MASS SPECTROMETRIC PROFILING OF EPIGENETIC DNA MODIFICATIONS IN AN *EPICOCCUM NIGRUM* STRAIN

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Bioactive compounds produced by endophytic fungi have great potential and can be utilized by the pharmaceutical and food industries, as well as in agriculture. However, it was observed that the genes responsible for the production of bioactive metabolites from fungal endophytes are usually not expressed in axenic cultures [1,3]. The media used for the culture of fungal endophytes in the laboratories do not contain the nutrients available/found in their native hosts. As a result of the lack of the host stimulus in the medium, many genes encoding biosynthetic pathways become silent, limiting their production of secondary metabolites [1]. Silent biosynthetic pathways of new bioactive secondary metabolites can be induced using epigenetic modifiers due to the change of the genomic chromatin structure. DNA methyltransferase (DNMT) and/or histone deacetylase (HDAC) inhibitors can be a possible treatment in the laboratory to activate these secondary metabolites to modify the chromatin structure of the coding genes [2,3]. The use of these epigenetic modifiers can increase the amount of medicinally useful compounds and improve the efficiency of the microbial production of these compounds [2].

In our research, we selected an endophytic fungus that had already been characterized as a hypericin producer. However, during sub-cultivations, this secondary metabolite production ability of the fungus was lost. To recover the disappeared character and to reveal the possible altered secondary metabolite set, valproic acid (VPA) and sodium-butyrate (SB) were applied as epigenetic modifiers. Both VPA and SB are also members of HDAC inhibitors. The effect of both compounds was examined by the mass spectrometric metabolic profiling of the treated strains. Initially, the concentrations of the epigenetic treatments were defined by the inhibition zone determination of both VPA and SB in agar plate assays. Based on our results, it was observed that there was not any inhibition of the compounds within the applied concentration range. Thus, a relatively high concentration (1000 µM) was applied to form enough high epigenetic pressure on the fungal strain. After the cultivation with the epigenetic modifiers, both the ferment broth and mycelium of grown fungal cultures were subsequently extracted using ethyl acetate and mixture of chloroform-methanol (4:1). The resulted extracts were evaporated and high-resolution mass spectrometric analysis was performed to investigate the possible alterations of the produced metabolites.

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References

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