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Metabolic response of *Aspergillus sydowii* to OSMAC modulation produces acetylcholinesterase inhibitors

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ABSTRACT

Fungi are an extraordinary source of bioactive metabolites especially because their metabolic profile can be modulated according to the conditions under which they are grown, a strategy known as OSMAC. *Aspergillus sydowii* has been showing to be a fungal species with a series of interesting biosynthetic pathways, but production of acethylcholinesterase inhibitors was still not sufficiently explored from this fungal species. In this work, *A. sydowii* was grown in three different media aiming at awaking biosynthetic routes silent in the works already reported in the literature, in order to produce acethylcholinesterase inhibitors. Using sucrose (150 g/L) and peptone (12 g/L) as sources of carbon and nitrogen respectively, a very active extract was obtained (84.5 ± 2.2% of acethylcholinesterase inhibition). Metabolites **1–5** were isolated from this extract. These metabolites are described from *A. sydowii* by the first time, to the best of our knowledge. Compound **2** was the most active AChE inhibitor (93.25 ± 0.41%). The results show how small changes in culturing conditions can modulate the production of bioactive secondary metabolites that are silent in other cultivation conditions.

1. Introduction

Filamentous fungi are microorganisms widely distributed in nature and have been used for various applications, highlighting the production of metabolites with biological activity (Hoffmeister and Keller, 2007). Among the existing fungal genera, *Aspergillus* genus comprises species able to cause both positive and negative economic and environmental impacts (Klich, 2002). Many species of this genus are responsible for producing important secondary metabolites to pharmaceutical, food and cosmetics industries. *Aspergillus sydowii* is a species that has been used for production of secondary metabolites with chemical and pharmaceutical applications, such as indole alkaloids (He et al., 2012), sesquiterpenoids (Chung et al., 2013; Wang et al., 2014) and xanthones (Trisuwan et al., 2011), as well as enzymes of industrial interest, such as lipases (Byndyia and Ramana, 2012), xylanases (Ghosh and Nanda, 1994) and endoglucanases (Mushtaq et al., 2014). Sesquiterpenoids with antimicrobial and antiviral activity were also obtained from *A. sydowii* (Wang et al., 2014). Therefore, *A. sydowii* is a fungal species with a broad scope of pharmacological applications. In a study conducted by Song et al., new xanthone derivatives from *A. sydowii* were obtained (Song et al., 2013) and some of them presented immunosuppressive activity. From a marine-derived *A. sydowii* strain, isolated in East China Sea, were obtained three news sydowiols, trispyrogallol ethers which exhibit activity against *Mycobacterium tuberculosis* protein tyrosine phosphatase A (PtpA) (Liu et al., 2013). The effect of an epigenetic modifier over a *A. sydowii* strain isolated from a Taiwan marine sediment led to the production of sesquiterpenoids that exhibited anti-diabetic and anti-inflammatory activity (Chung et al., 2013). In a recent work, Li et al. isolated a new alkaloid with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE). Among compounds isolated by these authors, a diketopiperazine cyclo-(L-Trp-L-Phe) was obtained and showed moderate inhibition of MRSA and MRSE (Li et al., 2017).

Our research group has been looking for acetylcholinesterase inhibitors produced from natural sources (plants and fungi) (Schurmann et al., 2010; Teles and Takahashi, 2013; Dos Santos et al., 2017; Lima et al., 2018). In a series of screenings conducted on our group, a strain showed outstanding preliminary results for acetylcholinesterase inhibition and was identified as *A. sydowii.* Although *A. sydowii* has been showing to be a fungal species with a series of interesting biosynthetic pathways, production of acetylcholinesterase inhibitors was still not sufficiently explored from this fungal species. Many biosynthetic routes

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can be silent in fungi and a strategy that has been widely used to induce the expression of those silent metabolites from microorganisms is OSMAC (**O**ne **S**train, **MA**ny **C**ompounds) (Bode et al., 2002). This approach consists in varying cultivation conditions, like physico-chemical (for instance temperature and pH), nutritional (type of substrate used), or biological factors, such as the presence of another microorganism (biotic stress) or addition of gene expression modifiers, to obtain different metabolites, or increase their yields, from a single microorganism species (Bills et al., 2008; Takahashi et al., 2013; Hewage et al., 2014; Bertrand et al., 2014). Applying OSMAC approach, using three different culture media, Zhang et al. (2013) isolated different bioactive indole diketopiperazines from the fungus *Aspergillus fumigatus*. In another example, Wang et al. (2011) obtained different profiles of cytochalasins from the fungus *Spicaria elegans*, varying the type of amino acid used in the fermentation. Therefore, the present study aimed at inducing a metabolic response of *A. sydowii* using OSMAC approach, for production of metabolites with AChE inhibition capacity.

2. Results and discussion

2.1. Identification of the fungal isolate 622

The morphological analysis of the fungal isolate 622 showed smooth conidiophores with subglobose and spinose conidia (diameter 2.5–3 μm), vesicle (5–10 μm), and biseriatephialides (length = 2.2–3.0 μm and breadth = 3.4–6.1 μm). The isolate 622 showed similar morphological characteristics with the literature associated with *Aspergillus sydowii* (Fig. 1) (Samson and Pitt, 2000). The morphological analysis allowed to identify the fungal isolate 622 as *Aspergillus cf. sydowii* (Fig. 1). *Aspergillus sydowii* is classified in section Versicolores; *A. sydowii* subclade contains *A. sydowii, A. creber, A. venenatus, A. tennesseensis, A. cvjetkovicii, A. jensenii* and *A. puulaauensis* (Jurjevic et al., 2012). Since to confirm the identification is necessary a combination of morphology and multiloccus DNA sequences analysis for strains in this clade (Jurjevic et al., 2012), the isolate 622 was identified as *Aspergillus cf. sydowii*. A voucher specimen has been deposited in AleloMicro, a culture collection localized in Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais, Brazil, under access code CMPC 717.

2.2. Chemical and biological profiles of extracts

The influence of variations in carbon and nitrogen sources on *A. sydowii* fermentation in three different media was analyzed by HPLC. These variations promoted changes in secondary metabolites biosynthesis, as observed in the chromatograms evaluated (Fig. 2). Extract M1 (180 mg) obtained from cultivation of *A. sydowii* using sucrose and yeast extract as sources of carbon and nitrogen, respectively, presented a significant amount of more polar compounds. Peaks from these compounds (relative retention time, RRt, between 1 and 5 min) can be observed in chromatogram of the extract (Fig. 2). Addition of glucose to the broth as a second source of carbon (M2) led to decrease in the yield of this extract (104.5 mg), when compared with the other two extracts obtained. In addition, this alteration also promoted polar compounds reduction which was observed in the HPLC profile of the extract obtained from M1. Replacement of yeast extract for peptone as a nitrogen source (M3) led to the significant increase in the yield of extract (261 mg). This change also stimulated expression of secondary metabolites which were not observed when *A. sydowii* was growing in M1 and M2. This condition was the one that provided improvement of metabolic expression by *A. sydowii*. When comparing the three culture broths, M3 promoted the expression of two compounds (RRt 16 min and 18 min) in greater yield. These compounds were not detected in M2 and showed very low production in M1. In addition, compounds with RRt 13 min and 22 min, which were present in M3, were not detected in M1 and M2. Therefore, it was established that peptone, an important amino acid complex, is a key ingredient for production of secondary metabolites from *A. sydowii*. In a study conducted by Mao et al. (2005), high yield of cordycepin, an important metabolite with high pharmacological potential, was obtained by optimizing the culture broth of *Cordycepts militaris*. The highest yield of cordycepin (345.4 mg/L) was obtained using peptone as nitrogen source. Chen and Johns (1993) related improved pigments production from *Monascus purpureus* fermentation. In that study, production of monascorubramine, an important red pigment, was obtained in higher concentration (200 mg/L) when peptone was employed as nitrogen source. In addition, ankaflavin, a yellow pigment, has been only detected in crude extracts when the same culture condition was employed for *M. purpureus* fermentation. These studies corroborate with the results obtained in the present work.

The extracts prepared in M1, M2 and M3 were evaluated as AChE inhibitiors. AChE is an enzyme targeted by most of the drugs currently used by Alzheimer's patients. The extract obtained when *A. sydowii* was cultivated in culture medium M3, besides presenting the most interesting HPLC profile, was also responsible for the higher AChE inhibition. As shown in Fig. 3, this extract was able to inhibit 84.5 ± 2.2% AChE under the bioassay condition. When comparing this result with percentages of inhibition found for the other two extracts (M1 73.8 ± 1.76% and M2 46.3 ± 2.79%), it was observed that the use of peptone as nitrogen source in M3 promoted expression of AChE inhibitors. Under M2 condition, some metabolic pathways in *A. sydowii,*

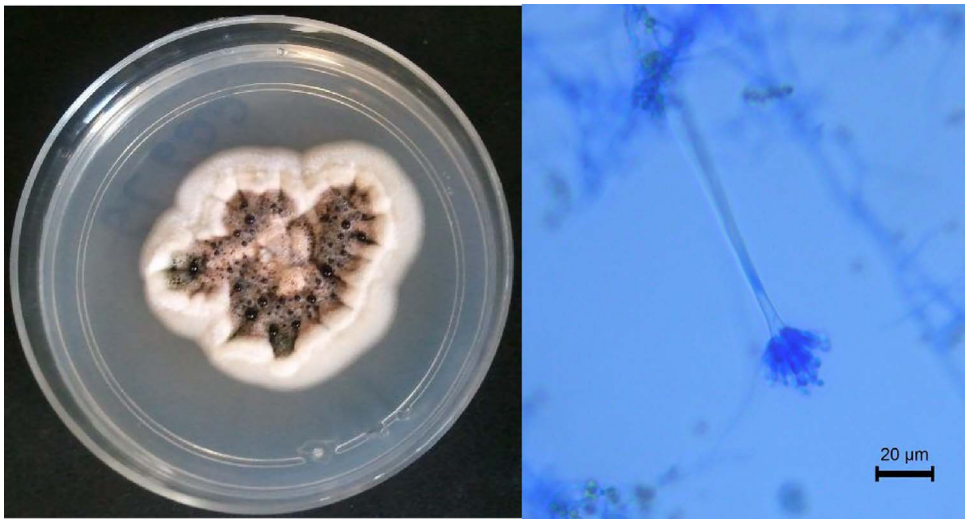

Fig. 1. *Aspergillus* cf. *sydowii*. A. culture plate, BDA colonies grown at 25 °C for 7 d. b. Subglobose vesicle and conidia, bar = 20 μm.