Área: QPN

Co-culture of *Penicillium setosum* and *Cryptococcus neoformans* reveals the production of antifungal diketopirerazine

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Highlights

A mixed fermentation strategy involving *P. setosum* and *C. neoformans* was employed to induce the production of secondary metabolites. The compound bisdethiobis(methylthio)acetylaranotine demonstrated antimicrobial activity against *C. neoformans*. This study focuses on the discovery of other dicetopiperazine compounds biosynthesized by this strain during mixed fermentation, aiming to identify compounds with therapeutic potential against pathogens such as *C. neoformans.*

Abstract

Co-cultures, also known as mixed fermentations, involves the growth of two or more microorganisms. This approach has emerged as an effective strategy to increase the production of natural products and to boost the activation of cryptic or silent gene clusters responsible for synthesizing new secondary metabolites. Many of these clusters are susceptible to biotic and/or abiotic environmental modifications what may further the production of bioactive compounds as a result of chemical and biological responses. The genus *Penicillium* represents one of the largest sources of fungal known chemodiversity, thus revealing a promising source for the discovery of new therapeutic agents through chemical and biological exploration of its secondary metabolome. In previous studies conducted by our research group (LaBiORG), with the *Penicillium setosum* CMLD 18 isolated from the *Swinglea glutinosa* plant, we identified the production of dicetopiperazines (DKPs) by the specie. These studies showed the detection of the compounds bisdethiobis(methylthio)acetylaranotine and fellutanine C. Their cytotoxic effects were confirmed, representing the first report of antileukemic activity of this dicetopiperazine to date[1]. Based on these findings, this work set out to investigate the interaction between the endophyte *P. setosum* and the pathogenic fungus *Cryptococcus neoformans*, seeking to determine whether this interaction could increase the production of other DKPs and trigger the expression of other silent biosynthetic pathways in *P. setosum*. Then, the cultivation *P. setosum* and *C. neoformans* was performed in a liquid Potato Dextrose (BD) culture environment, under static and agitated conditions. The extracts were analyzed by high-resolution liquid chromatography-mass spectrometry (HPLC-HRMS). Molecular dereplication is being performed through manual inspection, *in sillico* metabolomics tools, as well as data analysis on the GNPS platform. According to the obtained molecular annotations, it was possible to identify the induction of diketopiperazines in extracts from the co-culture, which were not detected in the axenic cultivation of *P. setosum* exposed under the same conditions. Bisdethiobis(methylthio)acetylaranotine was confirmed as one of the DKPs whose the production was induced by the applied co-culture. Interestingly, this class of compounds has antifungal activities described in the literature against *C. neoformans*[3,4]. Therefore, the compound bisdethiobis(methylthio)acetylaranotine (which had been previously isolated in our studies involving *P. setosum* and OSMAC approach[1,2]), was subjected to bioassays with *C. neoformans*, showing antifungal activity against the pathogen. This result highlights the potential of DKPs as candidates for the development of new antifungal agents to combat infections caused by *C. neoformans*.

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