution factor.

The results were expressed with the number of spores per ml of the initial suspension.

Iron uptake by the screened fungal strains

The screened fungal strains which showed the maximum capacity towards iron were further used for this study according to Levinskaitė *et al.*, (2009). Two ml from the fungal suspension with 10⁶ /ml of the screened strains was transferred to each tube containing different concentrations of Fe (2000, 3000, 4000, 6000, 8000, 10000, 12000 and 14000 ppm). The tube that contains the same components except iron was used as a control. The cultures were incubated on a rotary shaker at 25 ± 2° C for one week (Levinskaitė *et al.*, 2009). After incubation time, fungal biomass was collected by centrifugation at 9000 rpm for 20 min. The pellet thus obtained was washed three times with normal saline, dried at 105 °C for 3 hours and weighed.

The dry biomass of each strain (150 mg) was introduced into digestion vessels with 0.9 ml of nitric acid (HNO₃) and 2.7 ml of hydrochloric acid (HCL). After the digestion period (30 min), the containers were cooled to room temperature (about 30 min) and the solution volumes were supplemented up to 15 ml for each sample with deionized water and analyzed using atomic absorption spectroscopy (AAS) "AA-6200 SHIMADZU".

RESULTS AND DISCUSSION

Screening of iron tolerant strains

Three isolated strains: *Cladosporium cladosporioides*, *Penicillium citrinum* and *Aspergillus niger* were able to continue their growth in presence of high concentrations of iron in the laboratory (Fig. 1), this may be in agreement with the results of Gorbunova and Terekhova (1995), who found that 61% of the moulds are likely to grow on soils affected by ferric wastes. De Groot and Woodward (1999) have shown that the survival of the fungal species in the polluted soils can be related to their extrinsic characteristics, including cell wall composition, extracellular polysaccharides and the elimination of metabolites, leading to the binding or precipitation of metals such as iron (this normally occurs in the soil). Levinskaitė *et al.* (2009) reported in their study that the fungal strains: *Aspergillus niger* and *Penicillium oxalicum* were able to tolerate up to 20 mM of iron. Other researchers (Anahid *et al.*, 2011) have demonstrated that the fungal strain *Penicillium simplicissimum* can tolerate iron up to 2500 ppm. The values obtained from their study are lower than ours.

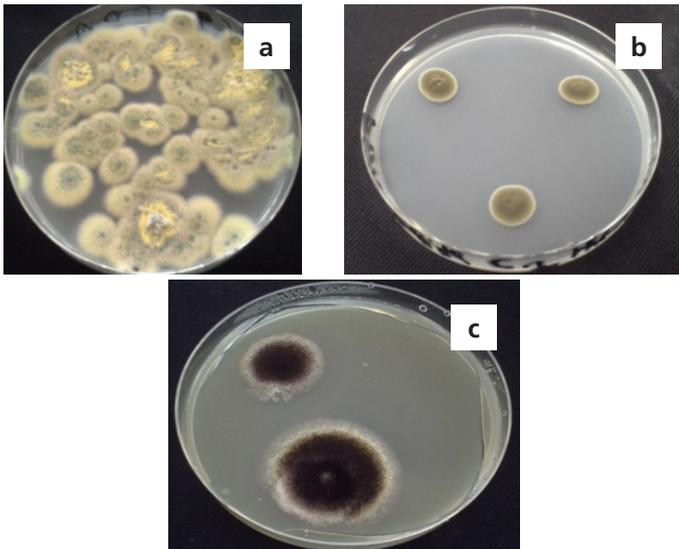


Figure 1 – 7 days-old fungal colonies of the isolated strains on PDA plates with 0 ppm of Fe concentrations: a. *Penicillium citrinum*, b. *Cladosporium cladosporioides*, c. *Aspergillus niger*

Effect of iron concentrations on fungal sporulation

This test allowed us to study the germination of the selected fungal strains in absence and in presence of different iron concentrations (Fig. 2).

The obtained results are consistent with the results of Wazeer and colleagues (2014) who studied the effect of heavy metals on the germination of *Isaria javanica* strain isolated from the soil and found that the spore germination was low when the medium is supplemented with iron. However, this result affirmed the used of screened strains in their vegetative form.

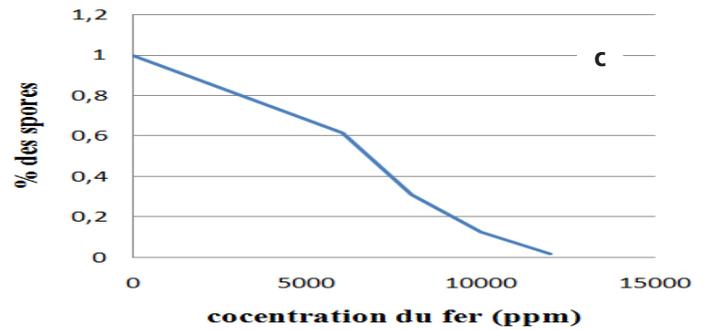
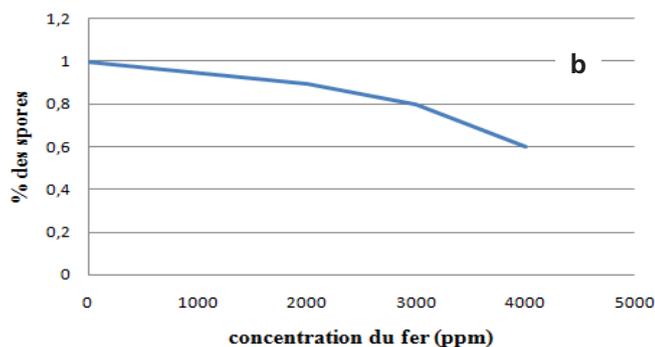
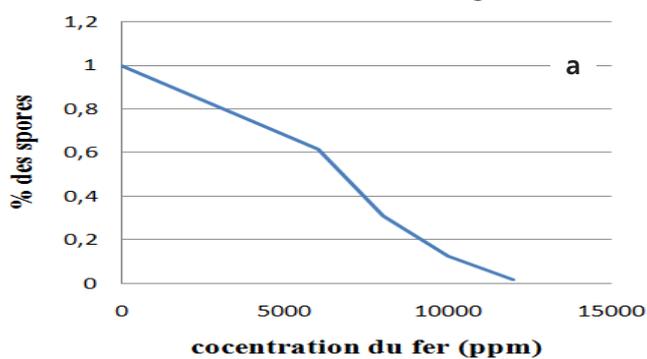
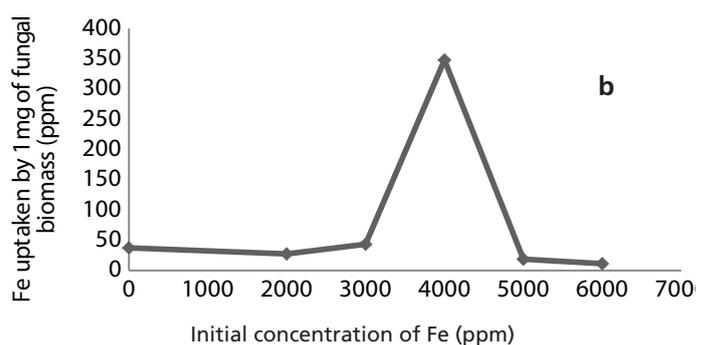
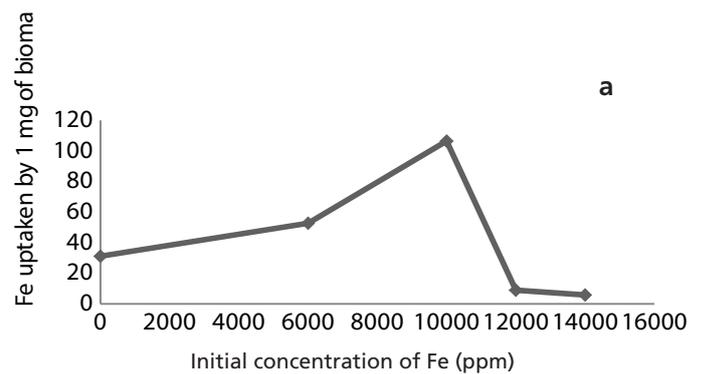


Figure 2 – Variation of fungal sporulation rate with iron concentration: a. *Cladosporium cladosporioides*, b. *Penicillium citrinum*, c. *Aspergillus niger*.

Iron uptake by the isolated fungal strains

This test studies the process of iron bioaccumulation by the three selected strains: *Cladosporium cladosporioides*, *Penicillium citrinum* and *Aspergillus niger* in absence and in presence of different initial concentrations of iron, the objective to assess the most effective accumulation capacity among them. The values obtained are expressed in ppm as shown in Figure 3.

The results of Levinskaité and his collaborators (2009) are in agreement with ours, they noted that the fungal strain *Penicillium oxalicum* is capable of accumulating up to 94.8% iron present in the medium, this high accumulation capacity means that 1 mM of iron added previously to the medium has no negative effect on the growth of the fungal biomass, this value is lower than ours due to the type of medium, the type of mould (species) and the range of the used iron concentrations.



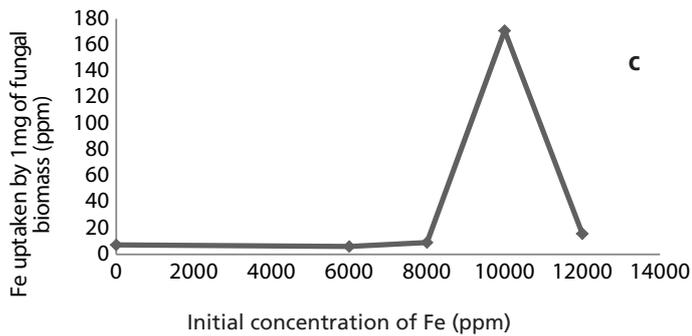


Figure 3 – Amounts of the uptaken Iron (Fe) by the screened fungal strains: a. *Penicillium citrinum*, b. *Cladosporium cladosporioides*, c. *Aspergillus niger*.

CONCLUSION

In this study, we isolated and screened three fungal strains that are able to uptake iron ions. It was found that the spore germination was low when the medium is supplemented with iron. It could retain relatively high quantities of iron derivatives with increased capacity towards the adsorption of the amending metal ion, although its specific mechanisms of the removal metal have not been completely clarified. These results indicate the potential applicability of these fungi for the remediation of heavy metals from polluted soils and water. Therefore, this study offers new agents for eco-friendly elimination of heavy metal from the environment.

CONFLICTS OF INTEREST

The authors hereby declare that we do not have any conflict of interest in regard to the information provided in this study.

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