Susceptibility of airborne fungi to the mycocins produced by *Wickerhamomyces anomalus*

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ABSTRACT
Atmospheric air is the most used and most successful dispersion medium for fungi. Airborne fungi can establish themselves and contaminate the air, reducing the quality of life of the organisms that circulate there. The broad spectrum of antimicrobial activity and great stability led to the use of *Wickerhamomyces anomalus* as a biocontrol agent, since it could be classified as a low-risk microorganism, rarely traced in human samples. In addition, the antimicrobial action of mycocins produced from this yeast in eukaryotic and prokaryotic microorganisms is already proven. The aim of the present study was to evaluate the inhibition of airborne fungi by mycocins. The passive sedimentation technique was used in Petri dishes exposed in an external environment. The exposed plates consisted of a control group, consisting only of Sabouraud Dextrose Agar medium, and another test, composed of medium and supernatant of mycocins from *Wickerhamomyces anomalus*. The growth of 6 genera of fungi was observed on the control plates: *Cladosporium spp., Penicillium spp., Alternaria spp. Aspergillus spp., Chrysosporium spp.* and *Fusarium spp*. While, on the test plates, there was no growth of microorganisms. Therefore, we concluded that the mycocins produced by *Wickerhamomyces anomalus* were able to inhibit the growth of airborne fungi.

Keywords: airborne fungi, *Wickerhamomyces anomalus*, mycocins, air quality.

RESUMO
O ar atmosférico é o meio de dispersão mais utilizado e mais bem sucedido para fungos. Os fungos transportados pelo ar podem se estabelecer e contaminar o ar, reduzindo a qualidade de vida dos organismos que ali circulam. O amplo espectro da atividade antimicrobiana e grande estabilidade levou ao uso da *Wickerhamomyces anomalus* como agente biocontrolador, uma vez que poderia ser classificado como um microorganismo de baixo risco, raramente rastreado em amostras humanas. Além disso, a ação antimicrobiana de micocinas produzidas a partir desta levedura em microrganismos eucarióticos e procarióticos já está comprovada. O objetivo do presente estudo foi avaliar a inibição de fungos transportados pelo ar por micocinas. A técnica de sedimentação passiva foi utilizada em placas de Petri expostas em um ambiente externo. As placas expostas consistiam de um grupo de controle, consistindo apenas do meio Sabouraud Dextrose Agar, e outro teste, composto de meio e sobrenadante de micocinas de *Wickerhamomyces anomalus*. O crescimento de 6 gêneros de fungos foi observado nas placas de controle: Cladosporium spp., Penicillium spp., Alternaria spp. Aspergillus spp.,

Chrysosporium spp. e Fusarium spp. Enquanto, nas placas de teste, não houve crescimento de microorganismos. Portanto, concluímos que as micocinas produzidas por *Wickerhamomyces anomalus* foram capazes de inibir o crescimento de fungos transportados pelo ar.

Palavras-chave: fungos transportados pelo ar, *Wickerhamomyces anomalus*, micocinas, qualidade do ar.

1 INTRODUCTION

Fungi are able to colonize different substrates and habitats. They are ubiquitous organisms widely distributed in the environment, it can be found in the plants, animals, soil, water, and air. Atmospheric air is the most used dispersion medium for these organisms. These microorganisms are carried through water, insects, humans, and animals, having the ability to disperse through the atmospheric air, being called airborne (GONÇALVES et al., 2018; NAGEEN et al., 2021).

They produce mycotoxins and, when in contact with the human organism, are capable of triggering allergic processes, irritation of mucous membranes and skin, fungal infections and promoting the exposure of sensitive individuals to their propagules and toxigenic metabolites (LOBATO; VARGAS; SILVEIRA, 2009; MEZZARI et al., 2003).

With their ability to establish themselves and contaminate the air, reducing the quality of life of organisms that circulate there, anemophilous fungi are considered indicators of air pollution (DO NASCIMENTO et al., 2019).

The rowing interest in allergenic microorganisms and the search for new environmental indicators has aroused interest in the knowledge of airborne fungi and ways to control them, since their frequency and diversity are associated with air quality (LOBATO; VARGAS; SILVEIRA, 2009; MEZZARI et al., 2003).

Some killer yeasts have been explored for possible applications in the biological control of these pathogens (CALAZANS et al., 2021; MANNAZZU et al., 2019; NASCIMENTO et al., 2020).

The broad spectrum of activity antimicrobial and great stability led to the use of *Wickerhamomyces anomalus* as a biocontrol agent, being classified as a low-risk microorganism, rarely traced in human samples (GIOVATI et al., 2018). In addition, *Wickerhamomyces anomalus* was the first mycocin-producing yeast (also called killer yeast) discovered to be able to inhibit the growth of both eukaryotic organisms and pathogenic prokaryotes (POLONELLI et al., 2011; VIEIRA et al., 2021).

The yeast in question is capable of producing different groups of mycocins with different molecular masses. Antimicrobial activity assays confirm the ability of this killer yeast to act on cell wall glucans (CECARINI et al., 2019). The mechanism of action not yet fully elucidated describes activity on the cell wall of yeast degrading β-glucans through the action of β-1,3-glucanase (GIOVATI et al., 2018; JUNGES et al., 2020).

The aim of the present study was to evaluate the inhibition of airborne fungi by mycocins.

2 MATERIALS AND METHODS

The passive sedimentation technique was used in Petri dishes exposed in an external environment - parking lot of the Hospital Universitário do Oeste do Paraná. They were exposed 1 meter away from the walls for 20 minutes. The exposed plates consisted of a control group, consisting only of Sabouraud Dextrose Agar, and another test, consisting of Sabouraud Dextrose Agar and mycocin supernatant from *Wickerhamomyces anomalus*, being incorporated into 50% Sabouraud Dextrose Agar, 50% of supernatant containing mycocins. Then, the growth and/or inhibition of the collected fungi was observed after incubation for 10 days at 25°C.

After detection of fungal growth on plates, the topography, texture, and pigmentation of each specific type of colony are noted in order to identify the fungi accurately. The identities of these fungi were identified using cultural and morphological characteristics and by also comparing them with confirmed representatives of different species in relevant texts.

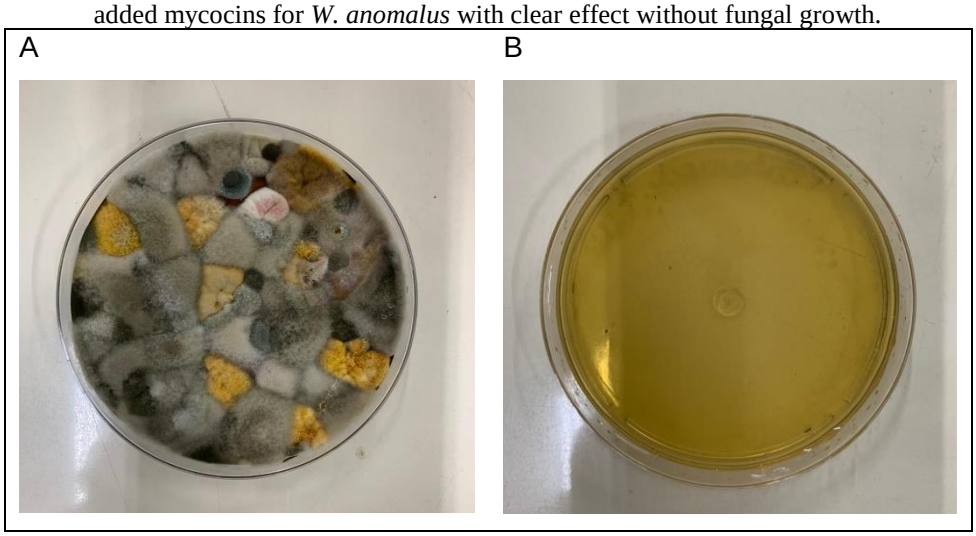
3 RESULTS AND DISCUSSION

The occurrence of airborne fungi in outdoor environments as well as their abundance, their diversity, and their impact on human, animal, and plant health is gaining attention globally (ODEBODE et al., 2020).

Six genera of fungi were observed the growth in the control plates: *Cladosporium spp., Penicillium spp., Alternaria spp. Aspergillus spp.*, *Chrysosporium spp.* and *Fusarium spp.*

The control plate and the test plate were exposed to the same period. On the control plate, the growth of the sedimented fungi can be observed (A); on the test side (B), the supernatant containing *Wickerhamomyces anomalus* mycocins was added to the culture medium, thus verifying the total inhibition of airborne fungi (Figure 1).

Figure 1. Fungal growth. A) Fungal growth on Sabouraud Dextrose Agar medium. B) Sabouraud Dextrose Agar added mycocins for *W. anomalus* with clear effect without fungal growth.



4 CONCLUSIONS

Therefore, this study showed the potential antimicrobial activity of mycocins produced by *Wickerhamomyces anomalus*, which in test in solid media proved to be efficient against airborne fungi.

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