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Penicillium citreosulfuratum Isolated from Corroded Parts of a Hydroelectric Power Plant: Characterization and Control with Essential Oils

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HIGHLIGHTS

- A strain of *Penicillium citreosulfuratum* was isolated from corroded metal.
- The fungus formed biofilm on the surface of aluminum wire.
- This strain grew in the presence of 50 mM Al^{3+} in liquid medium.
- Thyme, oregano, and melaleuca essential oils inhibited this strain growth.

Abstract: A *Penicillium* sp. strain was isolated from corroded parts in the Coaracy Nunes hydroelectric power plant in Amapá State, Brazil. Morphological and molecular techniques identified this strain as *Penicillium citreosulfuratum* Biourge, whose 5.8S-ITS rDNA sequence grouped well in a phylogenetic tree with other *Exilicaulis* section species of the *Penicillium* genus. The obtained strain of *P. citreosulfuratum* ability to form biofilm on the surface of aluminum wires was confirmed by optical and scanning electron microscopy. In plates containing PDA or T&K media, the *P. citreosulfuratum* strain growth was inhibited around a paper disk containing a highly concentrated solution of copper, iron, or nickel. However, there was no inhibition halo around the paper disk containing aluminum and there was a faint halo around the paper disk containing zinc. This fungus was able to grow in a modified T&K liquid medium containing 50 mM of Al^{3+} and less efficiently in the same medium but containing 50 mM Zn^{2+}. The essential oils of melaleuca, mint, thyme, and oregano at 100% inhibited the fungus growth. Oregano essential oil inhibited the *P. citreosulfuratum* strain growth in concentrations as low as 10%. In conclusion, the results show that the obtained strain of *P. citreosulfurarum*

can form a biofilm on aluminum wires, it grows in the presence of a high concentrations of aluminum and zinc, and essential oils can control its growth.

Keywords: *Penicillium citreosulfuratum*; Biofilm; Metal ions; Essential oils.

INTRODUCTION

Corrosion is a metal deterioration resulting from chemical reactions between it and the surrounding environment. It is a natural process that converts a refined metal into a chemically stable form, such as oxide, hydroxide, or sulfide. It occurs by chemical and or electrochemical oxidation of the metal (anodic reaction) in reaction with an oxidant, such as oxygen or sulfates (cathodic reaction). Therefore, corrosion is a genuine concern, with an economic cost reaching annual values of approximately US$2.5 trillion of direct costs, which corresponds to 3.4% of global gross domestic product (GDP), and about US$875 billion annually to prevent damage and services [1–3].

Biocorrosion, or Microbiologically Influenced Corrosion (MIC), designates microorganisms' involvement in the corrosion process, which may accelerate, induce, or even inhibit it [1–8]. These organisms are generally microscopic, and the involvement occurs by a chemical or electrochemical mechanism. In the biocorrosion process, the metabolic microorganisms' activities supply insoluble products that can accept electrons from the base metal. This biotic and abiotic reactions sequence produces a kinetically preferred pathway of electron flow from the metal anode to the universal electron acceptor, oxygen [2, 8]. Thus, microorganisms might accelerate corrosion by a chemical mechanism using their potentially surface-aggressive metabolites.

MIC can be associated with biofilm, a microorganism community or consortium that adheres to abiotic surfaces [1, 5]. Among the diverse organisms that may influence corrosion, we can cite bacteria, such as sulfur-oxidizing and iron-reducing bacteria, fungi, and algae in a lower number [2–5, 7]. Fungi are well-known organic acid producers and can contribute to MIC [1, 5]. In addition, much of the published work on aluminum biocorrosion and its alloys has implicated fungal contaminants, such as *Cladosporium*, *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. [2, 4].

Biocorrosion is also economically significant. It may affect nuclear plants, oil, gas, and other industries [6, 9–11], and 10-20% of the total corrosion damages are microbiologically influenced [4, 6]. For example, hydroelectric plants, the water from a dam runs through tapering pipes, thus rotating the turbines and creating electricity. In principle, the potential energy held in the dam converts into kinetic energy when it flows through the pipes. In these facilities, corrosion can mainly affect waterwheels, turbines, and associated piping systems [2, 6].

Many methods used to control biocorrosion are harmful to the environment, for instance, biocides and antibiotics. Those compounds are a common choice when controlling organisms involved in biocorrosion becomes necessary. However, knowing the preponderant species is essential for the best effectiveness of growth control [1, 4, 5]. Thus, environmentally friendly measures have been proposed, and natural products have gained prominence because some do not present corrosive action, are biodegradable, and effectively control microorganisms' growth. Essential oils, which have been generally recognized as safe (GRAS) and are innocuous for humans and plants, have been described as helpful in controlling biocorrosion [12, 13].

In this work, a *Penicillium citreosulfuratum* strain was obtained from corroded metallic structures of the Coaracy Nunes hydroelectric plant in Amapá State, Brazil. This strain biofilm formation capacity was characterized, and growth in metal presence was analyzed. In addition, some plant essential oils' ability to control this strain growth was tested.

MATERIAL AND METHODS

Sample collection

Corroded fragments of pipelines, valves, heat exchangers, and refrigeration systems were obtained in 2007 from the Coaracy Nunes Hydroelectric power plant, located on the Araguari River course, in Ferreira Gomes city, Amapá state, in the Brazilian Amazon region, at coordinates 00º54'10''N, 51º15'35''W. The crusts were removed with a porcelain instrument and stored in sterile 15 mL falcon tubes containing 0.3 g sterile vermiculite. In order to obtain fungi, 0.2-0.5 g of the collected samples, including the vermiculite, were inoculated in 10 mL of sterile T&K modified liquid medium [0.625 g/L $(NH_4)_2SO_4$; 0.625 g/L K_2HPO_4; 0.625 g/L $MgSO_4 \cdot 7H_2O$; 15 g/L glucose; 1 g/L yeast extract] [14]. The inoculated T&K medium was incubated under stationary condition at 30 °C for 24 hours. After this incubation, aliquots of this 10-mL culture were transferred and spread with a sterile loop onto Sabouraud agar and Dextrose & Mycosel agar plates, which were

incubated for 10 days at 25 °C. Finally, the obtained fungi colonies were transferred to Potato Dextrose Agar (PDA) slants, cultured at 25 °C for 7 days, and kept at room temperature.

The microorganism morphological and molecular characterization

A fungal colony (sample 6) was submitted to monosporic isolation [15]. The monosporic isolate was cultured in Potato Dextrose Agar (PDA) at 25 and 37 °C for 5 days, and macro- and micro-morphological characteristics were used in traditional classification keys [16]. This microorganism is being kept in PDA slants with transfers every four months and in PDA slants under mineral oil.

The monosporic isolate was also identified by DNA barcoding as follows. Mycelia were obtained from a culture performed in potato dextrose liquid medium for DNA extraction. The inoculum was prepared with a 1 cm^3 fragment from a PDA slant monosporic culture, which was smashed and shaken in 50 mL of distilled water. An aliquot of 2 mL of the obtained suspension (5.1×10^6 spores) was used as inoculum in 125 mL Erlenmeyer flasks containing 25 mL of liquid potato dextrose medium. The flask was incubated for 5 days without shaking at 25 °C, with a photoperiod of 12 h. The mycelium was collected by filtration in sterile gauze and macerated in a mortar with liquid nitrogen. The genomic DNA was extracted from the macerated mycelium using the protocol described by Koenig and coauthors [17]. The DNA was quantified in a spectrophotometer at 260 nm and by fluorometry using the Qubit Quantitation Fluorometer and the Quant-it™ dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). The DNA final concentration was adjusted to 100 ng/µL in TE buffer, and the DNA was kept frozen at -20 °C.

The amplification of the 5.8S-ITS rDNA region was performed in a thermocycler Techne TC-312 (Techne, Cambridge, UK) in a PCR tube containing 50 µL of the following reaction mixture: 1× enzyme buffer (20 mM Tris-HCl, pH 8.4, 50mM KCl); 1.5 mM $MgCl_2$, 3.0 U of Platinum *Taq* DNA polymerase (Thermo Fisher Scientific, USA); 0.2 mM of each dNTP; 50 pmol of each ITS4 and ITS5 primers [18], and 400 ng of the DNA sample. The PCR reaction consisted of 25 cycles of 1 min and 30 s at 94 °C, 1 min and 30 s at 50 °C, and 2 min at 72 °C. Before cycling, samples were heated for 5 min at 94 °C, and after cycling, samples were incubated for 10 min at 72 °C and frozen at -20 °C until use. Ten microliters of the PCR reaction were analyzed in a 1.5% agarose gel containing ethidium bromide (0.25 µg/mL). An amplicon of approximately 500 bp was obtained. The rest of the PCR reaction was purified with the PureLink™ PCR purification kit (Thermo Fisher Scientific, USA).

The amplified DNA was sequenced in the Human Genome and Stem-Cell Research Center (HUG-CELL) at the University of São Paulo (USP), Brazil, using the ITS4 and ITS5 primers [18] in order to sequence both strands. After trimming the 5´ and 3´ extremities, the resulting sequences were compared with sequences deposited in data banks using pairwise analysis. The rDNA gene partial sequence was deposited in GenBank (AN MZ540436).

For the phylogenetic analysis, 5.8S-ITS rDNA sequences of other *Penicillium* spp. were retrieved from databanks. All sequences were first aligned using Clustal Omega and trimmed at the 5´and 3´ends to have identical initial and final sequences and similar sizes in base pair numbers. Next, a phylogenetic tree was built in the MEGA11 program [19], using the Neighbor-Joining method [20] and checking the confidence limits of the branching with the Bootstrap analyses with 1000 replications [21]. Finally, the Maximum Composite Likelihood method [22] was used to compute the evolutionary distances, with the number of base substitutions per site serving as units.

Biofilm formation

In order to analyze the monosporic isolate's ability to grow on metals' surfaces, a 1 cm^3 fragment of a PDA slant monosporic culture was smashed and shaken in 20 mL of distilled water. An aliquot of 500 µL of the obtained suspension containing 3.2×10^6 spores was used as inoculum to 25 mL of the sterile modified T&K medium in 125 mL Erlenmeyer flasks. Each flask contained a 3-mm diameter and 3-cm long aluminum or copper wire piece. The control flasks contained the T&K medium with the wire pieces but were not inoculated with the fungi. After 40 days at 25 °C, with a photoperiod of 12 hours, without agitation, the wire pieces were analyzed by optical microscopy. Those that presented mycelium growth were further evaluated by scanning electron microscopy.

Scanning electron microscopy

The metal wires incubated or not with the *P. citreosulfuratum* strain were frozen in liquid nitrogen and lyophilized at -55 ºC in a freeze dryer (Martin Christ, Germany) for 6 h to preserve any biological material

morphologic characteristics. After that, the material received a gold cover. Micrographs were obtained in a Shimadzu scanning electronic microscope, model SS-550 Superscan, at 12 keV.

Metal ion resistance

The monosporic isolate's ability to withstand high concentrations of copper, zinc, aluminum, iron, or nickel, was tested in 9-cm diameter PDA or solid T&K media plates. For this purpose, 100 µL of a spore solution containing 2.55×10^5 spores were spread with a Drigalski spatula on the media's surfaces, and 1.6 cm diameter Whatman® Grade 1 paper disks were placed on the plate's center (Figure 1A). Then, sterile 50-µL aliquots of 1.0 M $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $Al_2(SO_4)_3 \cdot 16H_2O$, $FeCl_3$, or $NiCl_2 \cdot 6H_2O$ solution were applied to the paper disks. The plates were incubated at 25 °C with a 12 h photoperiod. The PDA and T&K plates were photographed after 2 and 7 days and 3 and 6 days of culture, respectively. Water in the paper discs was used as a control. This analysis was done in only one dish for each treatment.

A test was carried out to assess the isolate's growth in 25 mL of liquid T&K modified medium in 125 mL Erlenmeyer flasks added of 50 mM of $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $Al_2(SO_4)_3 \cdot 16H_2O$, $FeCl_3$, or $NiCl_2 \cdot 6H_2O$. The ions solutions were prepared separately in more concentrated solutions, autoclaved or 0.22 µm filter-sterilized ($FeCl_3$ only), and added to the sterile basal medium to reach the 50 mM concentration. Control flasks contained the basal medium, with no extra ion addition. The inoculum consisted of 500 µL of sterile deionized water containing 1.28×10^6 spores. After 12 days of stationary culture, at 25 °C, with a photoperiod of 12 hours, the mycelium was collected by vacuum filtration on filter paper and dried overnight at 45 °C. The mycelia dry weight was used to evaluate the fungus growth. This essay was carried out in three culture flasks.

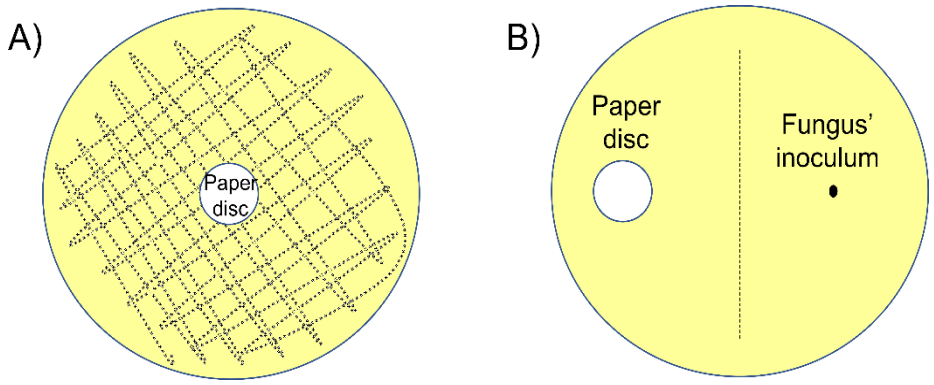

Figure 1. Inoculum methodology for metal resistance and essential oil growth inhibition analysis. A) The fungus spore's solution was spread on the top of the media, and a sterile paper disk was placed in the center. The PDA or modified T&K medium was used for metal resistance analysis, and the metal solution at 1.0 M (50 µL) was added to the top of the paper. Water was added to the control plate paper. The PDA medium was used for the diluted essential oil analysis, and 20 µL of the diluted essential oil was added to the paper's top. The control plate contained Vaseline in the paper disk. B) The essential oil growth inhibition analysis. A T&K medium plate was divided in the middle with an imaginary line. The paper disk was placed on one side of the plate and had 20 µL of the non-diluted essential oil added to its top. The fungus was inoculated on the other plate´s side. The colony diameter was measured after culture.

Growth inhibition by essential oil

Essential oils from sucupira (*Pterodon emarginatus*), copaiba (*Copaifera langsdorffii* Desf.), clove (*Eugenia caryophillata* Thunb.), oregano (*Oregum vulgare* L.), thyme (*Thymus vulgaris* L.), lemon (*Citrus limon* L.), mint (*Mentha piperita* L.), melaleuca (*Melaleuca alternifolia* Maiden & Betche), and rosemary (*Rosmarinus officinalis* L.), obtained from local markets, were evaluated regarding their ability to inhibit the fungus growth. For this purpose, 9-cm diameter plates containing solid T&K medium were used. A line was drawn in the middle of each plate. On a side, 1.6 cm diameter Whatman® Grade 1 paper disks were placed and soaked with 20 µL of each sterilized and pure essential oil (Figure 1B). Liquid Vaseline was used as a control. On the opposite plate side, the monosporic isolate was inoculated by a touch of a sterile wood stick covered with viable spores. After 7 days of culture at 25 °C, with a photoperiod of 12 h, each colony diameter was measured with a ruler. This analysis was carried out in triplicates. The growth inhibition determination was achieved employing the method described by Edgington and coauthors [23], in which the following formula determines the percentage of growth inhibition (PGI):

$$PGI = \frac{[\text{Average control colony (cm)} - \text{Average treatment colony (cm)}]}{\text{Average control colony (cm)}} \times 100 \quad (1)$$

In order to determine the essential oil minimum concentration able to retard the isolate's growth, a different approach was used. A 100 µL-aliquot of a solution containing 2.55×10^5 spores was spread on 9 cm diameter plates containing PDA. On the center of the plates, 1.6 cm diameter Whatman® Grade 1 paper disks were placed. A 20 µL-aliquot of the best growth inhibitor diluted essential oils (1, 5, 10, and 20%, v/v in Vaseline) was applied to the disks (Figure 1A). Pure liquid Vaseline was used as a control. After 4 days of cultivation at 25 °C, with a photoperiod of 12 hours, the plates were photographed and compared with the control. This analysis was done in only one dish for each treatment.

Statistical analysis

Statistical analyses were carried out by calculating the means and standard deviations of the results. Data were submitted to an ANOVA and compared using the Tukey test ($\alpha = 0.01$) using the program SASM - Agri [24].

RESULTS

The microorganism morphological and molecular characterization

The monosporic isolate's cultural and morphologic characteristics (Figures 2A, B, and C) showed it belongs to the *Penicillium* genus, with a characteristic yellow-colored reverse colony (Figure 2B) and a monoverticillated conidiophore (Figure 2D). Furthermore, the DNA barcoding identification, using the 5.8S-ITS rDNA region sequence, showed 100% of sequence identity and coverage with 5.8S-ITS rDNA sequences of *Penicillium citreonigrum* and *Penicillium citreosulfuratum*. These two species are very related, and both belong to the *Penicillium* genus section *Exilicaulis*, but they can be easily distinguished because only the *P. citreosulfuratum* grows at 37 °C [25]. Therefore, as our isolate grew well when cultured in BDA at 37 °C, it was considered a *P. citreosulfuratum* isolate, and its 5.8S-ITS rDNA sequence was deposited in GenBank with the accession number MZ540436.

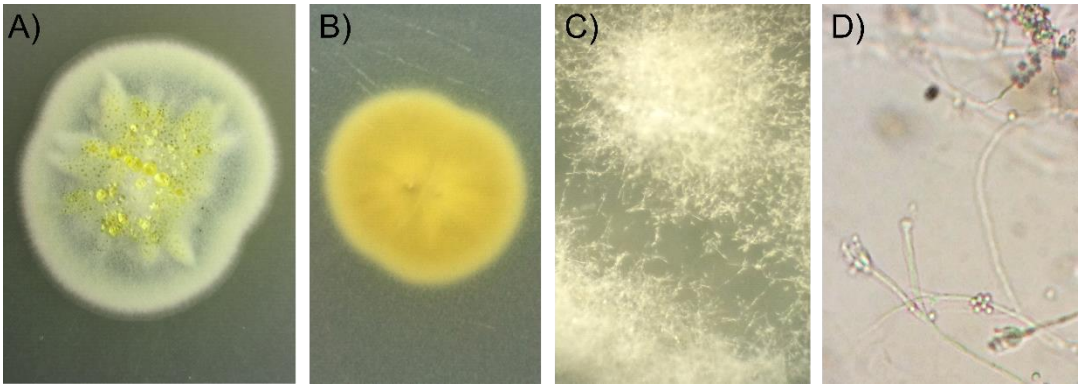

Figure 2. The *P. citreosulfuratum* morphologic characteristics. A) A colony of the fungus in PDA, after 5 days of culture at 25 °C, with a photoperiod of 12 h. B) The yellow reverse of a colony grown in the same conditions in PDA. C) The fungus is seen under a stereoscopic microscope. Numerous hyphae and conidiophores can be seen. D) The fungus is seen at optical microscopy showing the characteristic monoverticillated conidiophore of this species.

The obtained phylogenetic tree shows the grouping of the 5.8S-ITS rDNA sequence of the *P. citreosulfuratum* isolated in this work with other *Exilicaulis* section species sequences (Figure 3). The identity percentages of the sequences used to build the phylogenetic tree with the *P. citreosulfuratum* strain 5.8S-ITS rDNA sequence obtained in this work were higher than 88%.

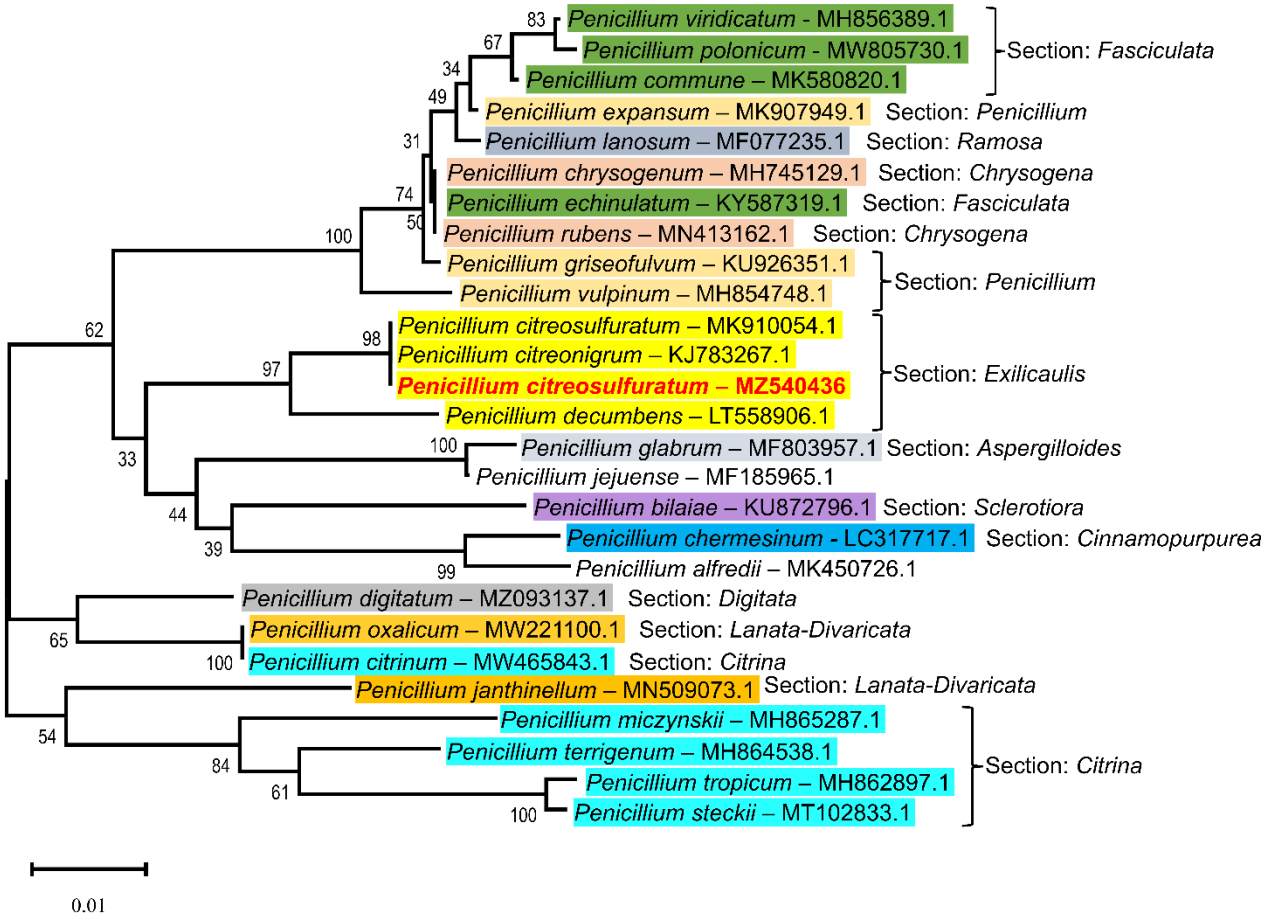

Figure 3. A phylogenetic tree with the 5.8S-ITS rDNA sequences. The obtained sequence of the *P. citreosulfuratum* studied in this work was compared with sequences retrieved from GenBank of other *Penicillium* spp. The replicate trees percentage in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 27 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 482 positions in the final dataset. The species names and GenBank access numbers are colored according to the *Penicillium* genus sections [26]. Species that have no attributed section were not colored.

Biofilm formation

The *P. citreosulfuratum* ability to form biofilm on aluminum and copper wires was verified in culture in T&K medium, for 40 days, in the presence of the 3-mm wide and 3-cm long wire pieces. The *P. citreosulfuratum* strain hyphae growth was observed on the aluminum wire under optical microscopy (Figure 4B) but not on the copper wire. The fungus growth on the aluminum wire was confirmed by scanning electron microscopy (Figure 4D).

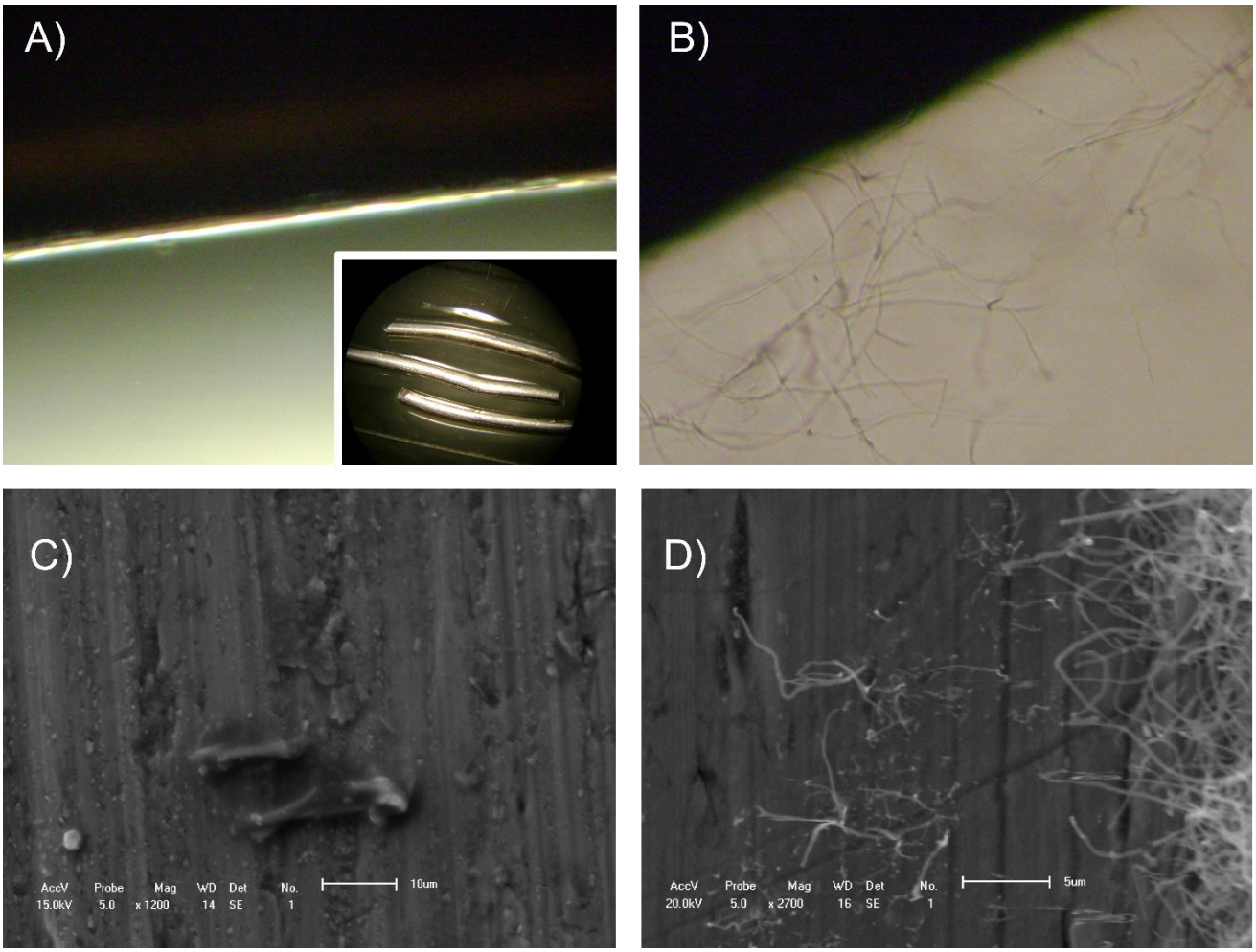

Figure 4. Optical and scanning electron microscopy of the aluminum wire that was incubated with the obtained isolate of *P. citreosulfuratum*. A) The aluminum wire that was not incubated with the fungus, i.e., the negative control, was observed under optical microscopy. An insert shows the aluminon wires with a naked-eye view. B) The aluminum wire that was incubated with the fungus was observed under optical microscopy. C) Scanning electron microscopy of the aluminum wire that was not incubated with the fungus, i.e., the negative control. The scale bar is at 10 µM. D) Scanning electron microscopy of the aluminum wire incubated with the fungus but with increased magnification (scale bar is at 5 µM). In B) and D), note the metallic structure surrounded by the fungus hypha.

Metal ion resistance

When the obtained strain of *P. citreosulfuratum* was cultivated on PDA medium plates containing paper disks soaked with 1.0 M $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $Al_2(SO_4)_3 \cdot 16H_2O$, $FeCl_3$, or $NiCl_2 \cdot 6H_2O$ at the center, a visible inhibition halo was observed with copper, iron, and nickel, but not with aluminum after 2 and 7 days of culture (Figure 5A). Zinc has caused an inhibition halo after 2 days of growth (Figure 5A). Similar results were obtained when T&K media plates were used (data not shown). Aluminum at 50 mM in T&K liquid medium also did not inhibit, and zinc partially inhibited the *P. citreosulfuratum* strain growth after 12 days of culture (Figure 5B). Copper, iron, and nickel at 50 mM in the T&K liquid medium inhibited *P. citreosulfuratum* strain growth (Figure 5B).

Growth inhibition by essential oils

The plant's essential oils growth effect was tested on the *P. citreosulfuratum* strain in T&K medium agar plates (Figure 6A). After 7 days of cultivation, it was observed that the essential oils of thyme, oregano, mint, and melaleuca inhibited the *P. citreosulfuratum* growth ($\alpha = 0.01$) in 97, 90.3, 86, and 75.9%, respectively (Table 1). These essential oils' impact at lower concentrations on inhibiting the *P. citreosulfuratum* growth in PDA agar plates was evaluated after 4 days of cultivation. Only oregano and thyme essential oils could visibly retard the *P. citreosulfuratum* growth at concentrations as low as 10% and 20%, respectively (Figure 6B).

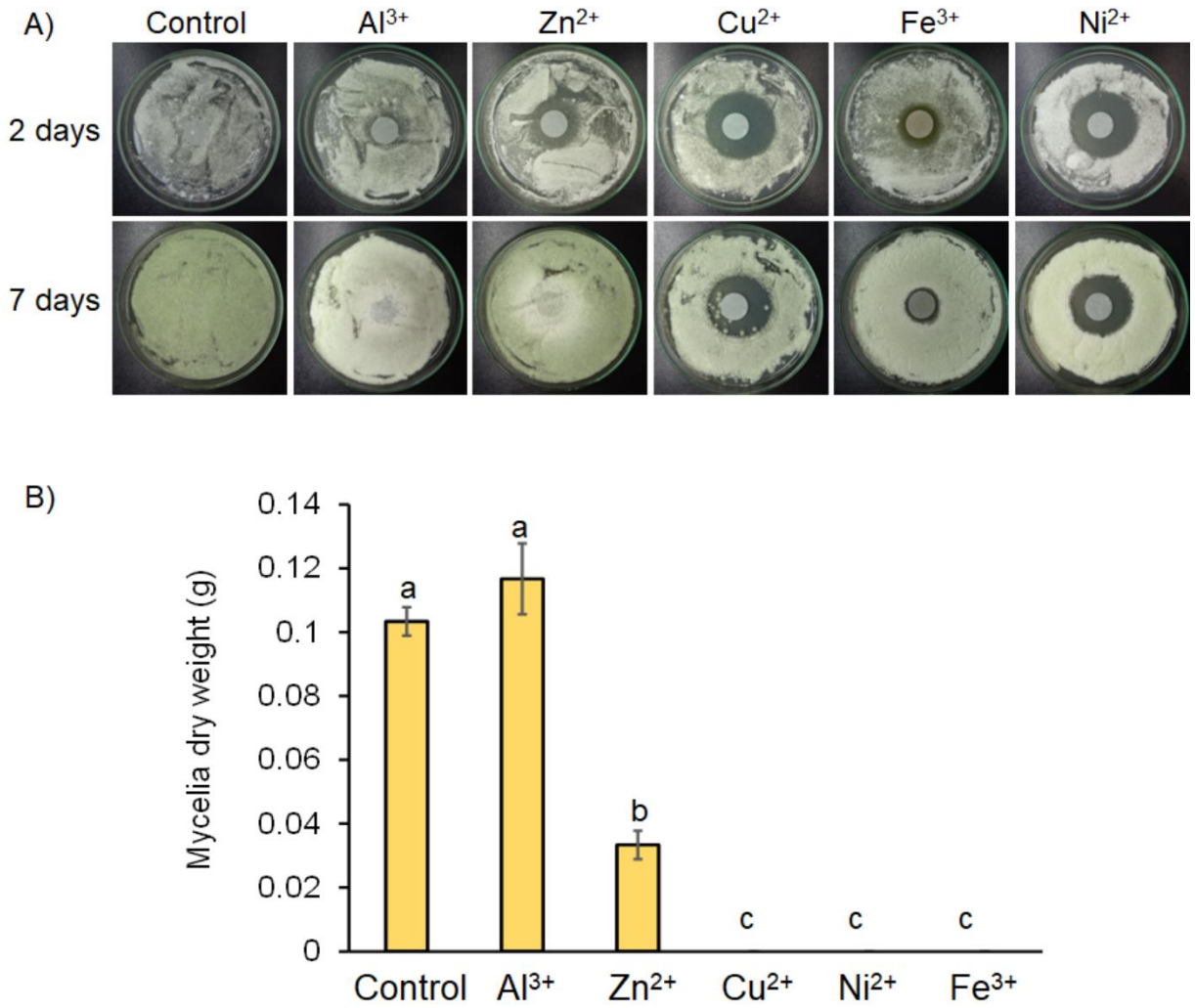

Figure 5. The metal resistance analysis in solid and liquid medium. A) Growth of the *P. citreosulfuratum* strain in PDA plates with 50 µL of 1 M ions solutions in the paper disk, after 2 and 7 days of culture at 25 °C, with a photoperiod of 12h. The control plate had water in the paper disk. B) The metal resistance analysis in a liquid medium. Growth of the *P. citreosulfuratum* strain in T&K medium in the presence of 50 mM of different ions after 12 days of stationary culture at 25 °C, with a photoperiod of 12h. The control had no ion addition in the medium. The data represent the average and standard deviation of triplicates. Different letters indicate different averages with statistical significance in the Tukey test (α = 0.01).

Table 1. *P. citreosulfuratum* growth in the presence of different essential oils.

Essential oil	Colony size (cm)*	PIG (%)[#]
Control	2.37 ± 0.11[a]	-
Lemon	2.43 ± 0.09[a]	0.0
Rosemary	2.33 ± 0.22[a]	1.7
Copaiba	2.23 ± 0.16[a]	5.9
Clove	2.30 ± 0.20[a]	2.9
Sucupira	2.27 ± 0.09[a]	4.2
Melaleuca	0.57 ± 0.36[b]	75.9
Mint	0.33 ± 0.04[b]	86.0
Oregano	0.23 ± 0.09[b]	90.3
Thyme	0.07 ± 0.09[b]	97.0

*The data is the average and the standard deviation of the results obtained in three T&K medium culture plates. Averages followed by the same letter are not statistically different by the Tukey test (α = 0.01)
[#]Percentage of growth inhibition

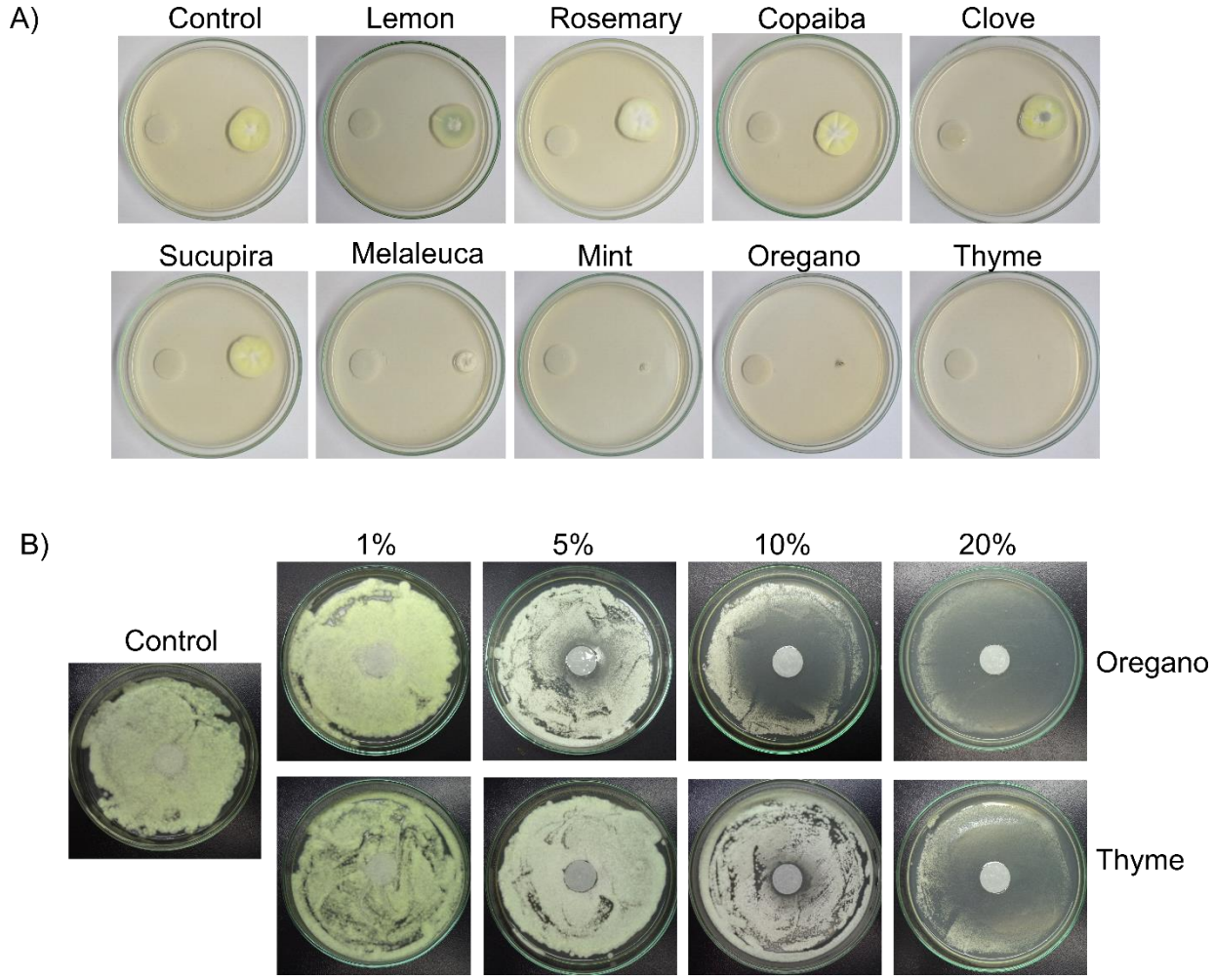

Figure 6. The essential oil inhibition of fungus growth. A) Representative dishes of the *P. citreosulfuratum* strain in modified T&K medium with sucupira, copaiba, clove, oregano, thyme, lemon, mint, melaleuca, and rosemary oils in the paper disk, after 7 days at 25 °C. The control plate has pure Vaseline in the paper disk. B) Dilution of oregano and thyme essential oils. Plates of PDA medium with the *P. citreosulfuratum* strain with a paper disk containing oregano and thyme diluted essential oils (1, 5, 10, and 20%) in Vaseline, after 4 days at 25 °C, with a photoperiod of 12h. The control plate has pure Vaseline in the paper disk.

DISCUSSION

The genus *Penicillium* is one of the most common fungi in various environments [16]. Its species have a universal distribution and a significant economic influence on human life and play essential roles in the food industry, as antibiotic producers, and in biomass decomposition [16]. *P. citreosulfuratum* is considered the correct name for *Penicillium toxicarium* [25]. This fungus was closely associated with cork bark (*Quercus suber* L.) in Portugal [27], and although not considered a health concern, it may contribute to moldy flavors in wine. Other species of the *Penicillium* genus *Exilicaulis* section, such as *P. citreonigrum*, are considered good producers of citreoviridin [28], a mycotoxin linked to yellow rice disease or acute cardiac beriberi [29]. However, it is still being determined whether the ex-type culture of *P. citreosulfuratum* (IMI 92228) produces citreoviridin [25].

Of the 25 described sections of the *Penicillium* genus [26], 11 were present in the generated phylogenetic tree (Figure 3). *Penicillium* in section *Exilicaulis* is characterized by non-vesiculate monoverticillate and biverticillate stipes. Species in this section are commonly found in terrestrial environments, such as air, soil, plants, and insects [25, 26]. However, it was also isolated from sea sand in Korea [30].

Biofilms consist of microorganism cells that stick to each other and surfaces. They are ubiquitous and can be formed by bacteria, archaea, and almost every species of microorganism [4]. In biofilms, the microorganism's cells are frequently embedded within a self-produced extracellular polymeric substance (EPS) matrix, such as lipids, polysaccharides, nucleic acids, and proteins, which causes adherence of cells to each other and (or) to a surface [4, 31]. The biofilm formation depends on the affinity between the metallic ions and the anionic groups of the extracellular polysaccharides (EPS) secreted by the microorganism [4,

32]. When biofilms develop on metallic surfaces, they may have a dual role, as they can inhibit corrosion or create microenvironments conducive to corrosion [32]. When causing corrosion, the probable mechanisms are that they can cause oxidation of sulfur or iron to obtain energy, produce inorganic and organic acids during their metabolism, and secrete degrading exoenzymes chelators, which will lead to corrosion [4]. The results of biofilm formation by *P. citreosulfuratum* (Figure 4), showed that it could adhere to the surface of aluminum wire. Considering that this microorganism was isolated from corroded material from a water energy plant, it is probable that the formed biofilm would lead to corrosion. Although other *Penicillium* species, for instance, *Penicillium commune* and *Penicillium frequentans*, have been responsible for MIC acceleration of metals [33, 34], to the best of our knowledge, this is the first report of the *P. citreosulfuratum* species linked to MIC.

However, biofilm formation did not occur on copper wires. This finding agrees with the growth inhibition that occurred when *P. citreosulfuratum* was cultured with solid and liquid media containing copper (Figures 5A and B). In agreement with the biofilm formation on aluminum wires, aluminum did not inhibit the *P. citreosulfuratum* strain growth in the solid and liquid medium analysis (Figures 5A and B). The partial inhibition of the fungus growth caused by zinc in the solid culture medium agrees with the results obtained in liquid media, where a partial inhibition of the *P. citreosulfuratum* growth was observed (Figures 5A and B).

The pollution effects on the environment caused by human and industrial activities provoke heavy metal accumulation into the water resources, threatening human health and the ecosystem. Biosorption is the process of passive pollutants binding by living or dead biomass and represents a potentially low-cost way of removing toxic heavy metals from industrial wastewater [35, 36]. Microorganisms are known to detoxify metals by several mechanisms, including ion exchange, chelation, adsorption, crystallization, valence transformation, extra and intracellular precipitation, and active uptake [37]. Among the microorganisms, fungi are more metal tolerant and have a higher microorganism surface-to-volume ratio than bacteria or actinomycetes [37]. Among fungi, the genus *Penicillium* has been described in metal biosorption analyses [37–42]. The *P. citreosulfuratum* growth in aluminum and zinc's presence could also be significant for this strain's role in MIC and biosorption.

In plants, essential oils function as antifungals, insecticides, and antibacterials, besides their effect against herbivores. Their components consist of 20-60 substances, some occurring in high amounts (20-70%), which are generally responsible for the biological effect. The major constituents of essential oils, which may be involved in the antifungal activity, are terpenes (e.g., pinene and limonene), terpenoids (e.g., geraniol), aromatic phenols (e.g., carvacrol, thymol, and eugenol), and cinnamaldehyde [13, 43]. Usually, essential oils with phenols and aldehydes as significant components, such as carvacrol, eugenol, thymol, and cinnamaldehyde, are the most effective, followed by those with terpene alcohols [44]. Accordingly, the two most efficient essential oils against *P. citreosulfuratum* were thyme, which contains thymol, and oregano, which contains thymol and carvacrol.

Essential oils have been shown to control other species of *Penicillium*, mainly in foodborne isolates [45–48]. In agreement with our results, oregano and thyme essential oils have also inhibited the growth of *Penicillium verrucosum* and *Penicillium digitatum*, respectively [45, 46]. However, in contrast to our results, clove essential oil has inhibited the *P. digitatum* and *Penicillium expansum* growth, and rosemary essential oil has inhibited the *P. expansum* growth [45, 47]. These contrasting results indicate that essential oils may act differently in different species of *Penicillium.* The plant essential oil's main antifungal properties are cell membrane disruption, cell wall formation alteration and inhibition, mitochondria dysfunction, and efflux pumps inhibition [12, 13]. It is possible that all these processes somehow affected the *P. citreosulfuratum* strain growth.

CONCLUSION

In conclusion, a *P. citreosulfuratum* strain has been isolated from corroded fragments of the Brazilian hydroelectric power unit Coaracy Nunes. This strain was able to form biofilm on aluminum wires and withstand high concentrations of aluminum and zinc in solid and liquid media. Furthermore, the thyme, oregano, melaleuca, and mint essential oils have been shown to be very efficient in curtailing this fungus growth, which could be helpful in its control.

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REFERENCES

1. Procópio L. The role of biofilms in the corrosion of steel in marine environments. World J Microbiol Biotechnol. 2019;35:73. Available from: https://doi.org/10.1007/s11274-019-2647-4
2. Beech IB, Gaylarde CC. Recent advances in the study of biocorrosion: an overview. Rev Microbiol. 1999;30:117–90. Available from: https://doi.org/10.1590/S0001-37141999000300001
3. Procópio L. Microbially induced corrosion impacts on the oil industry. Arch Microbiol. 2022;204:138–143. https://doi.org/10.1007/s00203-022-02755-7.
4. Loto CA. Microbiological corrosion: mechanism, control and impact - a review. Int J Adv Manuf Technol. 2017;92:4241–52. Available from: https://doi.org/10.1007/s00170-017-0494-8
5. Little BJ, Hinks J, Blackwood DJ. Microbially influenced corrosion: towards an interdisciplinary perspective on mechanisms. Int Biodeterior Biodegradation. 2020;154:105062. Available from: https://doi.org/10.1016/j.ibiod.2020.105062
6. Coetser SE, Cloete TE. Biofouling and biocorrosion in industrial water systems. Crit Rev Microbiol. 2005;31:213–32. Available from: https://doi.org/10.1080/10408410500304074
7. Emerson D. The role of iron-oxidizing bacteria in biocorrosion: a review. Biofouling. 2018;34:989–1000. Available from: https://doi.org/10.1080/08927014.2018.1526281
8. Beech IB, Sunner JA, Hiraoka K. Microbe-surface interactions in biofouling and biocorrosion processes. Int Microbiol. 2005;8:157–68. Available from: https://scielo.isciii.es/pdf/im/v8n3/03%20Beech.pdf
9. Skovhus TL, Eckert RB, Rodrigues E. Management and control of microbiologically influenced corrosion (MIC) in the oil and gas industry - Overview and a North Sea case study. J. Biotechnol. 2017;256:31–45. Available from: https://doi.org/10.1016/j.jbiotec.2017.07.003
10. Kokilaramani S, Al-Ansari MM, Rajasekar A, Al-Khattaf FS, Hussain A, Govarthanan M. Microbial influenced corrosion of processing industry by re-circulating waste water and its control measures - a review. Chemosphere. 2021;265:129075. Available from: https://doi.org/10.1016/j.chemosphere.2020.129075
11. Chajduk E, Bojanowska-Czajka A. Corrosion mitigation in coolant systems in nuclear power plants. Prog Nucl Energy. 2016;88:1–9. Available from: https://doi.org/10.1016/j.pnucene.2015.11.011
12. Nazzaro F, Fratianni F, Coppola R, De Feo V. Essential oils and antifungal activity. Pharmaceuticals. 2017;10:86. Available from: https://doi.org/10.3390/ph10040086
13. Hossain SMZ, Razzak SA, Hossain MM. Application of essential oils as green corrosion inhibitors. Arab J Sci Eng. 2020;45:7137–59. Available from: https://doi.org/10.1007/s13369-019-04305-8
14. Tuovinen OH, Kelly DP. Studies on the growth of *Thiobacillus ferrooxidans*. Arch Mikrobiol. 1973;88:285–98. Available from: https://doi.org/10.1007/BF00409941
15. Nelson PE, Toussoun TA, Marasas WFO. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park, 1983.
16. Pitt JI, Hocking AD. Fungi and food spoilage. Springer, New York, 2009.
17. Koenig RL, Ploetz RC, Kistler HC. *Fusarium oxysporum* f. sp. cubense consists of a small number of divergent and globally distributed clonal lineages. Phytopathology. 1997;87:915–23. Available from: https://doi.org/10.1094/PHYTO.1997.87.9.915
18. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Ed.), PCR Protocols, a Guide to Methods and Applications. Academic Press Inc., New York, 1990, pp. 315–22.
19. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7. Available from: https:// doi. org/ 10. 1093/ molbev/msab120
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25. Available from: https://doi.org/10.1093/oxfordjournals.molbev.a040454
21. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783. Available from: https://doi.org/10.2307/2408678
22. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA. 2004;101:11030–5. Available from: https://doi.org/10.1073/pnas.0404206101
23. Edgington LV, Khew KL, Barron GI. Fungitoxic spectrum of benzimidazole compounds. Phytopathology. 1971;61:42–4. Available from: https://doi.org/10.1094/Phyto-61-42
24. Canteri MG, Althaus RA, Virgens Filho JD, Giglioti E, Godoy CV. SASM-AGRI - System for analysis and mean separation in agricultural assays using Scott-Knott, Tukey and Duncan methods. Braz J Agrocomp. 2001;1:18–24. Available from: https://agrocomputacao.deinfo.uepg.br/dezembro_2001/Arquivos/RBAC_Artigo_03.pdf

25. Visagie CM, Seifert KA, Houbraken J, Samson RA, Jacobs K. A Phylogenetic revision of *Penicillium* sect. *Exilicaulis*, including nine new species from fynbos in South Africa. IMA Fungus. 2016;7:75–117. Available from: https://doi.org/10.5598/imafungus.2016.07.01.06
26. Serra R, Peterson S, Venâncio A. Multilocus sequence identification of *Penicillium* species in cork bark during plank preparation for the manufacture of stoppers. Res Microbiol. 2008;159:178–86. Available from: https://doi.org/10.1016/j.resmic.2007.12.009
27. Peterson SW, Jurjević Ž, Frisvad JC. Expanding the species and chemical diversity of *Penicillium* section *Cinnamopurpurea*. PLoS One. 2015;10:e0121987. Available from: https://doi.org/10.1371/journal.pone.0121987
28. Kushiro M. Historical review of researches on yellow rice and mycotoxigenic fungi adherent to rice in Japan. Mycotoxins. 2015;65:19–23. Available from: https://doi.org/10.2520/myco.65.19
29. Visagie CM, Houbraken J, Frisvad JC, Hong S-B, Klaassen CHW, Perrone G, et al. Identification and nomenclature of the genus *Penicillium*. Stud Mycol. 2014;78:343–71. Available from: https://doi.org/10.1016/j.simyco.2014.09.001
30. Park MS, Kim SH, Lee JW, Kim JS, Cho Y, Lim YW. Two unrecorded species belonging to *Penicillium* section *Exilicaulis* in South Korea. Korean J Mycol. 2020;48:175–85. Available from: https://doi.org/10.4489/KJM.20200019
31. Mitchell KF, Zarnowski R, Andes DR. Fungal Super Glue: The biofilm matrix and its composition, assembly, and functions. PLOS Pathog. 2016;12:e1005828. Available from: https://doi.org/10.1371/journal.ppat.1005828
32. Kip N, Van Veen JA. The dual role of microbes in corrosion. ISME J. 2015;9:542-51. Available from: https://doi.org/10.1038/ismej.2014.169
33. De Leo F, Campanella G, Proverbio E, Urzì C. Laboratory tests of fungal biocorrosion of unbonded lubricated post-tensioned tendons. Constr Build Mater. 2013;49:821–7. Available from: https://doi.org/10.1016/j.conbuildmat.2013.08.071
34. Juzeliūnas E, Ramanauskas R, Lugauskas A, Samulevičienė M, Leinartas K. Microbially influenced corrosion acceleration and inhibition. EIS study of Zn and Al subjected for two years to influence of *Penicillium frequentans*, *Aspergillus niger* and *Bacillus mycoides*. Electrochem Commun. 2005;7:305–11. Available from: https://doi.org/10.1016/j.elecom.2005.01.012
35. Ayele A, Haile S, Alemu D, Kamaraj M. Comparative utilization of dead and live fungal biomass for the removal of heavy metal: a concise review. Sci World J. 2021;2021:1–10. Available from: https://doi.org/10.1155/2021/5588111
36. Dhankhar R, Hooda A. Fungal biosorption – an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ Technol. 2011;32:467–91. Available from: https://doi.org/10.1080/09593330.2011.572922
37. Leitão AL. Potential of *Penicillium* species in the bioremediation field. Int J Environ Res Public Health. 2009;6:1393–417. Available from: https://doi.org/10.3390/ijerph6041393
38. Mahi PK, Tiwari KL, Jadhav SK. Biosorption of lead by biomass of resistant *Penicillium oxalicum* isolated from industrial effluent. J Appl Sci. 2017;18:41–7. Available from: https://doi.org/10.3923/jas.2018.41.47
39. Fan T, Liu Y, Feng B, Zeng G, Yang C, Zhou M, et al. Biosorption of cadmium(II), zinc(II) and lead(II) by *Penicillium simplicissimum*: isotherms, kinetics and thermodynamics. J Hazard Mater. 2008;160:655–61. Available from: https://doi.org/10.1016/j.jhazmat.2008.03.038
40. Xu X, Zhang Z, Huang Q, Chen W. Biosorption performance of multimetal resistant fungus *Penicillium chrysogenum* XJ-1 for Removal of Cu^{2+} and Cr^{6+} from aqueous solutions. Geomicrobiol J. 2018;35:40–9. Available from: https://doi.org/10.1080/01490451.2017.1310331
41. Verma A, Shalu Singh A, Bishnoi NR, Gupta A. Biosorption of Cu (II) using free and immobilized biomass of *Penicillium citrinum*. Ecol Eng. 2013;61:486–90. Available from: https://doi.org/10.1016/j.ecoleng.2013.10.008
42. Sundararaju S, Manjula A, Kumaravel V, Muneeswaran T, Vennila T. Biosorption of nickel ions using fungal biomass *Penicillium* sp. MRF1 for the treatment of nickel electroplating industrial effluent. Biomass Convers. Biorefinery. 2022;12:1059–68. Available from: https://doi.org/10.1007/s13399-020-00679-0
43. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Antifungal activities of thyme, clove and oregano essential oils. J Food Saf. 2007;27(1):91-101. Available from: https://doi.org/10.1111/j.1745-4565.2007.00063.x
44. Perricone M, Arace E, Corbo MR, Sinigaglia M, Bevilacqua A. Bioactivity of essential oils: a review on their interaction with food components. Front Microbiol. 2015;6:76. Available from: https://doi.org/10.3389/fmicb.2015.00076
45. Yahyazadeh M, Omidbaigi R, Zare R, Taheri H. Effect of some essential oils on mycelial growth of *Penicillium digitatum* Sacc. World J Microbiol Biotechnol. 2008;24:1445–50. Available from: https://doi.org/10.1007/s11274-007-9636-8
46. Ozcakmak S, Gul O, Dervisoglu M, Yilmaz A, Sagdic O, Arici M. Comparison of the effect of some essential oils on the growth of *Penicillium verrucosum* and its ochratoxin A production. J Food Process Preserv. 2017;41:e13006. Available from: https://doi.org/10.1111/jfpp.13006
47. Vieira AMFD, Steffens CA, Argenta LC, Amarante CVT, Oster AH, Casa RT, et al. Essential oils for the postharvest control of blue mold and quality of “Fuji” apples. Pesq Agropec Bras. 2018;53:547–56. Available from: https://doi.org/10.1590/s0100-204x2018000500003

48. Jahani M, Beheshti M, Aminifard MH, Hosseini A. Effects of essential oils to control *Penicillium* sp. in *in vitro* and in *in vivo* on grapevine (*Vitis Vinifera* L.) fruit. Int J Fruit Sci. 2020;20:812–26. Available from: https://doi.org/10.1080/15538362.2020.1769526