

Phosphate-Solubilizing Fungi from caves in the Serra do Espinhaço Meridional, Minas Gerais, Brazil

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Abstract

Brazilian caves have a high diversity and richness of fungal species, but little is known about the potential application of these cave fungi in agriculture. Many fungi can promote plant growth by solubilizing phosphorus (P) sources. This is an interesting characteristic for the agricultural sector, which is increasingly striving for sustainability using biological inputs. The present study aimed to isolate and identify phosphate-solubilizing fungi from Brazilian caves. Air, soil, and animal feces samples were collected from two caves, in the Serra do Espinhaço Meridional, Minas Gerais, Brazil. Fungi sampled from the air were isolated by exposing open Petri dishes containing NBRIP culture medium at different points in the caves. The serial dilution method was used for the fungal isolation of soil and animal feces samples. The isolates selected were identified through amplification and sequencing of genic regions. Seventeen isolates exhibited an apparent halo formation in the NBRIP medium and therefore were selected. Among them, four can be new species of *Penicillium* and one of *Xylaria*. Brazilian caves in the Serra do Espinhaço Meridional preserve fungi with the potential to solubilize phosphorus. The description of new taxa and new tests of solubilizing phosphorus are among the future works.

Résumé

Au Brésil, une grande diversité et richesse des fungi de grotte a été mise en évidence, mais peu d'informations sont disponibles sur l'application potentielle de ces fungi en agriculture. Les fungi peuvent stimuler la croissance des plantes en solubilisant les sources de phosphore (P). Cette caractéristique représente un intérêt considérable pour le secteur agricole, qui s'efforce de devenir de plus en plus durable grâce à l'utilisation d'intrants biologiques. Cette étude est destinée à isoler et identifier les fungi solubilisant le phosphore provenant des grottes brésiliennes. Des échantillons d'air, de sol et de matières fécales animales ont été prélevés dans deux grottes situées dans la Serra do Espinhaço Meridional, Minas Gerais, Brésil. Les fungi ont été isolés dans des boîtes de Petri contenant un milieu de culture NBRIP. Les isolats sélectionnés ont été identifiés par amplification et séquençage de régions géniques. Dix-sept isolats ont présenté une formation apparente de halo dans le milieu NBRIP. Parmi eux, quatre pourraient représenter de nouvelles espèces de *Penicillium* et une de *Xylaria*. Les grottes brésiliennes abritent des fungi possédant un potentiel de solubilisation du phosphore. La description de nouveaux taxons et la réalisation de nouveaux tests de solubilisation du phosphore seront effectuées ultérieurement.

1. Introduction

Caves are stable environments with peculiar characteristics that favor the development of unique biodiversity. In Brazil, more than 20,000 caves are registered (MINISTERIO DO MEIO AMBIENTE, 2022), but unfortunately, many are subject to extinction due to anthropic actions (CONDE et al., 2023a). Recent studies have shown that Brazilian caves harbor a high diversity and richness of fungal species, which increases the need for their preservation (CUNHA et al., 2020, ALVES et al., 2022, CONDE et al., 2023b, LEÃO et al., 2024, OLIVEIRA et al., 2024).

Fungi are organisms with great taxonomic, metabolic, and functional diversity. More than 140,000 fungal species and fungus-like organisms are estimated to exist worldwide, and only a small proportion of them are known (HYDE et al., 2024). Research on fungi in

caves includes reports, taxonomic descriptions, and bioprospecting for industrial uses (KHATRI et al., 2024). However, little is known about the potential applications of these cave fungi in agriculture.

Fungi can promote plant growth through mechanisms such as solubilizing phosphorus (P) sources and making them available to the plant. This is an interesting characteristic for the agricultural sector since phosphorus is essential for plant development. Furthermore, this nutrient is not accessible to plants due to the high adsorption of P in tropical and subtropical soils (OLIVEIRA et al., 2020), which requires external inputs of this nutrient. To our knowledge, no study has ever been conducted on cave fungi with the potential to solubilize phosphorus sources. The present work aimed to study cave fungi with this feature.

2. Materials and methods

2.1. Isolation and screening

Air, soil, and animal feces samples were collected from the Teto de Seixos and Gruta da Viola caves in the Serra do Espinhaço Meridional, Minas Gerais, Brazil. The samples were processed at the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas at the Universidade Federal de Viçosa. Fungi sampled from the air were isolated by exposing open Petri dishes containing NBRIP culture medium (NAUTIYAL, 1999) at different points in the caves. The serial dilution method was used for fungal isolation from soil and animal feces samples using the same NBRIP medium. The plates were incubated at 25°C, and isolation was performed every two days. Fungi capable of solubilizing calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) produced a halo around the colony and were selected.

A mycelium plug of each selected fungus was transferred to a Petri dish containing NBRIP (in triplicate) and incubated at 25 °C in the dark. After seven days, isolates that formed more apparent halos were

identified. Photographs were taken using a Nikon D3400 camera of the fungal colonies that showed positive result for phosphate solubilization.

2.2. Identification

The isolates were grown on PDA for 7–21 days at 25 °C in the dark to prepare slides. The fungi were grown in PDA medium with cellophane for seven days, followed by total DNA extraction. The internal transcribed spacer 1 and 2 regions and intervening 5.8S rDNA region (ITS), partial beta-tubulin gene region (TUB), and calmodulin gene region (CAL) of the fungal genome were amplified and sequenced. The sequences obtained were edited using FinchTV software and compared with sequences from type cultures available in the GenBank database using the BLAST tool. All selected fungal isolates were stored in tubes containing sterile distilled water at room temperature and 10% glycerol at –20 °C.

3. Results

Thirty-six fungal isolates were obtained from the samples analyzed in NBRIP culture medium. Of the 36 isolates individually tested in NBRIP medium, 17 exhibited an apparent halo formation (Fig. 1) and were subsequently selected for identification. Of the 17 phosphate-solubilizing isolates, 10 were obtained from soil samples, 6 from air samples, and 1 from animal feces. *Penicillium* was the most frequently identified

genus. The only isolate belonging to the phylum Basidiomycota was CDA 4723, classified under the genus *Trametes*. Initially, 11 isolates, out of 17 solubilizers, were identified with DNA sequences. Figure 2 provides information on the 11 selected isolates and results of the comparative analyses of sequences using the BLAST tool.

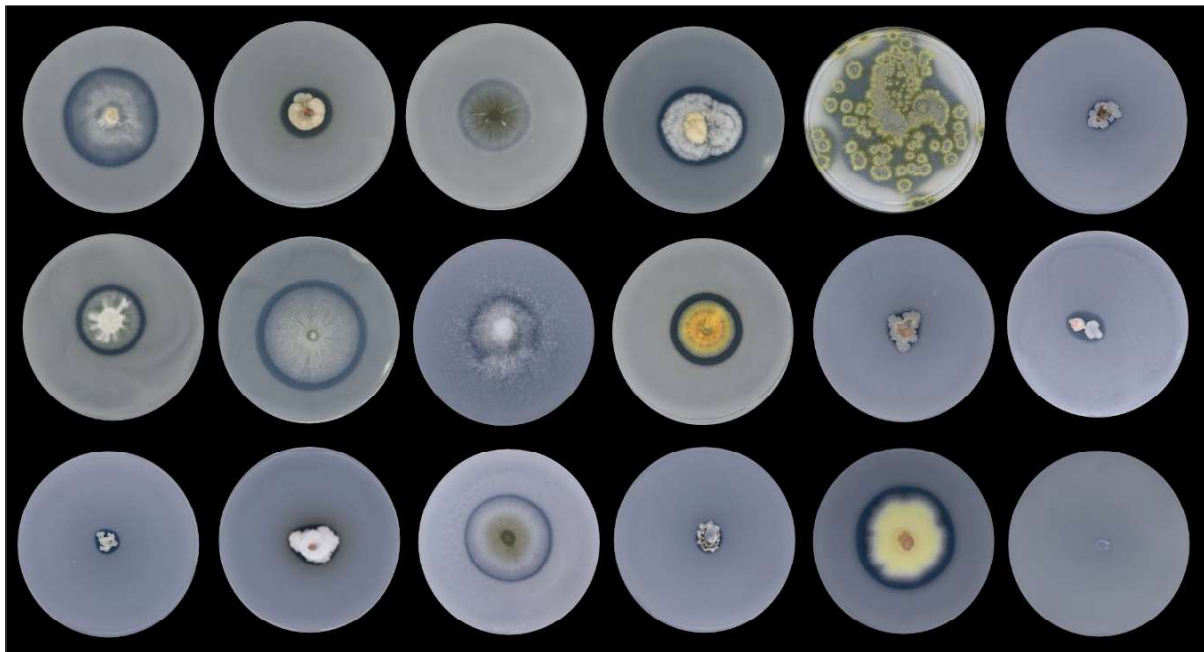


Figure 1: Seventeen selected isolates growing in NBRIP with halo formation and control plate. In the order of left to right: CDA 4717, CDA 4718, CDA 4702, CDA 4720, CDA 4701, CDA 4685, CDA 4722, CDA 4703, CDA 4723, CDA 4688, CDA 4724, CDA 4700, CDA 4725, CDA 4715, CDA 4710, CDA 4680, CDA 4716, Control.

Isolate	Cave	Sample	Blast	*Identity (%)
CDA 4717	Teto dos Seixos	Soil	<i>Purpureocillium lilacinum</i>	99,67 (TUB)
CDA 4718	Teto dos Seixos	Soil	<i>Penicillium jenningsiae</i>	97,85 (TUB)
CDA 4701	Teto dos Seixos	Soil	<i>Penicillium jacksonii</i>	94,10 (TUB)
CDA 4720	Teto dos Seixos	Soil	<i>Aspergillus chrysellus</i>	97,09 (CAL)
CDA 4703	Teto dos Seixos	Air	<i>Penicillium paneum</i>	82,37 (TUB)
CDA 4723	Teto dos Seixos	Air	<i>Trametes versicolor</i>	93,70 (ITS)
CDA 4688	Gruta da Viola	Soil	<i>Penicillium guanacastense</i>	95,16 (TUB)
CDA 4715	Gruta da Viola	Air	<i>Penicillium virgatum</i>	98,53 (TUB)
CDA 4710	Gruta da Viola	Air	<i>Penicillium paneum</i>	82,16 (TUB)
CDA 4680	Gruta da Viola	Air	<i>Xylaria hypoxylon</i>	92,14 (ITS)
CDA 4716	Gruta da Viola	Animal feces	<i>Talaromyces muroii</i>	98,91 (ITS)

Figure 2: Identification of fungal isolates obtained from air, soil and animal feces samples from caves with the potential to solubilize phosphorus sources. (*) The percentage of identity corresponds to a comparison of the gene regions of the isolates sequenced in the present study with reference isolate sequences available in the global GenBank database.

4. Discussion

In this study, 17 fungi with the potential to solubilize calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) were found, and this community was accessed for the first time in caves worldwide. Other researchers have conducted qualitative analyses in NBRIP medium, such as those presented in this study, and they are accepted as a starting point for screening P-solubilizing fungi (ZHANG et al., 2023, UGHAMBA et al., 2024).

The previous identification of the 11 isolates through consultation with GenBank highlighted a predominance of genera corresponding to the order Eurotiales, such as *Penicillium*, *Aspergillus*, *Talaromyces* and *Paecilomyces*. Studies have already shown that Brazilian caves harbor many fungi of this order. For example, new species of *Penicillium* and *Aspergillus* have already been reported in the caves of the Northeast region of Brazil (CONDÉ et al., 2022, LIMA et al., 2024).

According to MENDES et al. (2014), *Penicillium* was also one of the predominant genera among phosphate-solubilizing fungi using the NBRIP medium in their study. *Trametes* was the only genus identified within the phylum Basidiomycota, and this genus is associated with the degradation of lignocellulosic compounds, including causing white rot in wood (TIŠMA et al., 2021).

5. Conclusion

Brazilian caves in the Serra do Espinhaço Meridional, Minas Gerais preserve fungi with the potential to solubilize phosphorus obtained from air, soil, and animal feces samples. Furthermore, many of the

The identity percentages of isolates CDA 4701, CDA 4703, CDA 4688, and CDA 4710 indicate that they are possible new species of *Penicillium*, similar to the values found for CDA 4680, suggesting that they may be a new species of *Xylaria*. However, phylogenetic analyses including other informative gene regions must be performed to confirm this information.

KHATRI et al. (2024) highlighted the main studies focused on the bioprospecting of microorganisms in cave environments until now. However, little is still known about their potential applications in agriculture. The data presented here highlights the importance of more in-depth studies on fungi in caves and the need to preserve these environments. In addition, they provide new insights into the bioprospecting of microorganisms for agriculture.

Now, our 17 isolates comprise a pioneering collection of fungi with the ability to solubilize calcium phosphate, which will serve as the basis for future studies aimed at bioprospecting fungi to generate bioinputs. The next steps in this research will include verifying the amount of calcium phosphate and determining which other sources of phosphorus that each fungus is able of solubilizing.

selected isolates in this study represent possible new species that will be proposed subsequently.

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