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RESEARCH ARTICLE

Isolation and Identification of Iron, Chromium, and Manganese Tolerant Filamentous Fungi from Mining Soil

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Abstract: ***Background*:** In this study, filamentous fungi from iron mining soils in the Amazon were isolated and identified as *Talaromyces verruculosus*, *Trichoderma pseudoasperelloides*, *Penicillium rolfsii, Aspergillus cf. pseudoviridinutans*, *Aspergillus niger*, *Purpureocillium lilacinum*, and *Penicillium cf. guaibinense*.

***Objective*:** The objective of this study was to evaluate the isolated strains for radial growth rate (VCR) on solid media and liquid in the presence of metals.

***Methods*:** All these strains showed adaptive behavior in the presence of metals (Fe^{2+}, Mn^{2+}, and Cr^{3+}), but not significantly compared to controls.

***Results*:** The *T. verruculosus* strain was selected to evaluate its growth capacity in solid and liquid media, enriched with 1, 10, and 20 mg/L of iron, chromium, and manganese, respectively.

***Conclusion*:** *T. verruculosus* strain showed tolerance to the concentrations of the metals studied. Therefore, we can suggest that this characteristic of metal tolerance (Fe^{2+}, Mn^{2+}, and Cr^{3+}) exhibited by fungi isolated from Amazonian environments may indicate the potential for bioremediating areas polluted by heavy metals.

Keywords: Amazon fungi, heavy metal pollution, metal contamination, extreme environments, *Talaromyces verruculosus*, microorganisms of soil.

1. INTRODUCTION

Increasing industrialization and population growth around the world (anthropogenic action) have increased human exposure to environmental pollution [1]. Currently, pollution caused by heavy metals is one of the major issues worldwide [2]. Most of the pollution caused by heavy metals is severe, long-term, and non-reversible in nature [3]. The most common heavy metals that act as pollutants include arsenic (As), lead (Pb), mercury (Hg), chromium (Cr), zinc (Zn), cadmium (Cd), copper (Cu), and nickel (Ni) [4]. These have been listed as pollutants of priority concern by the US Environmental Protection Agency (USEPA) [5].

Excessive exposure of the human body to heavy metals is deadly, given that their bioaccumulation in human cells and tissues causes neurological, cardiovascular, hematological, reproductive, and immunological disorders. Other risks promoted by heavy metals include hypertension, skin cancer, and diabetes [6-8].

The methods available for soil and water remediation can be categorized as chemical, physical, or biological. Biological methods can be implemented either in the polluted area or as remediation strategies [9].

Fungi can successfully break down or transform complex toxic contaminants into simpler or less toxic agents. This process can be achieved by using different strategies that fall into two general categories: biosorption, which includes binding metal to the surface, and bioaccumulation, which involves intracellular uptake of metals *via* cellular metabolism [6]. These transformations change the ionic state of metals, which may affect their solubility, mobility, and bioavailability [10].

The fungi commonly reported as capable of undertaking bioremediation of heavy metals include *Rhizopus oryzae*, *Aspergillus* spp. (*versicolor, terreus, niger* and *fumigatus*), *Penicillium chrysogenum*, and *Gloeophyllum sepiarium* [11]. For example, Khan *et al.* (2019) showed *A. fumigatus* and *A. flavus* to achieve high removal efficiencies for Pb of 99.20% and 99.30%, respectively, while 96% and 95.50% of Hg were removed by *A. niger* and *A. terreus*, respectively.

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The Amazon is the largest Brazilian biome and contains a wide biodiversity of fauna and flora. However, this biome is also the target of high levels of mineral exploration activity, seeking metals, such as gold, iron, manganese, and aluminum. Thus, this biome is highly relevant to studies addressing the diversity of microorganisms present in such soils. Fungi isolated from environments that are already contaminated by metals tend to adapt naturally to the environment and consequently express interesting enzymes that have the capacity to act in the process of bioremediation of micropollutants [12].

In the present study, different species of filamentous fungi present in the soil of an area undergoing iron ore exploration activity in the state of Amapá, Brazil, were isolated and identified. The isolates were characterized and their tolerance towards the metals Fe^{2+}, Cr^{3+}, and Mn^{+2} at different concentrations was tested.

2. MATERIALS AND METHODS

2.1. Reagents and Solvents

Salts of the metal to be tested were obtained as follows: $Cr(NO_3)_3.9H_2O$ (98%) from IMPEX® (São Paulo, Brazil); $FeSO_4.7H_2O$ (99%) from Reagen (São Paulo, Brazil); $MnSO_4.H_2O$ (99%) from Vetec (São Paulo, Brazil); and NaCl (99%) from Qhemis. The HCl (37%) and HNO_3 (65%) were acquired from Qhemis. Cotton blue lactophenol dye was purchased from Newprov and chloramphenicol (98%) from Sigma-Aldrich (São Paulo, Brazil). Malt extract was purchased from Kasvi (Paraná, Brazil) and Sabouraud dextrose agar from Himedia (São Paulo, Brazil).

2.2. Obtaining Soil Samples

Soil samples were collected on the premises of the company UNAMGEN *Mineração e Metalurgia SA*, located in the municipality of Mazagão (State of Amapá, Brazil). The samples were collected at four random locations inside the mine, with the following geographical coordinates: sample (A): 0.398175º, -51.755533°; sample (B): 0.399548º, -51.755179º; sample (C): 0.398958º, -51.754728º; and sample (D): 0.398143º, -51.755168º (Fig. **1**). The collection was carried out on May 28, 2021, in the rainy season and at the soil temperature of 28°C. They were obtained at a depth of 20cm from the surface, using a sterilized stainless pipe and spatula [13], with some modifications. The samples were then transferred to previously sterilized and labelled polyethylene containers. Approximately 100 g of soil was collected in triplicate from each sampling point. These samples were dried in an oven at 50°C for 24 hours and then homogenized and passed through sieves with a mesh size of 2.0 mm.

2.3. Determination of Metal Content in Soil Samples

In the soil samples, we used the method of digestion with aqua regia, following the methodology of Pelozato *et al.* [14]. Firstly, 1 g of soil was weighed and transferred to a test tube containing 3 mL of distilled water, followed by the addition of 10 mL of aqua regia (3:1). The test tubes were placed in the digester block at 80°C for 4 hours. After cooling, the mixture was filtered using a filter paper. The residues that adhered to the paper were washed with distilled water and the volume of the solution was measured to 45 mL, in accordance with the methodology of Kazi *et al.* [15]. For the negative controls, the same procedure as described above was followed, but without adding the soil sample. All the analyses were carried out in quintuplicate. The resulting solutions were stored in Falcon tubes (Corning) and kept at 4°C until analysis.

Lastly, the solutions were analyzed by means of atomic absorption spectrophotometry (Shimadzu, model AA-630), using mixtures of acetylene as the oxidizing and fuel gas. Hollow cathode lamps (Hamamatsu Photonics K.K, model A802AC, Shizuoka, Japan) for Ni, Cu, Mn, Cr, Mg, Co, Fe, and Pb were used in the analysis. The analytical curves were prepared from stock solutions of 1000 mg/L, with dilution to the specific concentrations of the curve for each element under analysis. A pH meter (pH Pro Line Lab, São Paulo, Brazil) was used to determine the pH of the soil collected.

2.4. Isolation of Fungi from Mining Soil

Filamentous fungi were isolated using the serial dilution technique. A stock solution, prepared with 10 g of each soil sample, was transferred separately to an Erlenmeyer flask (250 mL) containing 100 mL of saline solution (0.9% NaCl) that had been sterilized in an autoclave (Phoenix, model AV-75, São Paulo, Brazil) for 20 min at 121°C. The samples were homogenized in an orbital incubator (Shaker, Lucadema®, model Luca-222, São Paulo, Brazil) for 60 min at 150 rpm to break up all the soil aggregates and expose the microbial cells to the solution (1:10 w/v). Serial dilutions of 1:100, 1:1000, and 1:10,000 were prepared from the stock solution. Subsequently, using a micropipette, 300 µL of each solution was sown in Petri dishes containing Sabouraud dextrose agar medium (2%) at pH 5, containing chloramphenicol (100 mg/L) that had been autoclaved beforehand. Plating was carried out in triplicate for each dilution. The plates remained in B.O.D. (Lucadema®, model Luca-161/03, São Paulo, Brazil) for 5 days at 28 °C. After incubation, the microorganisms were successively plated using the central point technique in Sabouraud dextrose agar (2%, pH 5) to obtain pure colonies.

2.5. Morphological Study and Molecular Identification of Isolates

The isolates were initially identified based on their macroscopic characteristics, such as the morphology, color, and appearance of the colony, and microscopic characteristics, such as the septation of the mycelium and the shape and texture of the conidia under a phase-contrast microscope (Nikon E-200, São Paulo, Brazil) through microcultures on slides in accordance with the methodology of Sena *et al.* [16] with modifications. To confirm these identifications, molecular approaches were also applied at the Multidisciplinary Center for Chemical, Biological, and Agricultural Research (CPQBA), University of Campinas, Paulínia, SP, Brazil (http://webdrm.cpqba.unicamp.br/).

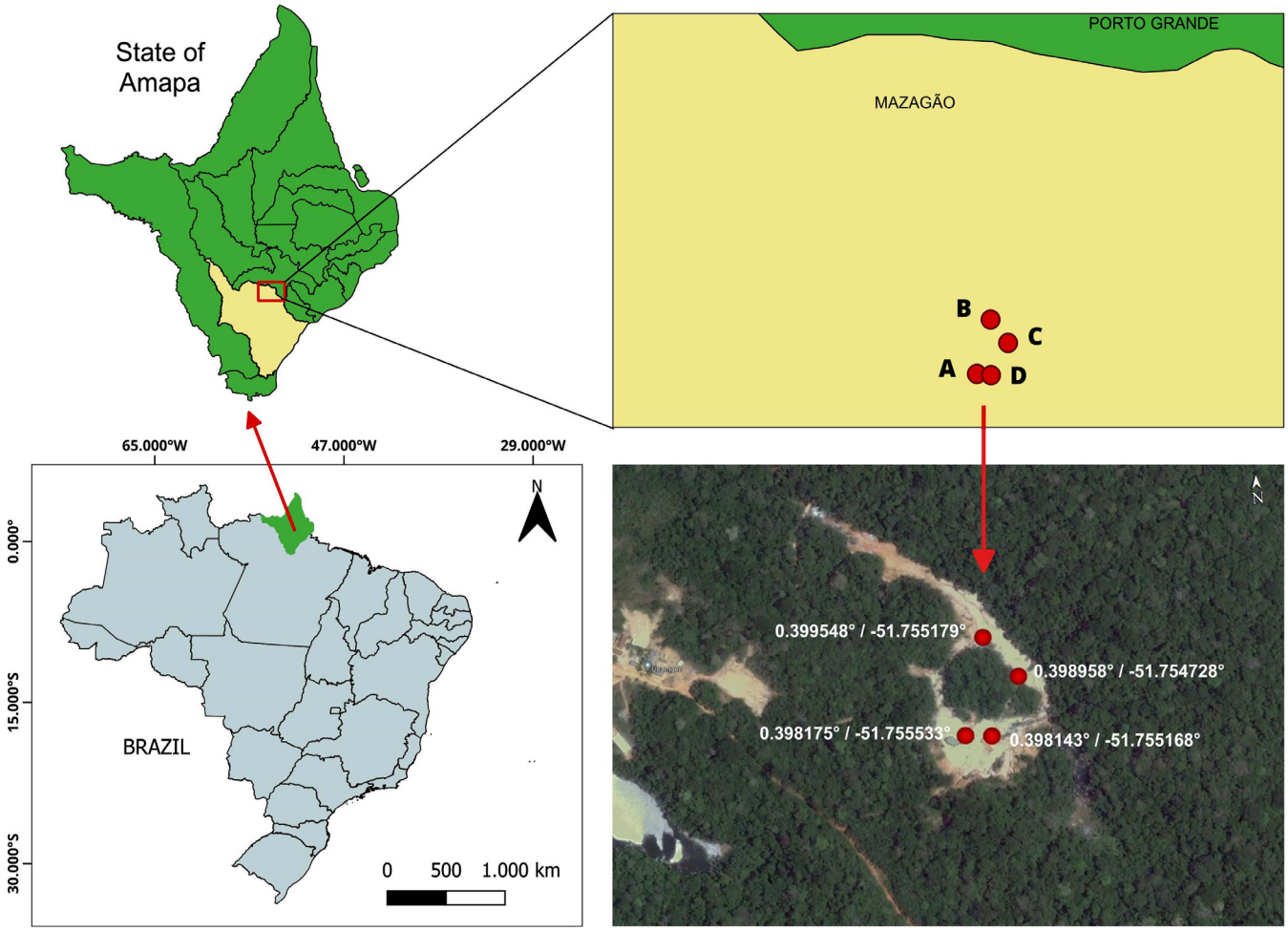


Fig. (1). Location of the collected area in Mazagão (State of Amapá, Brazil). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The genomic DNA from the culture was purified using the phenol DNA extraction protocol [17]. Amplification of the markers for the genes was carried out by means of PCR methodology, using the extracted genomic DNA as a template. The primers (synthetic oligonucleotides) used for the PCR reaction were the following: 728f and TEFI r, complementary to the TEF region, for the sample CBMAI 2752; ITS1 and ITS4, complementary to the ITS region, for the sample CBMAI 2755; and Bt2a and Bt2b, complementary to the beta-tubulin region for each isolate (CBMAI 2753, CBMAI 2754, CBMAI 2756, CBMAI 2757, and CBMAI 2758). The amplification product was column purified (GFX PCR DNA and Gel Band purification kit, GE Healthcare) and was subjected directly to sequencing using the ABI 3500XL series automatic sequencer (Applied Biosystems) (**supplementary material**).

To conduct genetic distance analysis, the partial sequences of the genes obtained through the primers used were assembled into a consensus (single consensus sequence combining the different fragments obtained). This was compared with the sequences of organisms represented in the GenBank (http://www.nebi.nlm.nih.gov/) and CBS (http://www.westerdijkinstitute.nl/) databases. Sequences of microorganisms related to the unknown sample were then selected to build a dendrogram. The DNA sequences were aligned using the CLUSTAL X program [18] within BioEdit 7.2.6 and genetic distance analyses were conducted using the MEGA program version 6.0 [19]. A distance matrix was calculated using the model of Kimura [20], and the dendrogram was constructed from the genetic distances using the neighbor-joining method [21], with bootstrap values calculated from 1,000 resampling, using the software included in the MEGA 6.0 program (**supplementary material**).

2.6. Tolerance Test on Fungal Isolates in Relation to Metals (Fe^{2+}, Mn^{2+}, and Cr^{3+}), Performed in a Solid Medium

An initial screening was carried out to assess the tolerance of radial growth of the isolates in the presence of the main metals determined in the soil analysis (Fe^{2+}, Mn^{2+}, and Cr^{3+}). Solid culture media containing Sabouraud dextrose agar and malt (2% and 2%, respectively) with a mixture of solutions of $FeSO_4.7H_2O$ (9.91 mg/L), $Cr(NO_3)_3.9H_2O$ (1.67

mg/L) and $MnSO_4.H_2O$ (2.39 mg/L) at pH 5, which had previously been sterilized in an autoclave (121ºC for 20 min), were inoculated into the isolates using an inoculation loop through a central insertion point. Incubation was done at 28ºC (B.O.D.) for 9 days. The radial growth of each colony was monitored every 24 h. Plates of solid medium without metal supplementation were used as a control. The test was carried out in triplicate for each isolate.

2.7. Tolerance Test on the Fungus *Talaromyces verruculosus* (CBMAI 2754) in Relation to Metals (Fe^{2+}, Mn^{2+}, and Cr^{3+}) at Different Concentrations in Solid and Liquid Media

The fungus *Talaromyces verruculosus* (CBMAI 2754) was selected for evaluation of its tolerance to metals at different concentrations as follows: $FeSO_4.7H_2O$ (1, 10, and 20 mg/L); $MnSO_4.H_2O$ (1, 10, and 20 mg/L); and $Cr(NO_3)_3.9H_2O$ (1, 10, and 20 mg/L).

The solid culture medium, which was sterilized in an autoclave at 121 °C for 20 min, was prepared from Sabouraud dextrose agar (2%) and malt (2%), with solutions of the selected metals and pH adjusted to 5. The plates were inoculated using an inoculation loop through a central insertion point and incubated at 28 ºC (B.O.D.) for 7 days. The radial growth of each colony was monitored every 24 h. The solid medium without metal supplementation was used as a control. The experiment was conducted in triplicate.

The tolerance of the isolate *Talaromyces verruculosus* CBMAI 2754 to the selected metals was also assessed in a liquid medium. Erlenmeyer flasks (125 mL) containing 50 mL of medium from malt (2%) at pH 5 and the same amounts of metals as the solid medium mentioned above were sterilized in an autoclave at 121°C for 20 min. Inoculations were carried out with six standardized circular fragments (0.5 cm in diameter) from solid fungal cultures, and incubation was done for 7 days at 28 °C (B.O.D.), with metals and without metals. The flask was kept under orbital agitation for 7 days at 30°C and 150 rpm. Lastly, the mycelia were vacuum-filtered on quantitative filter paper and dried in an oven for 30 hours at 40 °C to determine the fungal mass. All experiments were carried out in triplicate.

2.8. Statistical Analysis

Data have been expressed as mean with the standard error of the mean (SD). For statistical analysis, one-way ANOVA (analysis of variance) was used, followed by the Tukey-Kramer post-hoc test. $P \leq 0.05$ was considered to represent a statistically significant difference. The GraphPad Prism® (version 5.03) software was used.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Soil Samples

All the soil samples had a clay texture. In addition, the pH of the samples was slightly acidic, around 5.1 ± 0.4. A pH of less than 7 in soils was attributed to the presence of fulvic, humic, carbonic, and organic acids. Acidic soil pH increases the dissolution and mobility of metal cations [22].

The concentrations of metals (Ni, Cu, Mn, Cr, Mg, Co, Fe, and Pb) in the samples collected were determined by means of atomic absorption, as described in Fig. (**2**). As expected, Fe was present in all the samples collected. Sample A showed a high concentration of Fe (9.916 mg/L). In sample B, there was a high concentration of Mg (7.880 mg/L), while in samples C and D, the metal with the highest concentration was Mn, with values of 3.091 mg/L and 2.393 mg/L, respectively. Pb was only determined in sample C at 0.046 mg/L, while Ni was only present in samples C and D, with values of 1.633 mg/L and 0.559 mg/L, respectively.

Penicillium rolfsii CBMAI 2753 was the species most present in the samples. The greatest diversity of species was obtained in soil sample D, while in sample B, only the species *Trichoderma pseudoasperelloides* CBMAI 2752 was isolated, as shown in Fig. (**3**).

A total of 42 isolates were obtained from the soil samples. The isolates were classified into five genera: *Trichoderma, Penicillium, Talaromyces, Aspergillus,* and *Purpureocillium*. All the isolates obtained were identified based on their morphological and microscopic characteristics, and their identities were confirmed through sequencing and genetic distance analysis based on fragments of the beta-tubulin, Translation Elongation Factor (TEF), and ribosomal gene spacer (ITS) genes of all the fungal isolates. These were assessed using the nucleotide BLAST tool (NCBI), and the fungi were then classified according to the similarity of their sequences to those in the GenBank database. As an example, as shown in Fig. (**4**), the sequences of the β-tubulin region of the sample showed 99-100% similarity to the sequence in the same region of the ribosomal operon of the species *Talaromyces verruculosus*, deposited in the GenBank database and the CBS-Knaw database.

The genetic distance analysis (Fig. **4**) facilitated the recovery of this sample in a cluster with 95% resolution with the strain *Talaromyces verruculosus*. The other species were identified as follows: *Trichoderma pseudoasperelloides* (CBMAI 2752), *Penicillium rolfsii* (CBMAI 2753), *Aspergillus sp. cf. pseudoviridinutan* (CBMAI 2755), *Aspergillus sp. series nigri close to A. niger* (CBMAI 2756), *Purpureocillium lilacinum* (CBMAI 2757), and *Penicillium guaibinense* (CBMAI 2758).

As shown in Table **1**, *Trichoderma pseudoasperelloides* CBMAI 2752 has hyaline hyphae with several branches and a smooth wall, containing ovoid-shaped conidia (spores). *Penicillium rolfsii* CBMAI 2753 presents septate hyphae and conidiophores that appear as branches of the mycelium. The structure of the conidiophore consists of a stipe that can be narrow or broad. At the apex of the stipe is the "*Penicillus*", which may consist of conidiogenous cells called phialides. *Talaromyces verruculosus* presents branched, septate hyaline hyphae, conidia, and spores. *Aspergillus niger* CBMAI 2756 has septate hyaline hyphae, conidiophores with radial heads, branched and septate ends, and chain-like

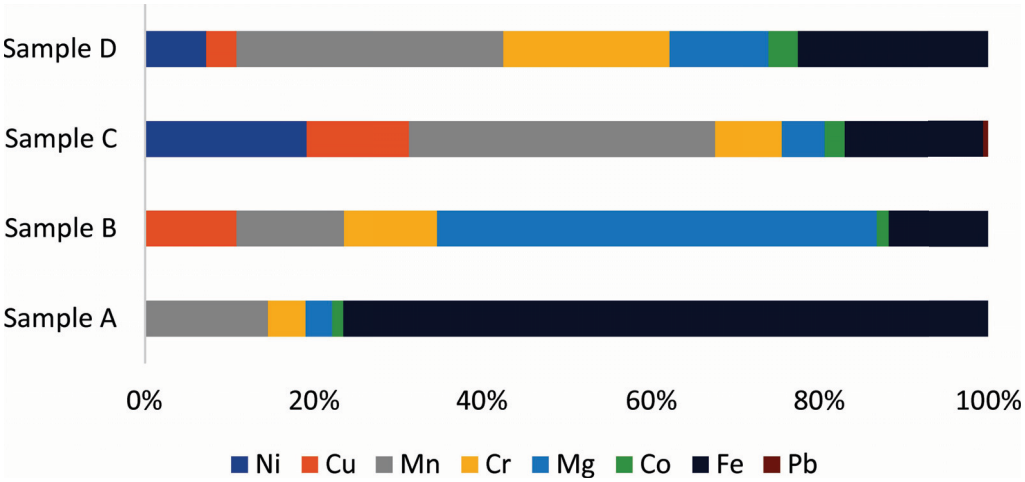


Fig. (2). Determination of the concentrations of metals present in the soil samples collected. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

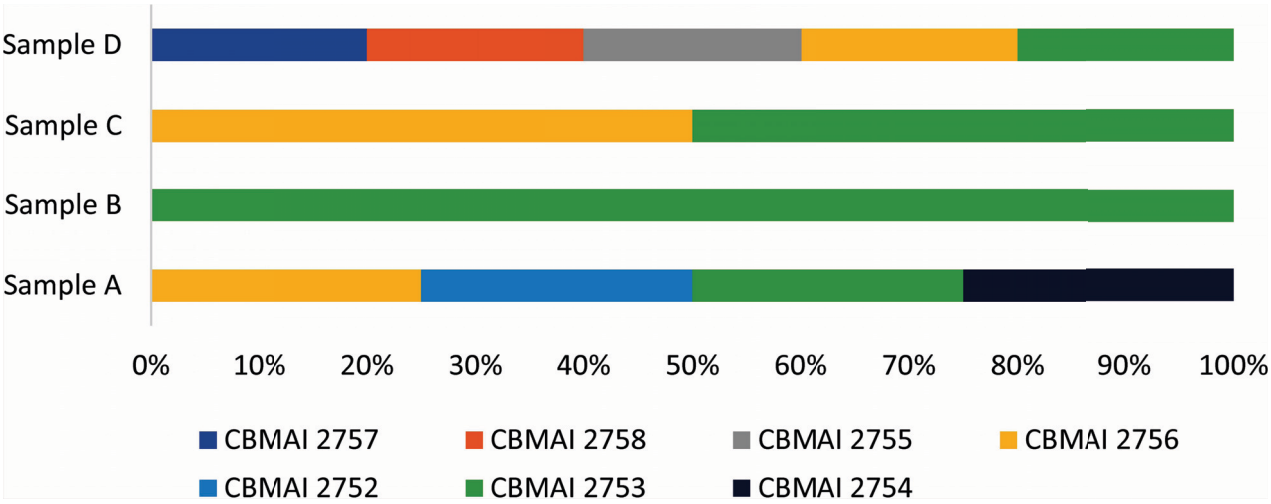


Fig. (3). Presence of the microorganisms isolated in each soil sample. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

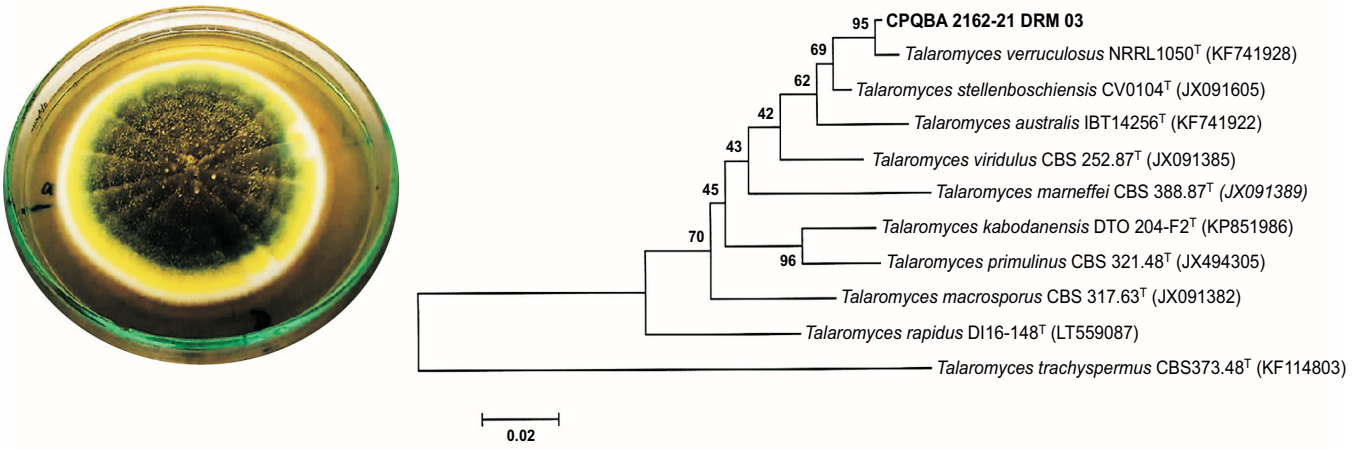


Fig. (4). Culturing of the fungus *Talaromyces verruculosus* on solid medium and dendrogram based on genetic distance using the neighbor-joining method, showing the relationship between the β-tubulin of sample CPBBA 2162-21 DRM 03 and the sequence of the lineage of related microorganisms found in the Mycobank and GenBank databases. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

spores with a brown to black color [23]. *Purpureocillium lilacinum* CBMAI 2757 has septate, hyaline hyphae, and branched conidiophores at the end, presenting a brush-like appearance. *Penicillium* cf. *guaibinense* CBMAI 2758 has a structure with hyphae, conidiophore, phyllids, conidia, and septa.

Based on the analysis of the soil samples, the metals Fe, Mn, and Cr were commonly found in samples with moderate or high concentrations. Therefore, it was decided to evaluate the adaptive behavior of the isolates in a solid medium enriched with a mixture of the metals Fe, Mn, and Cr. In doing this, it was considered that the presence of metals at certain concentrations can lead to changes in the metabolic activity of microorganisms [24], including decreased ATP production or extracellular enzyme activity [25].

3.2. Tolerance Test on Fungal Isolates in Relation to Metals (Fe^{2+}, Mn^{2+}, and Cr^{3+}), Performed in Solid Media

Natural selection occurs more strongly for fungi isolated from soil, as the process of adaptation to metal stress is probably accompanied by the exclusion of fungal species sensitive to such metals. This consequently influences the composition of the soil microbial community [26].

In general, from the growth rate, all the isolates showed adaptive behavior in the presence of a mixture of heavy metals (Fe, Mn, and Cr). However, the isolates *T. verruculosus* CBMAI 2754 and *P. rolfsii* CBMAI 2753 showed better behavior when cultured in a medium enriched with metals (Fig. **5**). It is worth mentioning that *T. verruculosus* CBMAI 2754 and *P. rolfsii* CBMAI 2753 were isolated from samples with high concentrations of iron, manganese, and chromium. These species are resistant to these metals, which in some cases are toxic, and these components are used as a source of energy for their own growth [27].

The Radial Growth Velocity (RGV) is the angular coefficient of the line obtained from linear regression on the radii of the colonies as a function of time. Thus, the greater the slope of the line is, the greater the radial growth velocity and the greater the growth potential of the fungus will be [28]. From the growth rate results, it was observed that in this regard, all the isolates in the presence of the medium containing metals showed higher RGV than the control medium (without metals), with the exception of *P. guaibinense* CBMAI 2758, which had an RGV of 2.0584 cm/d for the medium enriched with metals and 2.7097 cm/d for the medium in the absence of metals. In this isolate, the medium supplemented with metals caused a delay in the value of radial growth velocity on the fourth day. The fungus *T. pseudoasperelloides* CBMAI 2752 showed the highest RGV value of 4.1088 cm/d, followed by CBMAI 2756 with 4.1060 cm/d, although there was no significant difference between the respective controls. Other *Trichoderma* and *Aspergillus* species also showed good tolerance against lead, zinc, copper, nickel, and cadmium [12]. The isolate CBMAI 2757, on the other hand, showed a lower RGV, with 1.2171 cm/d and 1.2108 cm/d, for the enriched medium and the control, respectively (Fig. **5**).

Metal tolerance is usually attributed to sites of isolation as fungi have more tolerance ability to contaminated sites over other non-native species [29]. However, a variety of morphological responses under metal stress have been reported in fungi, such as a change in the color of the fungal colony; constricted, ruptured, flattened, and swelled mycelium; outward expansion of mycelia, *etc*. [30].

Talaromyces species have a wide distribution and can be isolated from various substrates. Initially, this genus was described as a teleomorphic species with a *Penicillium* or *Penicillium-like* anamorph that produces soft-walled ascomata covered in hyphae [31]. However, based on phylogenetic and phenotypic characteristics, following the concept of single-name nomenclature [23], all the accepted species of *Penicillium* subg. *Biverticillium* were transferred to *Talaromyces*.

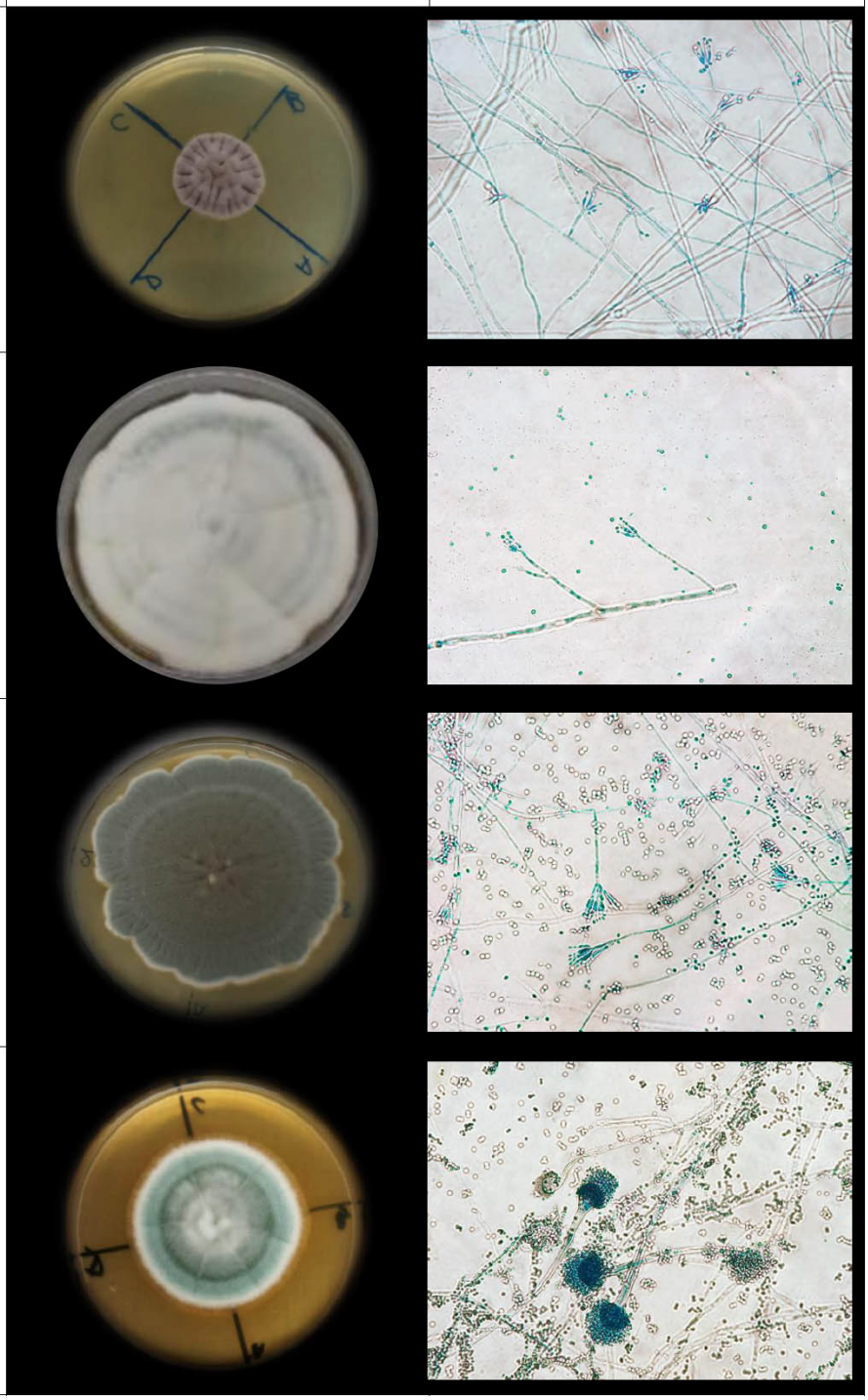
In recent literature, some properties of extracts from or use of total cells of the fungus *T. verruculosus* are presented, such as the production of pigments with antioxidant and antibacterial viability [32]. In another study, *T. verruculosus* isolated from Amazonian soil (Marabá, PA, Brazil) was used to hydrolyze lignocellulosic biomass from babassu residue and sugarcane bagasse; it was found to increase the production by 80% when *T. verruculosus* cellulases were applied for 72 h [33]. In addition, *T. verruculosus* strain PF157-2, isolated from agricultural soil in different regions of Iran, was used as a biofertilizer to solubilize elemental phosphorus from rock phosphate. In that study, the *T. verruculosus* species proved capable of improving the soil quality, and thus represented an alternative to chemical fertilizers that would promote ecologically correct and sustainable agriculture [34]. *T. verruculosus* is, therefore, a promising species with the potential for industrial bioprospecting.

Based on the previous results, *T. verruculosus* CBMAI 2754 was chosen for further evaluation of the behavior of this isolate in a solid medium enriched with 1, 10, and 20 mg/L of iron, chromium, and manganese, respectively. As described in Table **2**, the isolate *T. verruculosus* CBMAI 2754 in the medium enriched with individual metals did not show any significant difference regarding the concentrations of metals used or the types of metals.

The mycelial growth of *T. verruculosus* CBMAI 2754 in a liquid medium was also evaluated in the presence of Fe, Mn, and Cr at different concentrations (1, 10, and 20 mg/L). As shown in Fig. (**6**), chromium was the metal with the greatest influence on the mycelial growth of *T. verruculosus* CBMAI 2754, with significant differences at all the concentrations evaluated.

On the other hand, as shown in Table **2**, the growth rate of the isolate *T. verruculosus* CBMAI 2754 in the presence of the metals iron, manganese, and chromium in Sabouraud dextrose agar (2%) and malt (2%), at pH 5 and 28ºC, the amount of dry mycelial mass of *T. verruculosus* CBMAI

Table 1. Morphology and microscopy of filamentous fungi isolated from iron mine soil: (a) *Purpureocillium lilacinum*; (b) *Penicillium* sp. cf. *guaibinense*; (c) *Penicillium rolfsii*; (d) *Aspergillus sp.* cf. *pseudoviridinutans*; (e) *Aspergillus sp. series nigri clote to A. niger*; (f) *Trichoderma pseudoasperelloides*; (g) *Talaromyces verruculosus.*. In Sabouraud dextrose agar medium (2%) and malt (2%), pH 5, 28 °C for 9 days of culturing.

Species isolated	Macromorphology[a]	Micromorphology
Purpureocillium lilacinum (CBMAI 2757) (a)		
Penicillium sp. cf. *guaibinense* (CBMAI 2758) (b)		
Penicillium rolfsii (CBMAI 2753) (c)		
Aspergillus sp. cf. *pseudoviridinutans* (CBMAI 2755) (d)		

(Table 1) contd….

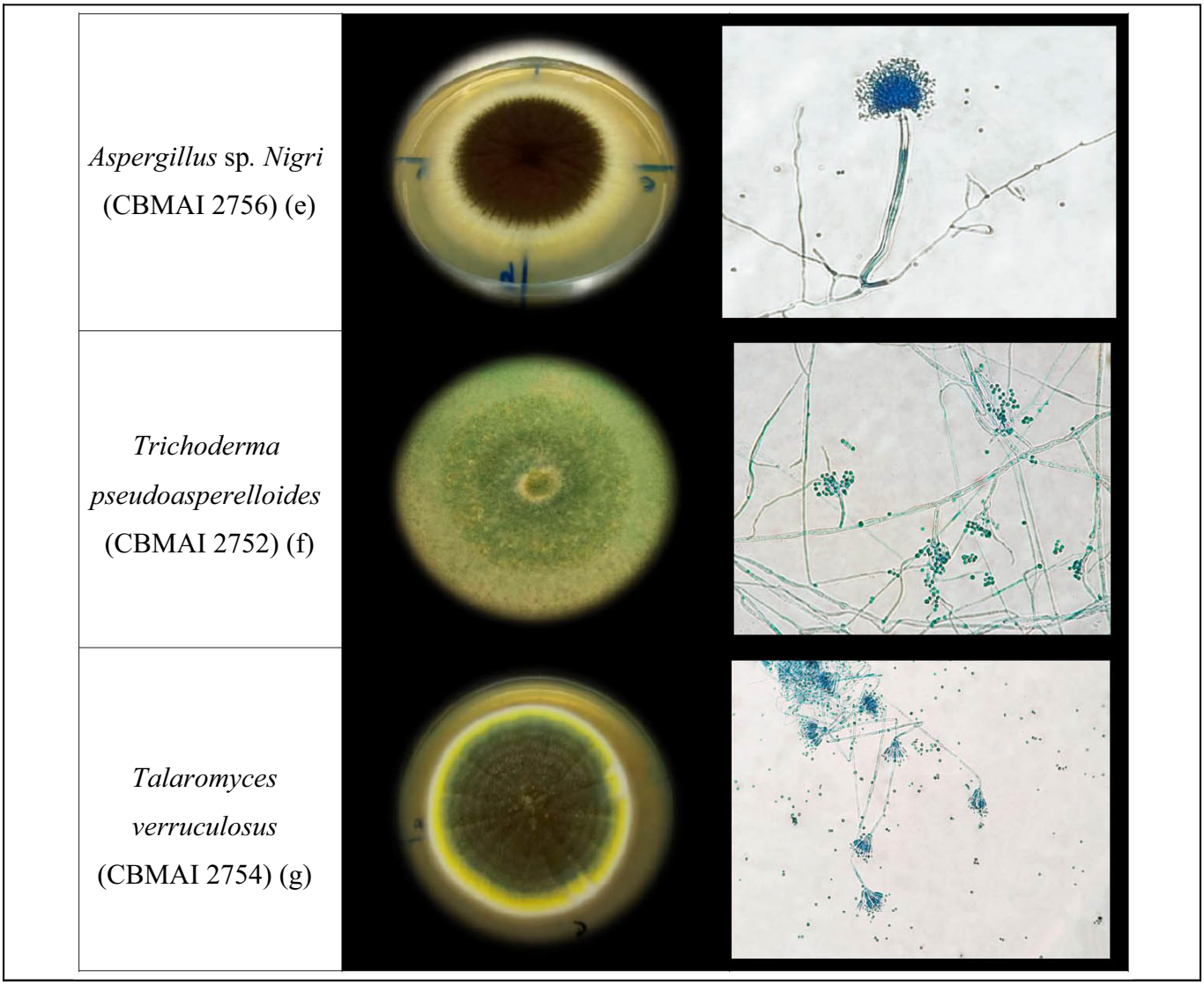


Table 2. The growth rate of the isolate *T. verruculosus* CBMAI 2754 in the presence of the metals iron, manganese, and chromium in Sabouraud dextrose agar (2%) and malt (2%), at pH 5 and 28ºC, assessed at 7 days.

Metal (mg/mL)	Equation	R^2	Speed of Growth (cm/d)
Iron			
1	Y = 0.7518*X - 0.1643	0.9972	2.5359
10	Y = 0.8357*X - 0.5071	0.9965	2.7599
20	Y = 0.8054*X - 0.4964	0.9966	2.6334
Manganese			
1	Y = 0.8741*X - 0.7536	0.9957	2.62194
10	Y = 0.8000*X - 0.5893	0.9997	2.64899
20	Y = 0.8420*X - 0.6214	0.9988	2.698986
Chromium			
1	Y = 0.8214*X - 0.5036	0.9958	2.73266
10	Y = 0.8429*X - 0.6929	0.9973	2.58419
20	Y = 0.8455*X - 0.6929	0.9985	2.70127

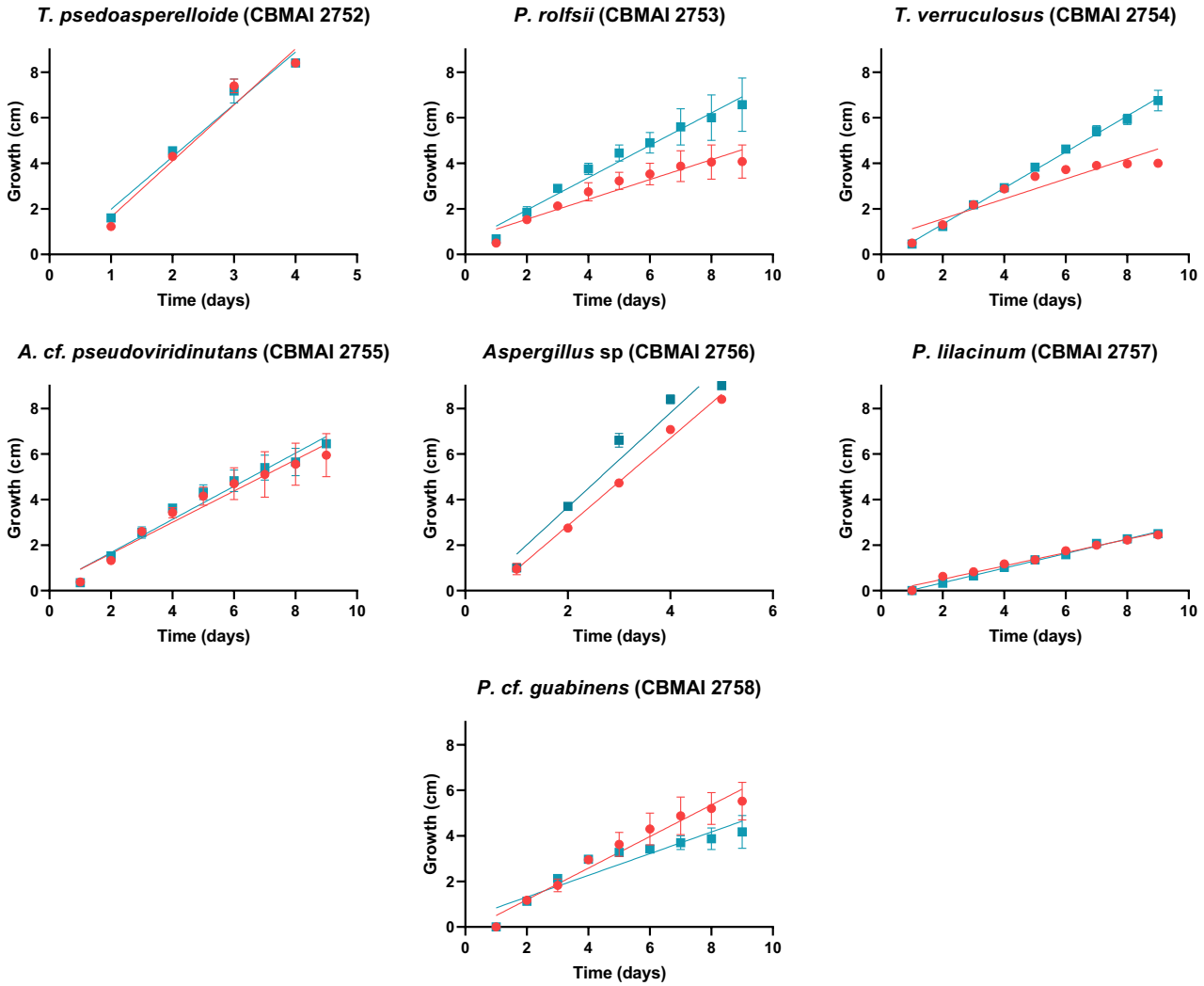


Fig. (5). Radial growth kinetics of fungal isolates in medium enriched with iron, manganese, and chromium metals and their respective negative controls. Culturing conditions: Sabouraud dextrose agar medium (2%) and malt (2%) at 28 °C and pH 5. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

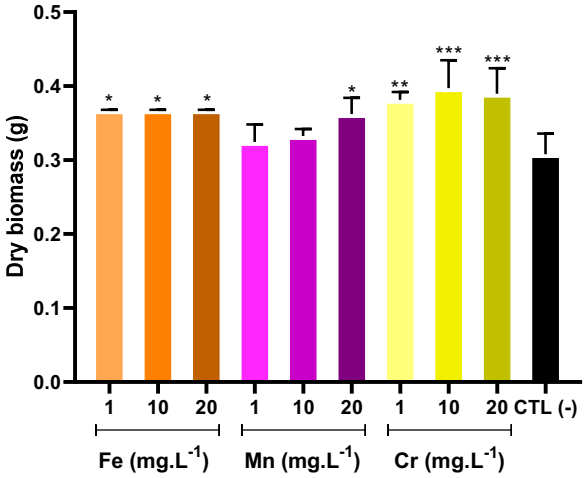


Fig. (6). Culturing of the filamentous fungus *T. verruculosus* CBMAI 2754 in liquid medium with different concentrations of the metals Fe, Mn, and Cr, at 28°C and pH 5 for 9 days of culturing. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2754 did not vary between the concentrations used, but it showed a significant gain in mass in comparison to the negative control. Moreover, the presence of manganese in the liquid culture medium did not significantly influence the gain in mass of the fungus *T. verruculosus* CBMAI 2754 at concentrations of 1 and 10 mg/L, and the gain in mycelial mass was only significant at the higher concentration of 20 mg/L. Microorganisms tend to respond to heavy metals in a biphasic manner, with low metal concentrations stimulating growth and higher metal concentrations inhibiting it [35], as observed in this experiment.

The process of filamentous fungal tolerance to high concentrations of metals starts in the cell wall containing glycoproteins and polysaccharides, attracting sequester metals within the biomass [35]. Different mechanisms of interaction and sorption of metals by fungi have been classified based on cell metabolism and the location of metal detoxification. Initially, metal ions are trapped in the cell wall through biosorption, a process independent of metabolism. Various processes, such as chelation, ion exchange, reduction, complexation, precipitation, and surface adsorption, have been suggested to be active during the passive binding of metal ions [30]. Several extracellular products of fungi, including organic acids, polymers, and anions, such as sulfides and phosphates, participate in precipitation.

CONCLUSION

This paper has described the identification of seven strains of filamentous fungi isolated from the soil of an iron ore mining area in the Amazon region. The fungi isolated were of different genera and species, as follows: *Talaromyces verruculosus, Trichoderma pseudoasperelloides, Penicillium rolfsii, Aspergillus* sp. cf.*pseudoviridinutans, Aspergillus* sp. series nigri, to close *A. niger, Purpureocillium lilacinum*, and *Penicilliumsp*. cf *guaibinense*. The strains isolated were used in adaptation tests in solid media with supplementation of metals (Fe, Mn, and Cr), and showed tolerance to the metals tested. Overall, *Talaromyces verruculosus* CBMAI 2754 demonstrated the best adaptation to the medium supplemented with the metals. This indicates the possibility that these fungi could be used in bioremediation studies on medium contaminated with Fe, Mn, and Cr.

AUTHORS' CONTRIBUTIONS

Patrícia de A. Nóbrega was responsible for the methodology, formal analysis, data curation, writing of the original draft, and writing, review, and editing of the manuscript. Beatriz L. Ferreira contributed to the methodology, formal analysis, data curation, writing of the original draft, and writing, review, and editing of the manuscript. Lucas S. Sá took part in conceptualization, methodology, formal analysis, data curation, writing of the original draft, and writing, review, and editing of the manuscript. Francinaldo S. Braga was responsible for the formal analysis, data curation, and writing. Roberto M. Bezerra contributed to the conceptualization, project administration, and funding acquisition. Irlon M. Ferreira contributed to the conceptualization, project administration, funding acquisition, writing of the original draft, and writing, review, and editing of the manuscript.

LIST OF ABBREVIATIONS

RGV	=	Radial Growth Velocity
TEF	=	Translation Elongation Factor
USEPA	=	US Environmental Protection Agency

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this study can be found in the online version of the article.

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