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Resistant fungi isolated from contaminated uranium mine in Brazil shows a high capacity to uptake uranium from water

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HIGHLIGHTS

- Fifty seven fungi were isolated from the uranium mine area.
- In tolerance tests, 38% of the fungal isolates were tolerant to uranium.
- The majority of tolerant fungi were isolated from soil samples.
- Some fungi showed morphological changes and pigment (melanin) production.
- Biosorption tests showed species with high potential for uranium uptake from water.

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ABSTRACT

The Osamu Utsumi uranium mine occupies a 20 km^2 area in the city of Caldas, which is located in the state of Minas Gerais, Brazil. Since mining activities ended at Osamu Utsumi 24 years ago, the surrounding area has become contaminated by acid effluents containing high concentrations of uranium. Thus, the aim of this study was to assess the uranium bioremediation capacity of 57 fungi isolated from the mine area. In tolerance tests, 38% (22) of the fungal isolates were considered tolerant to uranium, including 10 *Penicillium* species. At a uranium concentration of 2000 mg L^{-1} 48 fungi did not exhibit mycelial growth index inhibition. Minimal inhibitory concentration (MIC) analysis showed growth of 25 fungi above a uranium concentration of 8000 mg L^{-1}. At high uranium concentrations, some fungi (i.e., *Talaromyces amestolkiae* and *Penicillium citrinum*) showed morphological changes and pigment (melanin) production. Among the fungal isolates, those considered to be more tolerant to uranium were isolated from soil and sediment samples containing higher concentrations of heavy metal. When comparing the results of resistance/tolerance tests with those for uranium biosorption capacity, we concluded that the fungi isolated from the Osamu Utsumi mine with the best potential for uranium bioremediation were *Gongronella butleri*, *Penicillium piscarium*, *Penicillium citrinum*, *Penicillium ludwigii*, and *Talaromyces amestolkiae*. Biosorption tests with live fungal biomass showed that 11 species had a high potential for uranium uptake from contaminated water.

1. Introduction

The Osamu Utsumi uranium mine occupies a 20 km^2 area in the city of Caldas, which is located in the state of Minas Gerais, Brazil, and was the first uranium mine in Brazil. Mining activities at this site began in 1982 for the production of yellowcake [triuranium octoxide (U_3O_8)] and ceased in 1995 with the depletion of uranium deposits (Souza et al., 2013).

Since the closure, the mine entered a decommissioning phase due to high levels of contamination by heavy metals in the water, soil and sediment (Cipriani, 2002; Fagundes, 2008).

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The primary problem in the Osamu Utsumi mine area is the acid mine drainage (AMD) from the waste tailings piles, which led to the formation of an acid lake (pH 2.5–3.5) in the open-pit mine (OPM). In addition to the low pH, this water is contaminated with high levels of uranium and other heavy metals. Currently, the treatment of acid water is performed by the addition of calcium hydroxide and flocculants, with the monthly cost of treatment ranging from US$ 200,000 to US$ 250,000 (Nóbrega et al., 2008).

The chemical treatment with calcium hydroxide forms an alkaline precipitate, and this dark mud with high concentrations of heavy metals is dumped back into the OPM (Ferrari, 2010). Even after water treatment, the results of analyses have indicated that high levels of heavy metals remain, with uranium concentrations of up to 0.3 mg L^{-1}, which may cause problems for the health of the population living in the region (CNEN, 2012).

Water-soluble uranium is characterized by its toxic potential, being harmful to aquatic organisms, plants, and humans, as well as all organisms exposed to effluents that are derived from uranium mines (Cumberland et al., 2016).

Conventional treatment methods for water contaminated with toxic metals suffer efficiency and cost limitations. An interesting alternative to these conventional treatments is a bioremediation strategy based on the use of indigenous fungi isolated from the contaminated site to remove uranium and other heavy metals from water (Hernahadini et al., 2014; Mani and Kumar, 2014).

In addition, according to Boopathy (2000), the use of biological-based methods avoids the risks associated with hazardous waste production related to the use of chemicals, providing greater safety and decreasing the disturbance to the environment.

Obtaining fungal biomass is a cheaper alternative to the conventional methods used to treat contaminated water, since fungi grow on cheap substrates and does not require many nutrients. Conventional methods for the removal of metal ions, such as chemical precipitation and membrane filtration, are extremely expensive when treating large amounts of water and do not show high efficiency at low concentrations of metal (1–100 ppm) (Yang et al., 2012). In addition, these methods generate large quantities of toxic products that require careful disposal.

For the application of fungal biomass, it is only necessary to add biomass to the contaminated water, partially or completely replacing the use of chemical agents. Moreover, using this remediation technique, it is possible to desorb uranium and reuse the biomass (Singh, 2006).

The fungal cell surface contains chitin, which has a high capacity for the removal of heavy metal ions (Bishnoi and Garima, 2005; Das et al., 2008; Gadd, 1986). *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp., *Saccharomyces* spp. and *Fusarium* spp. have been described as being excellent bioadsorbents of heavy metal ions, such as U, Th, Sr, Ni, Zn, Pb, Cr, As (Galun et al., 1983; Sheno Merrin et al., 1998; Tan and Cheng, 2003; Tsezos et al., 1997; Volesky et al., 1993).

There are four primary types of bioremediation that are relevant to contaminated uranium mine sites, including biosorption, biomineralization, bioreduction and bioaccumulation. The majority of studies on uranium bioremediation by fungi have focused on the mechanisms of metal biosorption by fungal biomass (Liang and Gadd, 2017; Qian et al., 2017; Vázquez-Campos et al., 2015).

Different local environments are colonized by distinct mycobiota, and microorganisms can have different biosorption capacities. Although the biosorption of other heavy metals by fungi has been widely studied, investigations regarding uranium biosorption by fungi are lacking.

Thus, the aim of this study was to conduct uranium resistance and tolerance tests on fungi isolated from the contaminated Osamu Utsumi uranium mine. Furthermore, the uranium biosorption capacity of the fungi was investigated using live fungal biomass, and the distribution of uranium on the surface of the cells was analysed.

The results obtained in this study will help to advance our understanding of the decontamination of environments contaminated with uranium and provide insight into how indigenous fungal communities can be used to support remediation efforts in the Osamu Utsumi mine. Finally, we highlight the pioneering work regarding the first mine built in Brazil, and we expect to provide tools for the use of biomass to treatment of contaminated water.

2. Materials and methods

The 57 fungi used in this study were isolated from the contaminated former Osamu Utsumi uranium mine. The fungi were isolated on Petri dishes containing Potato Dextrose Agar (PDA) (Oxoid, Basingstoke, UK) with or without tartaric acid 3.5% (used to acidify the media to pH 3.5) (Clesceri, 1998; Silva and Junqueira, 1995). Fungal identification was performed using previously described classical and molecular methods (Pitt and Hocking, 2009; Visagie et al., 2014; Yilmaz et al., 2014).

2.1. Uranium concentration measurement

The identification and quantification of uranium from samples was performed at the Chemistry and Environment Centre in the Nuclear and Energy Research Institute (CQMA/IPEN-SP).

The water samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES), while the soil and sediment samples were analysed by wavelength dispersive X-ray fluorescence spectroscopy (WDXRF).

2.2. Tests of resistance and tolerance to uranium

Initially, the isolated fungi were inoculated onto PDA in petri dishes and cultured in an incubator at 25 °C for 7 days. Subsequently, tolerance index (TI-U), minimum inhibitory concentration (MIC-U), and mycelial growth index (MGI-U) tests were performed. These tests have been shown to be well suited for determining the resistance of fungi to heavy metals (Anahid et al., 2011; Zafar et al., 2007).

2.2.1. Tolerance index (TI-U)

To determine the uranium tolerance index (TI-U) values, after growing the fungi as described above, conidia were suspended in phosphate-buffered saline (PBS) containing 0.1% Tween 80 and counted in a Neubauer chamber. Subsequently, the final suspension was adjusted to a concentration 1×10^5 conidia mL^{-1}, and 10 μL of the fungal suspension aliquoted onto the centre of the Petri dishes (Assunção et al., 2015). For the fungi that did not produce conidia, 5-mm diameter discs were aseptically cut from the mycelia of each fungus used as inocula (Jo et al., 2009).

For each fungus, 10 μL of the conidial suspension or a single 5-mm disc was inoculated onto the centre of a Petri dish containing PDA supplemented with 100 mg L^{-1} of uranium nitrate. Fungi were also inoculated onto PDA without uranium as a control. The plates were then placed in an incubator set at 25 °C for 7 days. Subsequently, the TI-U values were calculated by dividing the radius of the colony exposed to uranium by that of the control colony.

2.2.2. Mycelial growth index (MGI-U)

An MGI-U analysis was performed to study the effects of different uranium concentrations on fungal growth. First, 10 μL of a conidial suspension (1×10^5 conidia mL^{-1}) or a single 5-mm disc

was inoculated onto the centre of PDA plates supplemented with 0, 100, 500, 1000, 2000, 4000, or 8000 mg L^{-1} of uranium nitrate. Measurements of the colony diameters were performed every 24 h during 7 days of incubation at 25 °C, and the resulting values were used to calculate the MGI-U values (Dias et al., 2005) as follows:

$$\text{MGI-U} = \Sigma \frac{(D-Da)}{N}$$

Where MGI-U = mycelial growth index; D = current mean diameter of the colony; Da = diameter of the colony from the previous day; and N = number of days after inoculation.

All of the tests were performed in triplicate as previously described (Akhtar et al., 2013; Anahid et al., 2011; Fazli et al., 2015; Reisinger et al., 2008) with slight modification.

2.2.3. Minimum inhibitory concentration (MIC-U)

For the MIC-U analysis, 10 μL of conidial suspension of each fungus (1×10^5 conidia mL^{-1}) was inoculated onto PDA plates supplemented with 0, 100, 500, 1000, 2000, 4000, and 8000 mg L^{-1} of uranium nitrate. The medium was adjusted to a pH of 5.6, and MIC-U was defined as the lowest concentration of heavy metal that inhibited the visible growth of the analysed fungus.

2.3. Biosorption test

Biosorption tests using live fungal biomass were performed to determine if the fungi with the highest tolerance and resistance towards uranium also had the best capacity to remove uranium from water. To this end, the parameters used included a pH of 3.5 and a temperature of 25 °C, which are close to the values observed in the basins formed in the mine area.

2.3.1. Production of live biomass

All fungi isolated on PDA were also cultivated in liquid medium (Potato Dextrose Broth; PDB) to obtain live biomass. The cultures were grown for 7 days in a horizontal orbital shaking incubator set at 25 °C with shaking 150 rpm. After the growth of the fungal biomass, the cultures were centrifuged and the biomass was separated from the liquid medium by filtration using a paper filter. The biomass was then washed 5 times with ultrapure water before being used in the biosorption tests (Ahmad et al., 2005; Sana et al., 2015; Volesky and May-Phillips, 1995).

2.3.2. Contact of fungal biomass with uranium

After obtaining the biomass, a stock solution of 100 mg L^{-1} of uranium nitrate was prepared and the pH of the solution was adjusted to 3.5 using sodium hydroxide (0.1 N NaOH) or hydrochloric acid (0.1 N HCl) solutions as appropriate. The pH was measured using a digital pH meter (Kasvi).

The live biomass was weighed (0.2 g) and added to sterile 50-mL falcon conical tubes containing 10 mL of stock solution. The tubes were placed on a horizontal orbital shaker set to 150 rpm and shaken for 1 h at 25 °C. After mixing, the suspensions were vacuum filtered through 0.2-μm SFPES membranes (Allcrom) (Salvadori et al., 2014; Volesky and May-Phillips, 1995).

The concentration of uranium in the filtered solution before and after contact with the fungal biomass was determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

The amount of uranium retained by each fungus was expressed in terms of milligrams of metal per gram of biomass (Cabral et al., 2010; Salvadori et al., 2014; Yi et al., 2016), which was calculated using equation (1), while the biosorption efficiency was calculated using equation (2):

$$q = \left(\frac{Ci - Cf}{m}\right) V \quad (1)$$

$$E(\%) = \left(\frac{Ci - Cf}{Ci}\right) 100 \quad (2)$$

where q = amount of biosorbed uranium (mg U g^{-1} biomass); Ci = initial concentration of uranium (mg L^{-1}); Cf = final concentration of uranium (mg L^{-1}); m = mass of fungal biomass (g); V = volume of the reaction mixture (L); and E = biosorption efficiency (%).

2.4. Identification of uranium associated with fungal biomass

The identification of uranium on the surface of the fungal cell was performed using scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS) (Bozzola and Russell, 1998).

2.5. Statistical analysis

To analyse the results, Microsoft Excel and OriginPRO 8 were used to perform regression analysis, determine correlation coefficients, and extensions of these techniques (Pimentel-Gomes, 2009).

3. Results and discussion

Tolerance and resistance tests are typically performed to screen for fungi that tolerate heavy metal stress, as the results can indicate the potential of fungi for use in bioremediation. In particular, indigenous filamentous fungi isolated from contaminated areas can undergo selective pressure that can increase their tolerance and biosorption capacity for heavy metals (Ahmad et al., 2005; Gola et al., 2016; Sayer and Gadd, 2014; Shazia et al., 2013).

Thus, the survival mechanisms used by fungi in heavy metal-contaminated habitats includes cell wall binding of metals, intracellular/extracellular enzyme production, intracellular metal sequestration, extracellular metal sequestration and precipitation, suppressed metal influx, enhanced metal efflux, and complexation (biosorption, biomineralization, bioaccumulation and redox transformations), with biosorption being of the primary mechanisms promoting the tolerance of microorganisms towards heavy metals (Fomina et al., 2008, 2007; Ogar et al., 2014; Vázquez-Campos et al., 2015).

3.1. Tolerance index (TI-U)

Among the 57 fungal isolates tested, 22 (38%) exhibited a uranium tolerance index value of ≥1 (Fig. 1), 10 of which were members of the genus *Penicillium*, including five *Penicillium citrinum* isolates.

The other 12 tolerant fungi included 3 *Talaromyces* spp., 2 *Aspergillus* spp., and 1 isolate each of *Pochonia chlamydosporia*, *Umbelopsis ramanniana*, *Metarhizium robertsii*, *Gongronella butleri*, *Purpureocillium lilacinum*, *Mucor fragilis*, and *Trichoderma asperellum*.

The growth rate of the remaining 35 isolates was affected at a uranium nitrate concentration of 100 mg L^{-1}. These isolates were considered to be non-tolerant according to the criteria used in this study, as although some fungal growth was observed, their TI-U values (growth compared to the uranium-free control) were below 1.

Comparing the tolerant fungi to the sample types from which

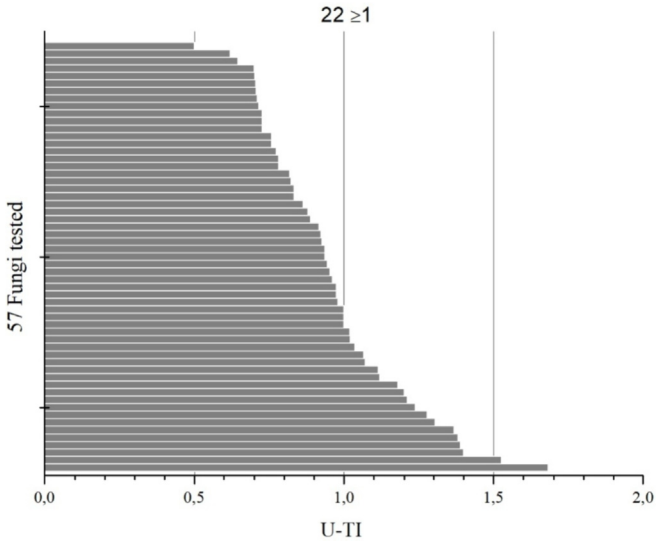

Fig. 1. Uranium tolerance index (TI-U) values of the 57 fungi isolated from the Osamu Utsumi uranium mine. Twenty-two fungi with TI-U values of ≥1 were considered tolerant to 100 mg L^{-1} of uranium nitrate.

these strains were isolated showed that the majority were isolated from soil (63.6%), followed by sediment (27.3%), and then water (9.1%). Similarly, the highest uranium concentrations were observed in the soil and sediment samples (50–245 and 268–577 mg kg^{-1}, respectively), while the uranium concentrations in the water samples ranged from 1.05 to 4.46 mg L^{-1}.

3.2. Mycelial growth index (MGI-U)

Measurements of the mycelial growth index (MGI-U) showed that despite displaying a reduced mycelial growth rate compared to the controls, all of the isolated fungi continued to grow on medium containing up to 500 mg L^{-1} of uranium. Furthermore, at 1000 mg L^{-1} of uranium nitrate, only one isolate (*Penicillium citrinum* no. 52) displayed completely inhibited growth, while at 2000 mg L^{-1}, nine isolates failed to show any visible signs of growth (Table 1, Supplementary data). These results showed that 56 and 48 isolates were able to grow in the presence of 1000 and 2000 mg L^{-1} of uranium, respectively. Considering that the highest uranium concentration measured in this study was 577 mg kg^{-1} in sediment samples, all fungi isolated appear to be capable of growing under representative conditions of the most contaminated environments at the Osamu Utsumi mine site.

In extreme environments with high metal concentrations, this stress can stimulate the development of tolerance mechanisms in fungi and other microorganisms to promote their survival. These strategies may include a decreased growth rate (which reduces energy and nutrient consumption) (Ayangbenro and Babalola, 2017), the production of organic particles, the production of melanin, or spore germination. Particularly for fungi, the impact this stress on growth may be observed as a decreased mycelial growth rate (Baldrian, 2003; Gadd et al., 2001; Gadd and Griffiths, 1980; Newby and Gadd, 1987), and the use of the abovementioned stress tolerance mechanisms can allow fungi to persist in the environment for long periods of time (Chávez et al., 2015; Magan, 2007).

3.3. Minimum inhibitory concentration (MIC-U)

Uranium concentrations of 1000, 2000, 4000, and 8000 mg L^{-1} inhibited the growth of 1, 3, 11 and 17 of the fungal isolates, respectively. Twenty-five isolates did not have an MIC-U determined, since they were able to grow at even at the highest concentrations tested (8000 mg L^{-1}; Fig. 2), and it was not possible to increase concentration further due to metal precipitation in the medium. The names of all the fungi described above are noted in Table 2 in Supplementary data.

Our results demonstrated that the fungi isolated from the Osamu Utsumi uranium mine are highly resistant to uranium. In addition, some fungi also demonstrated changes in morphology, pigmentation, and halo production around the colonies in culture medium supplemented with uranium concentrations above 2000 mg L^{-1}, including *Talaromyces amestolkiae* and *Penicillium citrinum* (Fig. 3).

Changes in the colour of the growth medium indicate the production of pigments by fungi, potentially in response to stress caused by exposure to toxic metals. The observed production of dark pigments (Fig. 3) suggests the synthesis of melanin ($C_{18}H_{10}N_2O_4$), which may have a role in the protection of fungi from chemical and radiological stressors (Ban et al., 2012; Barrow; Barrow and Aaltonen, 2001; Gruhn and Miller, 1991).

Fungi isolated from extreme environments have a high capacity for physiological adaptation and gene expression, traits aid in their survival under stressful conditions caused by heavy metals present in the environment (Dhar et al., 2013). These fungi may be more resistant to heavy metals than isolates of the same species isolated from uncontaminated sites, but because this capability will depend on the species tested and where it was isolated, some isolates may be tolerant and others react negatively to even low metal concentrations (Iram et al., 2013).

This ability to adapt quickly to the stresses caused by contaminated environments may increase the efficiency of the bioremediation processes compared to the use of fungi isolated from uncontaminated sites (Fazli et al., 2015). Therefore, the utilization of microorganisms isolated from the relevant contaminated site may be an important step in ensuring the efficacy of a bioremediation strategy (Ezzouhri et al., 2009; Fazli et al., 2015; Ferreira et al., 2010).

3.4. Biosorption tests

In addition to the uranium tolerance tests, experiments using live fungal biomass were performed to investigate the biosorption

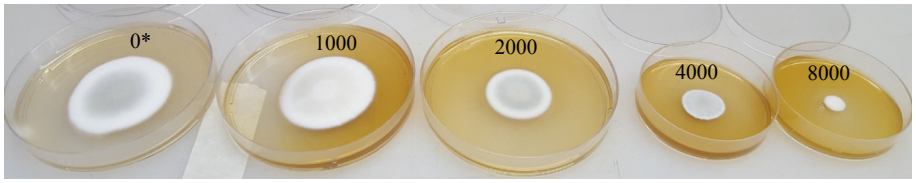

Fig. 2. Example of a fungus for which the MIC-U was not determined (MIC-U > 8000 mg L^{-1}). * Numbers indicate the concentration of uranium (mg L^{-1}) added to the medium (PDA).

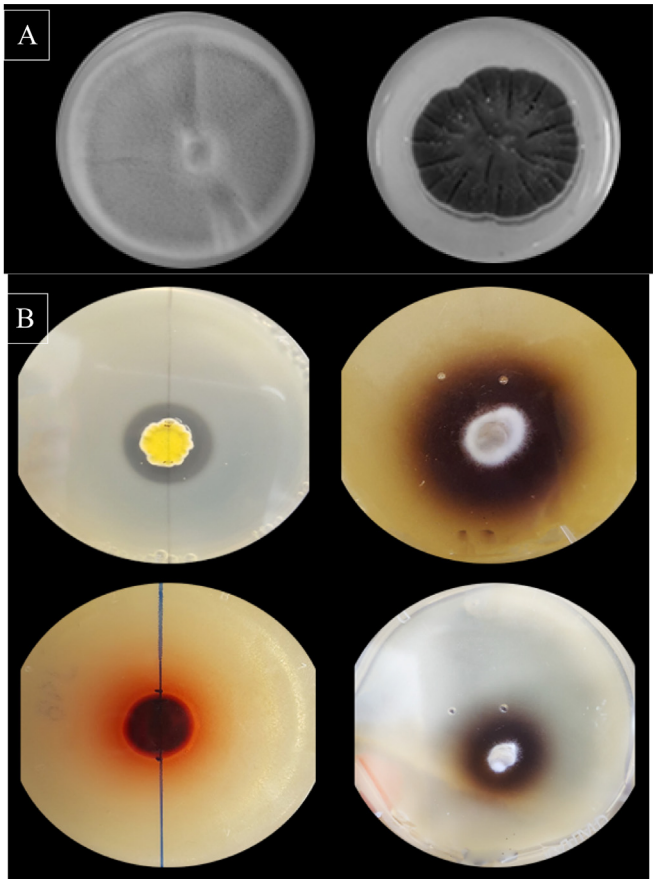

Fig. 3. (A) Images of *T. amestolkiae* and *P. citrinum* colonies on uranium-free PDA medium (controls) and (B) colonies grown on PDA medium supplemented with high uranium concentrations (above 2000 mg L^{-1}) showing changes in morphology, pigmentation and halo production around colonies.

capacity of the isolated fungi towards uranium. In the biosorption tests, all 57 fungal isolates were able to remove uranium from aqueous solution, but the amount removed was different for each isolate (Fig. 4). The parameter q (milligrams of uranium removed per gram of fungal biomass) was used to describe the ability of each isolate to remove uranium from solution, where fungi with q values greater than three were considered to be the best biosorbents (Table 2, Supplementary data).

Eleven of the fungal isolates were identified as promising candidates for bioremediation by having q values greater than 3 and being able to remove more than 60% of uranium from solution, including *Penicillium piscarium* isolates, a *Gongronella butleri* isolate, a *Phoma nebulosa* isolate, and a *Talaromyces amestolkiae* isolate.

Our results showed that fungal biosorption was high in solutions with a pH value of 3.5. Typically, the biosorption process become less effective in solutions with a pH $\leq$ 4 due to competition from hydrogen ions for binding sites on the cell wall, which leads to a decrease in the number of negatively charged binding sites available for the sorption of cations such as UO_2^{2+} (the predominant form of uranium in acidic and oxidizing environments) (Farhan and Khadom, 2015).

In acidic environments contaminated with uranium, remediation is challenging, particularly when concentrations are less than 100 mg L^{-1}, as conventional processes are ineffective at removing uranium at these relatively low concentrations. Fungi are known to be important for immobilizing small amounts of uranium dissolved in water (Ogar et al., 2014), and in this study, the isolated fungi tested were able to remove between 10 and 80% of uranium from solution, 100% of uranium remained in solution was not removed in the non-biological controls.

The filamentous fungi used in this study were able to accumulate between 0.3 and 4.0 mg metal/g biomass^{-1}, which is comparable to that observed other studies. For example, Fazli et al., (2015) observed that the filamentous fungi they investigated were able to accumulate between 2 and 7 mg metal/g biomass^{-1} when challenged with a solution of cadmium. Another study also showed that fungal species isolated from phosphate rocks with high concentrations of heavy metals were tolerant to high concentrations of uranium and were also excellent biosorbents of heavy metals (Abd El Hameed et al., 2015).

To assess for any possible correlation between the uranium tolerance (TI-U) and uranium biosorption (q) for the fungal isolates, the TI-U value was plotted against the q value for each isolate. The Pearson correlation coefficient of the data was 0.1, indicating that was not a linear correlation between uranium tolerance and uranium sorption under these conditions (Fig. 5).

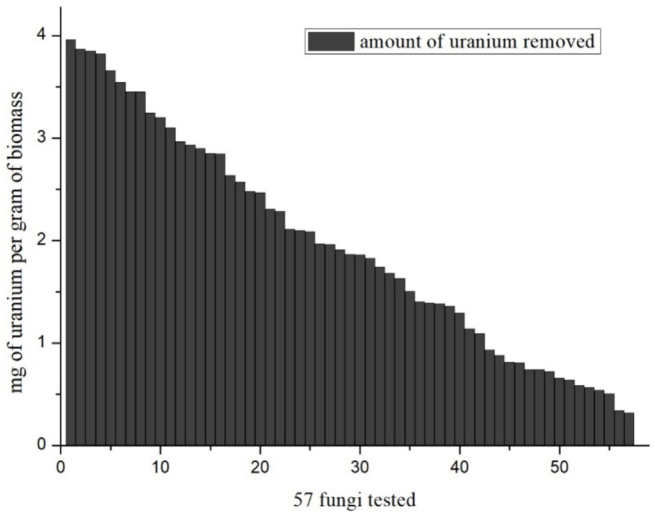

Fig. 4. Amount of uranium removed by each of the 57 fungal isolates from the Osamu Utsumi uranium mine.

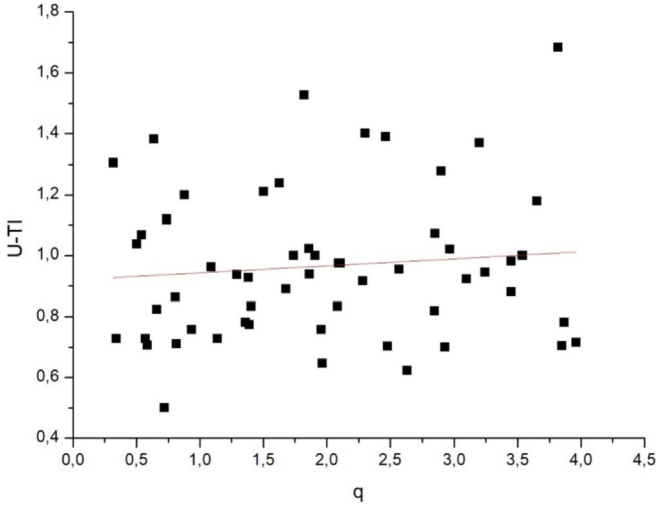

Fig. 5. Pearson correlation coefficient between uranium the tolerance index (TI-U) and uranium biosorption values (q) for the 57 fungi isolated from the Osamu Utsumi uranium mine.

Despite the lack of any clear trends across the whole dataset, focusing on fungi that met the criteria of having TI-U values greater than one and q values greater than three resulted in the identification of five fungal isolates that showed positive results across all experiments, including *Gongronella butleri*, *Penicillium piscarium*, *Penicillium citrinum*, *Penicillium ludwigii*, and *Talaromyces amestolkiae*.

Table 2 in the Supplementary data shows the results of the tolerance, MIC, and biosorption tests for each isolate.

3.5. Scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS)

Among the five fungal isolates identified as having the highest potential for biosorption, scanning electron microscopy (SEM) was used to evaluate *Penicillium piscarium* (no. 25, Table 2, Supplementary data) to test the hypothesis that adsorbed/precipitated uranium would be located on the cell surface.

The SEM images in Fig. 6 show hyphae of *P. piscarium* grown in the absence (Fig. 6a and c) and presence (Fig. 6b and d) of uranium. Compared to the uranium-free system, *P. piscarium* biomass grown in the presence of uranium appears to display additional features present on the hyphal cell surfaces that could be uranium-containing precipitates. Further analysis via energy dispersive spectroscopy (EDS; Fig. 7) confirmed the presence of uranium within the biomass matrix of *P. piscarium*, similar to the results of studies on fungal uranium precipitation performed by Liang et al. (2015).

Uranium immobilization on the fungal cell surface may occur due to biosorption or biomineralization processes (Majumder and Wall, 2017). Biosorption involves the direct binding of ions to cellular ligands, such as hydroxyl, carboxyl, amine, and/or phosphoryl groups (Vázquez-Campos et al., 2015). In contrast, biomineralization describes processes by which biological activity induces the nucleation and precipitation of new solid phases. For uranium, biomineralization typically involves the liberation of ligands (i.e., carbonate, oxalate, phosphate) from cellular or extracellular substances that precipitate out with the uranium or by redox manipulation (i.e. through the reduction of U(VI) to less soluble U(IV) phases) (Newsome et al., 2014).

Biomineralization generally requires the activity of live microorganisms, while either living or dead biomass can be used for biosorption. Both live and dead fungal biomass generally exhibits a high capacity for heavy metal uptake, even under extreme conditions of low pH values (Mani and Kumar, 2014).

4. Conclusions

The results of our study demonstrate that fungi isolated from the contaminated Osamu Utsumi uranium mine have a high resistance to uranium. This is the first study on fungal bioremediation conducted in the area of the mine, which is in the process of being decommissioned. The production of pigments, such as melanin, during fungal growth in the presence of high uranium concentrations may be an important tolerance mechanism to this heavy metal, and along with SEM images, indicates that the fungi may be capable of precipitating uranium.

The high uranium biosorption capacity of these fungi may

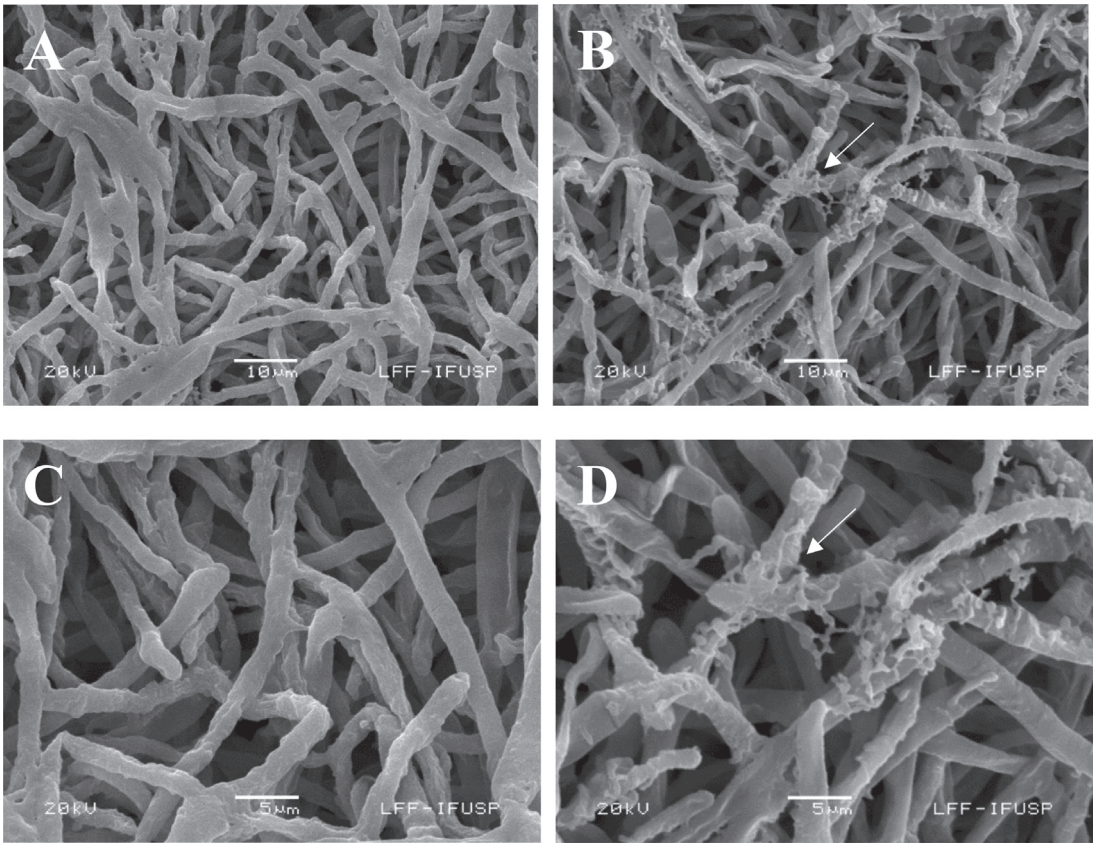

Fig. 6. Scanning electron microscopy (SEM) of live biomass of *Penicillium piscarium*. Micrograph A shows the biomass of the fungus that has not been exposed to uranium, whereas micrograph B shows biomass used for uranium biosorption (the arrows show the binding of uranium on the surface of the fungal biomass). Micrographs C and D are zoomed images of hyphae from panels A and B, respectively.

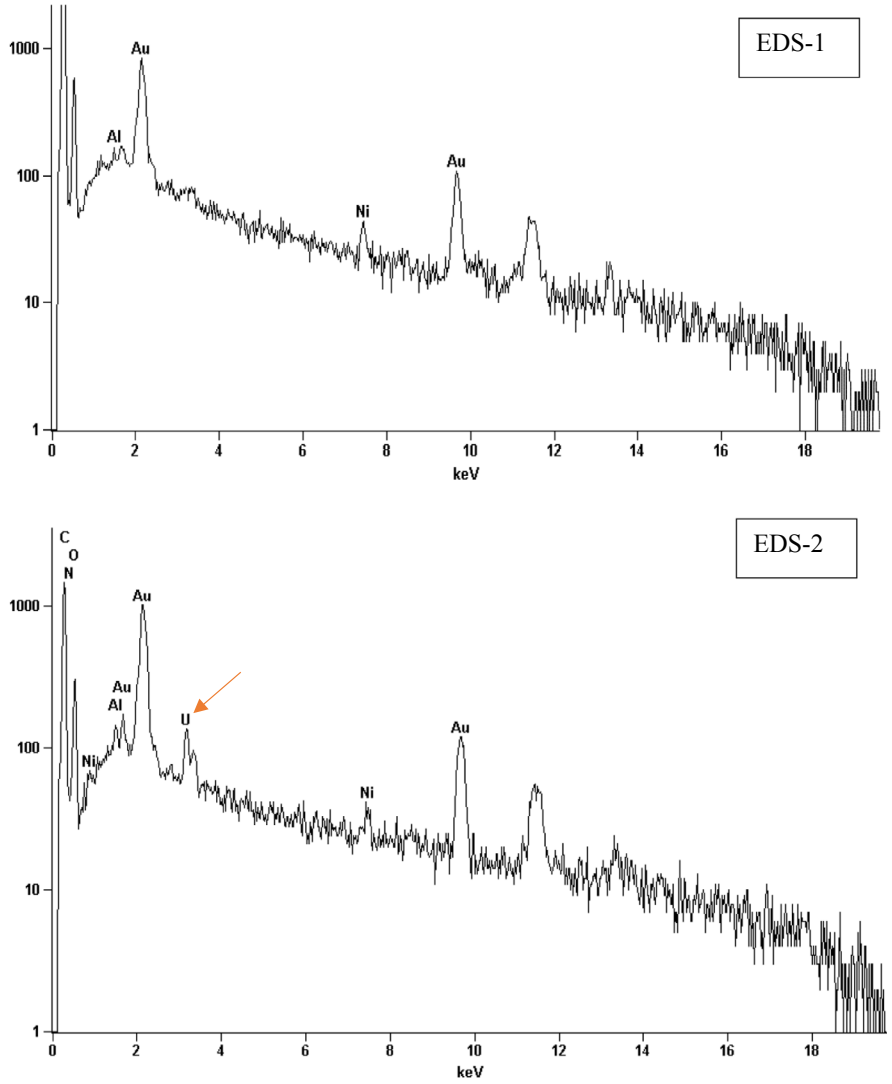

Fig. 7. Energy dispersive spectroscopy (EDS) analysis of the elements on the *Penicillium piscarium* hyphal surface.

represent an important advance in the field of biotechnology and offers a potentially efficient and cost-effective method to aid the decontamination of areas contaminated with uranium. Therefore, the application of fungal bioremediation processes could assist traditional decontamination processes, helping to solve one of the primary problems currently encountered in mining areas, which is the solubilization of uranium and other contaminants as a result of acid mine drainage. This ability would aid in improving the quality of the water that is discharged as effluents into the rivers of a given region, thereby protecting individuals and the environment from the harmful impacts of contamination.

We hope to apply this technique to support the decontamination of acid water formed in the Brazilian uranium mine in the near future.

Declaration of competing interest

The authors declare no competing financial interest.

EDS-1 represents the fungal biomass that was not in contact with uranium (gold metal was used for contrast), whereas EDS-2 represents the fungal biomass that took up uranium from solution (arrow shows the uranium trace).

CRediT authorship contribution statement

Ednei Coelho: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing - original draft, Writing - review & editing. **Tatiana Alves Reis:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Marycel Cotrim:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing. **Thomas K. Mullan:** Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Benedito Corrêa:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

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