Aromatic compounds from the endophytic fungus *Asordaria conoidea* and their allelochemical property using OSMAC strategy

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**ABSTRACT**

Endophytic fungi are biodiverse and alternative source of bioactive compounds, due their different abilities of genetic expression and alteration of biosynthetic pathway when submitted to different culture conditions. The metabolic profile of three different crude extracts (A, B and C), obtained from the endophytic fungus *Asordaria conoidea*, were evaluated by HPLC and \(^1\)H NMR. Antioxidant and allelochemical activity were also evaluated. OSMAC diversified the metabolic production, mainly in the solid culture, where the tyrosol, 4-hydroxybenzaldehyde, 2-phenylacetamide and vanillic acid were isolated. The structures of the compounds were elucidated mainly by NMR. Extracts had antioxidant potential, however, only Extract C showed allelochemical activity, as inhibition of 65.5% in growth. This study confirms the efficiency of the OSMAC platform in producing extracts of different properties and compounds. Herein the *A. conoidea* was isolated for the first time as an endophytic microorganism.

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1. Introduction

Several biologically active products can be produced by different species of fungi, such as the *Asordaria conoidea*. Naturally well distributed fungus, being commonly found in soils of tropical regions with coprophobic behavior in the feces of herbivorous animals (Cai et al. 2006). A microorganism of metabolic potential is still very little studied. It is a potential source of natural products with unique characteristics and diverse applications, from agriculture to human health (Nisa et al. 2015; Araujo et al. 2016).

Microorganisms are sensitive to culture media for production of secondary metabolites, and that changing the culture medium could lead to the changes in metabolite profiles (Hillman et al. 2017). An alternative form of fungi cultivation is called OSMAC (One Strain Many Compounds). The method stimulates the production of the secondary metabolites in a single microbial strain by manipulating cultivation parameters (Wijesekera et al. 2017). Herein we used the OSMAC strategy to evaluate the potential of the metabolic production of the endophyte *A. conoidea* in different culture media.

To identify major compounds, as well as to evaluate the antioxidant and allelochemical properties of the crude extracts obtained.

2. Results and discussion

The molecular identification, based on ITS DNA region, showed 100% similarity with the species *Asordaria conoidea*, then allowed the design of a phylogenetic tree (see Figure S1). The nucleotide sequence determined was deposited in the GenBank database under the access number MN394547. Herein, we report the species *A. conoidea* first time as an endophytic microorganism.

The chemical profile obtained by HPLC and 1H RMN reveals a complex metabolic profile. The Extract C showed a higher number of peaks in comparison to the liquid medium. So solid media was selected for large-scale culture for the isolation of the major compounds.

The antioxidant potential of the Extracts A, B and C was revealed in chromatoplates with a methanol solution of DPPH (0.2%). It was observed the presence of antioxidant substances in all extracts, evidenced by the appearance of yellowish spots on the chromatoplate (retention factors: 0.0/0.42/0.36 and 0.44). This activity may be attributed to the isolated substances tyrosol and 4-hydroxybenzaldehyde. For allelochemical potential of the extracts were evaluated the seedling elongation and germination of the seed of *Lactuca sativa* L (Table 1). Allelochemical activity for Extract C was exhibit in the root and lettuce seedlings development (Table 1, Figure S2). The Extract C responds with a variety of different allelochemical compounds, inhibiting plant development with the increase of the extract concentration. Which can be attributed to the cultivation of the same fungal strain in different media (OSMAC), which led to the formation of crude extracts with different chemical composition.

From Extract C, it was possible to isolate and identify four substances: tyrosol, 4-hydroxybenzaldehyde, 2-phenylacetamide and vanillic acid. The structure of the compounds was determined by NMR experiment and comparing the data with the literature (Antonovic et al. 1990; Moustafa et al. 2007; Christophoridou and Dais 2009; Uzor et al. 2016).
Tyrosol is a known phenolic compound (quorum-sensing) of fungal origin, also found in olive oils with antioxidant potential, antimicrobial and growth promoter in fungi (Brilhante et al. 2016). 4-hydroxybenzaldehyde, on the other hand, is considered an important chemical intermediate in the production of drugs, perfumes and in the synthesis of flavorings (Ha et al. 2000; Wang et al. 2017). 2-phenylacetamide is a known synthetic compound, but also isolated as a natural product in plants and endophytes (Moustafa et al. 2007; Conti 2012).

Vanillic acid is important in pharmacological application, used in the treatment of inflammatory diseases and chronic infections (Yrbas et al. 2015). This compounds have been isolated as a natural product in plants and microbial crops (Cheng et al. 2014; Uzor et al. 2016).

From the structural features of the compound isolated, all these compounds might have come from the same shikimate pathway, being the aromatic amino acids precursors in all proposals of biosynthetic routes, exemplified in Figure S3 (Yang et al. 2018).

It has been reported the isolation of metabolic class such as, flavonols, tannins, saponins, steroids and phenolic compounds from the roots of the host plant Cochlospermum regium (Andrade et al. 2008). Observing the similarity structural features of the aromatic compound isolated with the classes identified in the host species, we can suggest that the fungus A. conoidea may have a symbiotic relationship and co-evolved with its host plant biosynthesizing similar compounds.

3. Experimental

The experimental section is available in supplementary

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