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Fungi from Brazilian Savannah and Atlantic rainforest show high antibacterial and antifungal activity

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ABSTRACT

Brazilian biodiversity is among the biggest ones in the world, providing one of the major sources for bioprospection of new bioactive molecules. This study reports the isolation of new fungal strains from Brazilian biomes, aiming the biotechnological production of antimicrobial compounds against important microorganisms in the field of foodborne diseases, and difficult healing infections. First, 169 filamentous fungi were isolated from soil samples of Savannah and Atlantic Rainforest biomes in São Paulo State. The isolates were investigated about their inhibitory effect on the growth of five microorganisms (*Bacillus cereus*, *Staphylococcus aureus* subsp. *aureus*, *Escherichia coli*, *Salmonella enterica* subsp. *enterica* and *Candida albicans*), by microdillution assay. Around 93.5% of the isolates showed antimicrobial activity. Among them, 13% presented high activity and were evaluated about their minimal inhibitory concentration (MIC). Results revealed extracts with low MIC (0.31 mg mL^{-1}) and with bactericidal and bacteriolytic activity. These fungi were identified as *Trichoderma* spp, *Fusarium* spp., *Acremonium* spp., *Penicillium* spp., and *Paecilomyces* spp. The Brazilian biomes presented themselves as interesting bioprospection spots of new sources of antimicrobial compounds, and this is the first report of antimicrobial potential of fungi from entire Atlantic rainforest or from this geographical area of Brazilian Savannah.

1. Introduction

Natural products are typically secondary metabolites, in many cases with biological activities, broadly employed in pharmaceutical or agricultural field. Because of their high molecular diversity, they are inspiration for many therapeutic agents, representing almost 50% of newly approved drugs and related to almost one-third of the top selling drugs (Bolzani et al., 2012; Newman and Cragg, 2012; Strohl, 2000). Fungi are known for their metabolic potential to produce a great variety of enzymes and bioactive metabolites for food or pharmaceutical applications (Nigam and Singh, 2014). In this field, they play an important role in everyday life, producing antibiotics such as penicillin, anticancer drugs as taxol, immunosuppressants such as ciclosporin and cholesterol-lowering drugs as lovastatin (Gerke and Braus, 2014). However, the bioprospection of fungi from new or less investigated ecological niches is the key step to collect different fungal strains and, consequently, to discover new bioactive compounds (Bull et al., 2000; Demain and Zhang, 2005; Knight et al., 2003).

The most well known bioactive compounds from fungi are antibiotics (Awad et al., 2012). The continuous discovery of these compounds is one of the most important goals in biomedical research (Gerke and Braus, 2014; Salamoni et al., 2010) since infectious diseases are between the top ten causes of death worldwide (WHO, 2013). The main cause of this is the indiscriminate use of antibiotics in medical, food, veterinary and agriculture fields, what leads to the development of multi-resistant microorganisms. Due to the important need of new antimicrobials, and the lost of interest by pharmaceutical industry researching them (Demain, 2014; Wright, 2014), the main reason this study was carried out was to look for new efficient antimicrobial compounds that fungal biodiversity can provide.

Brazil is one of the most biodiverse countries, housing almost 10% of world's known species (Lewinsohn and Prado, 2005). Its huge extension and localization implies in different biogeographically zones or biomes, including Amazon, Atlantic rainforest, and Savannah area (Valencia and Chambergo, 2013). Located at southeast Brazil, the State of São Paulo is basically formed by Atlantic rainforest and Savannah

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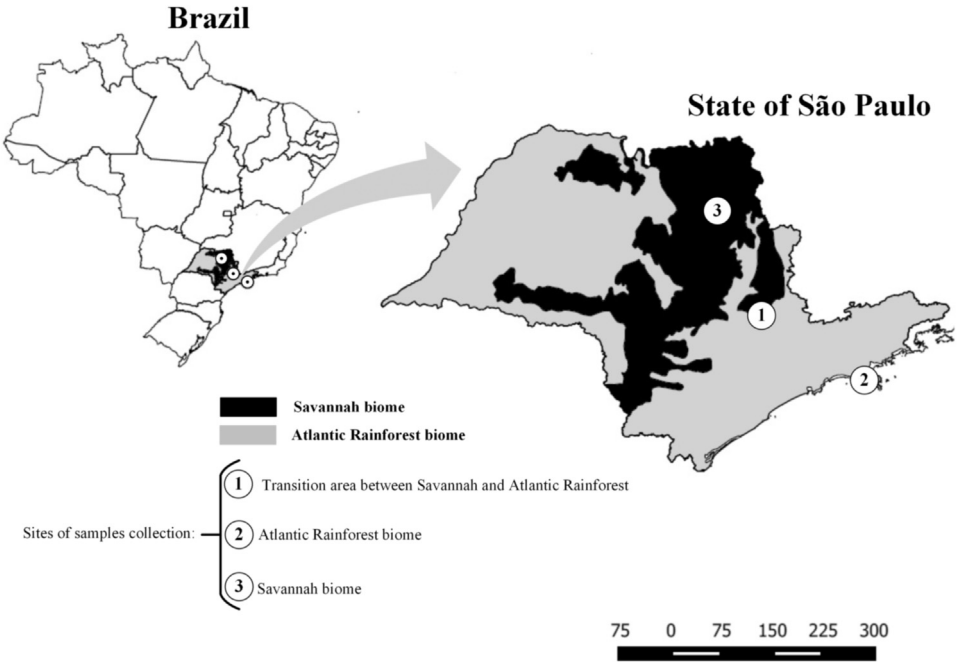

Fig. 1. Map of São Paulo State biomes with the three sites of soil samples were collected.

biomes. Although these regions are between the most biologically rich ecosystems on planet, they are mostly found in Brazil, and endangered to be extinct (Myers et al., 2000). The safe bioprospection of these areas can contribute to the discovery of new bioactive compounds and molecules with biotechnology applications, as shown by some studies (Alves-Prado et al., 2010; Colen et al., 2006; da Silva et al., 2015; Takahashi et al., 2008; Valencia and Chambergo, 2013). However, to the best of author's knowledge, there is no reported study that evaluated the antimicrobial properties of fungi isolated from the soil of São Paulo State biomes.

For the reasons presented above, the aims of the present study were to isolate new fungal strains from Atlantic rainforest region (23°82′ S, 45°44′ W), Savannah area (21°17′ S, 47°81′ W) and transition area between Savannah and Atlantic rainforest (22°80′ S, 47°05′ W), from Brazilian unexplored habitats (Fig. 1), to investigate the antimicrobial activity of these fungi and to identify, up to genres level, the most active isolates. Fungal extracts were tested against important microorganisms in the field of foodborne diseases and difficult healing infections.

2. Material and methods

2.1. Sampling

Samples of fruits, flowers, seeds, and soil (maximum depth of 5 cm) were collected from the litter of the areas presented in Fig. 1, from Atlantic rainforest (23°82′ S, 45°44′ W), Savannah biome (21°17′ S, 47°81′ W), and transition area between both biomes (22°80′ S, 47°05′ W). The environmental samples were collected with sterile lab tools, placed in plastic bags, then stored at 4 °C, until isolation procedures.

2.2. Fungal isolation

A portion of one gram of each environmental sample was diluted in 10 mL of sterile distilled water. Aliquots of each diluted sample were transferred to potato dextrose agar (PDA, Difco), supplemented with chloramphenicol (Sigma-Aldrich). The plates were incubated at 30 °C for five days, with daily observation. When pure filamentous colonies were observed (with clear zones around them) they were transferred to PDA slants. The pure cultures were overlaid with mineral oil and stored at 4 °C for further use (Kumar et al., 2010). These strains are deposited at The Brazilian Collection of Environmental and Industrial Microorganisms - CBMAI (Official website: http://webdrm.cpqba.unicamp.br/cbmai/). The access of biodiversity and its genetic resources was authorized by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and registered with the process number 010662/2013-8.

2.3. Fungal extracts

The isolates were cultivated on PDA for five days at 30 °C. After incubation, the culture growth was extracted from agar surface with sterile water. The extracts were filtered using a 0.22 μm membrane (Merck, Millipore) to remove the fungal cell mass and the cell-free supernatant was frozen to further evaluate their antimicrobial activity (Kumar et al., 2010).

2.4. Tested microorganisms

Five quality control strains were used in this study, including four bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* subsp. *aureus* ATCC 6538, *Escherichia coli* ATCC 11229 and *Salmonella enterica* subsp. *enterica* ATCC 14028) and one yeast (*Candida albicans* ATCC 10231). To prepare this microorganism's inoculum, Nutrient Broth (NB - Difco) was used for bacterial growth at 37 °C and YM Broth (YMB - Difco) was used to yeast growth at 30 °C. The concentration of the microorganisms' suspensions was standardized to 10^5 CFU mL^{-1}.

2.5. Fungal antimicrobial activity

The assay was based on an international microdilution method proposed by Eloff (1998), with some modifications. In general, the 96-well plates (Corning Costar) were prepared by dispensing into each well 50 μL of an appropriate medium (NB or YMB), 20 μL of extract, 50 μL of the microorganism inoculum and the final volume completed to 200 μL with sterile water. Medium control (water and medium), negative control (water, medium and inoculum), and the extracts control (water, medium and extract) were assayed simultaneously with samples. Microplates were incubated for 24 h at 37 °C and 30 °C, for bacteria and yeast, respectively. After incubation, 20 μL of

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