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Antimicrobial activity and acetylcholinesterase inhibition by extracts from chromatin modulated fungi

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ABSTRACT

Major health challenges as the increasing number of cases of infections by antibiotic multi-resistant microorganisms and cases of Alzheimer's disease have led to searching new control drugs. The present study aims to verify a new way of obtaining bioactive extracts from filamentous fungi with potential antimicrobial and acetylcholinesterase inhibitory activities, using epigenetic modulation to promote the expression of genes commonly silenced. For such finality, five filamentous fungal species (*Talaromyces funiculosus*, *Talaromyces islandicus*, *Talaromyces minioluteus*, *Talaromyces pinophilus*, *Penicillium janthinellum*) were grown or not with DNA methyltransferases inhibitors (procainamide or hydralazine) and/or a histone deacetylase inhibitor (suberohydroxamic acid). Extracts from *T. islandicus* cultured or not with hydralazine inhibited *Listeria monocytogenes* growth in 57.66 ± 5.98% and 15.38 ± 1.99%, respectively. Increment in inhibition of acetylcholinesterase activity was observed for the extract from *P. janthinellum* grown with procainamide (100%), when compared to the control extract (39.62 ± 3.76%). Similarly, inhibition of acetylcholinesterase activity increased from 20.91 ± 3.90% (control) to 92.20 ± 3.72% when the tested extract was obtained from *T. pinophilus* under a combination of suberohydroxamic acid and procainamide. Concluding, increases in antimicrobial activity and acetylcholinesterase inhibition were observed when fungal extracts in the presence of DNA methyltransferases and/or histone deacetylase modulators were tested.

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Introduction

Multiresistant microbial strains have emerged over the last decades[1,2] probably related to the extensive use of currently marketed antimicrobial agents. This scenario led to an increase on the search for novel sources of compounds with antimicrobial activity. Essential oils as lemongrass oil[3] are an example of new source of compounds with antibiotic activity. In addition, screening of fungal extracts has demonstrated that *Aspergillus* and *Penicillium* species produce metabolites with antibacterial activity against potentially pathogenic bacteria species such as *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium and *Pseudomonas aeruginosa*.[4,5]

Alzheimer's disease is the most common cause of dementia and affected approximately 35 million people around the world in 2010.[6] One of the mechanisms of Alzheimer's disease involves the reduction in acetylcholine production which implies in gradual loss of memory and learning ability along with other risk factors.[7] Based on the cholinergic hypothesis it was discovered that some molecules inhibit acetylcholine degradation by acetylcholinesterase enzyme, increasing the time of action of acetylcholine in the synaptic cleft.[8]

Fungi are important sources of bioactive secondary metabolites.[9,10] It is believed that the fungi represent the second largest kingdom in terms of diversity.[11] Ascomycota phylum, in which the genera *Penicillium* and *Talaromyces* pertain, has a greater genetic diversity for secondary metabolites production when compared to other fungi groups.[12]

Besides antimicrobial activity, fungi are important sources of bioactive molecules with acetylcholinesterase inhibition activity.[13] This high metabolite diversity observed in fungi could be due to the development of mechanisms for communication, competition and chemical defense over evolution process.[14]

Gene expression in filamentous fungi can be modulated by different conditions such as nutrients availability, light incidence, cultivation temperature, co-cultivation with other microorganisms, and epigenetic modifications.[15] The term epigenetics refers to chemical modifications in chromatin structure that can be able to change the conformation of this molecule in order to affect gene expression.[16] The basic structure of chromatin organization is given by the formation of nucleosomes, a protein octamer conglomerate (pairs of histone proteins H3, H4, H2A and H2B) in which the double-stranded deoxyribonucleic acid molecule is rolled.[17] Heterochromatic regions are less active in terms of gene transcription when compared to euchromatic regions.[18] Genes encoding secondary metabolites are usually organized in clusters[19] and silenced genes expression can be activated using epigenetic approaches.

Small chemicals, such as hydralazine, suberohydroxamic acid and procainamide are used as inhibitors of enzymes related to epigenetic modification mechanisms, including DNA methyltransferases (DNMT) and histone deacetylases (HDACs). Suberohydroxamic acid acts as a HDACs inhibitor while other epigenetic modulators, e.g. procainamide, inhibit DNMTs.[20] Despite results showing that secondary metabolism is affected by epigenetic modulators, few evidences prove a change in qualitative and quantitative patterns of metabolites with biological potential after epigenetic induction of the producing microorganisms.

In this context, the goal of the present study was to evaluate the effect of epigenetic modulation on the antimicrobial activity and acetylcholinesterase inhibition by extracts from fungal growth media of species pertaining to the *Penicillium* and *Talaromyces* genera.

Materials and methods

Microorganisms and inoculum preparation

Five filamentous fungi species (*Talaromyces funiculosus*, *Talaromyces islandicus*, *Talaromyces minioluteus*, *Talaromyces pinophilus*, *Penicillium janthinellum*) were isolated from soil and stored at the Laboratory of Biotechnology and Bioassays (Federal University of Minas Gerais, Belo Horizonte, MG, Brazil). They were reactivated in tubes containing potato dextrose agar (PDA, Himedia Labs) for 7 days to promote sporulation. Five milliliters of aqueous Tween 80 0.5% (w/v) were added to the tubes containing the reactivated fungal strains and mixed to suspend spores. Spores suspension was standardized using a Neubauer chamber to obtain about 1×10^7 spores mL^{-1}.

Epigenetic modulation

Suberohydroxamic acid, hydralazine and procainamide (Sigma–Aldrich, Darmstadt, Germany) were used as epigenetic modulators. All experiments were conducted in duplicate in Erlenmeyer flasks (500 mL) containing 125 mL of potato dextrose broth prepared in distilled water (PDB Himedia Labs) previously sterilized in autoclave. In a first group of experiments, epigenetic modulators were added individually (0.1 mM) before inoculation with 0.5 mL of pre-standardized spore suspension. The second set of experiments was conducted, also in duplicate, in which two epigenetic modulators were added together in the same concentration used in the first experiment (0.1 mM each). The following combinations were used: procainamide + suberohydroxamic acid; procainamide + hydralazine; and hydralazine + suberohydroxamic acid. As controls, experiments without addition of epigenetic modulators were run in parallel for each species and to exclude modulators interference in posterior chromatographic analysis, controls were carried without inoculation. Culture was carried out with shaking (100 rpm) at room temperature for 15 days. After this, liquid-liquid extraction was performed using ethyl acetate (25 mL) for three times. The organic solvent was evaporated down and the resulting extracts were kept in desiccator.

Chromatographic analysis of fungi extracts

Crude extracts were submitted to high performance liquid chromatography (HPLC UV/Vis – Shimadzu Prominence LC-20AT) in order to evaluate a possible effect on metabolite diversification promoted by epigenetic modulation by comparing the chromatographic profiles of the experimental and control assays. Methodology used was as described by Bracarense & Takahashi[21] by injecting 20 μL of each extract

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