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Preliminary Characterization of Hericium Species Sampled in Tuscany, Italy

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Abstract : Fungi of the genus Hericium contain various compounds with antibacterial activity, cytotoxic effect on cancer cells and bioactive molecules. Some of the active metabolites stimulate the synthesis of the Nerve Growth Factor (NGF). Recently, the effect of dietary supplement based on Hericium erinaceus on recognition memory and on hippocampal mossy fiber-CA3 neurotransmission was published. The aim of this study was to investigate the presence of Hericium species on Italian territory in order to isolate the strains for further studies and applications. The first step was to collect Hericium sporophores in Tuscany: H. alpestre Pers., H. coralloides (Scop.) Pers. and H. erinaceus (Bull.) Pers. were the species present. The strains of H. alpestre (H.a.1), H. coralloides (H.c.1) and H. erinaceus (H.e.1 & H.e.2) have been isolated in pure culture and preserved in the collection of the University of Pavia (MicUNIPV). The DNA sequences obtained from the strains were compared to other sequences found in international databases. Therefore, it was possible to construct a phylogenetic tree that highlights the clear separation in clades of the sequences and the molecular identification of our strains with the species of Hericium considered. The second step was to cultivate indoor and outdoor H. erinaceus in order to obtain as many sporophores as possible for further chemical analysis. All the procedures for H. erinaceus cultivation have been followed. Among the available recipes for indoor H. erinaceus cultivation, it was used a substrate formulation contained 70% oak sawdust, 20% rice bran, 10% wheat straw, 1% CaCO3 and 1% sucrose. The bioactive compounds present in the mycelia and in the sporophores of H. erinaceus were chemically analyzed in collaboration with the Centro Grandi Strumenti of the University of Pavia using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS). The materials to be analyzed were previously freeze-dried and then extracted with an alcoholic procedure. Preliminary chromatographic analysis revealed the presence of potentially bioactive and structurally different secondary metabolites such as polysaccharides, erinacins, ericenones, steroids and other terpenoids. Ericenones C and D (in sporophores) and erinacin A (in mycelium) have been identified by comparison with the respective standards. These molecules are known to have effects on the Central Nervous System (CNS) cells, which is the main objective of our studies. Thanks to the high sensitivity in the detection of bioactive compounds of H. erinaceus, it will be possible to use the To obtain lyophilized mycelium and the respective culture broth, 4 small pieces (about 5 mm2) of the respective H.e.1 or H.c.1 strains, taken from the margin of growing cultures (MEA), were inoculated into 1 liter of 2% ME (malt extract, Biokar Diagnostics). The static liquid cultures were kept at 24 °C in the dark chamber and fungi grew for one month. 10 replicates for each strain have been done. The method proposed as an analytical screening protocol to determine the optimal growth conditions of the fungus and to improve the production chain of H. erinaceus. These results encourage to carry out chemical analyzes also on H. alpestre and H. coralloides in order to evaluate the presence of bioactive compounds in these two species.

Keywords : Hericium species, Hercium erinaceus bioactive compounds, medicinal mushrooms, mushroom cultivation
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