

Endophytic Microorganisms from *Styrax officinalis* and their Antimicrobial Activities

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Aim of the study: Endophyte microorganisms reside in the living tissues of the host plant without causing a harmful effect to the host. Endophytes may contribute to their host plant by producing a wide variety of substances that provide protection against pathogenic microorganisms and herbivores. *Styrax officinalis* is a plant species that found in south and west Anatolia and benzoin resin, a dried exudation from pierced bark currently produced from this plant commercially. Therefore the aims of this study were to isolation and identification of the endophytic microorganisms from *Styrax officinalis* and determine their metabolites.

Material and Methods: Whole plants of *Styrax officinalis* was collected from İzmir Turkey in May 2016. Different plant parts including leaves, stems and roots were subjected to surface sterilization with bleach and ethanol to eliminate the surface microorganisms. Then the outer tissues are removed with a sterilized scalpel and inner tissues are sliced thinly than placed on isolation media. Plates were incubated at 27°C up to 6 weeks. Endophytic microorganisms were isolated as pure cultures. Endophytic isolates were identified using polyphasic taxonomy. Furthermore all isolates were subjected fermentation in 250 ml erlenmeyer flasks in order to assess their antimicrobial activities and other metabolites such as benzoin. Microorganisms were subjected to feed batch fermentation and cell free fermentation broths were used in antimicrobial activity studies after extracted with ethyl acetate. Antimicrobial activities of the extracts were determined by disc diffusion and broth microdilution methods against a panel of test microorganisms. Another 50 ml cell-free fermentation broth were lyophilised and stored at -20°C for metabolite analysis.

Results: A total of 8 bacterial endophytes and 11 fungal endophytes were isolated from the different tissues of the *Styrax officinalis*. All isolates were characterized using polyphasic approach. Different phenotypic and genotypic characteristics such as gram staining, morphology, oxidase, catalase, 16s rDNA, ITS and calmodulin sequence analysis were used for identification purposes. Bacterial isolates were identified at genus level as follows *Pseudomonas*, *Bacillus*, *Curtobacterium*, *Paenibacillus* and *Micrococcus*. *Penicillium* was the most abundant genus among fungi and all endophytic fungal starins were belonged to Ascomycota division. Only butanol extracts showed activity and 4 bacterial and 5 fungal isolates were found active against test microorganisms. The bacterial isolate G1b showed activity against *Enterococcus faecium*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and MRSA and MIC values were found as 16, 64, 128, 128 and 512 µg/ml respectively. Another bacterial isolate I5b has been shown to have activity against *C. albicans* and MRSA with a MIC value as 64 and 256 µg/ml respectively. This study presents the first report about the endophytic microorganisms of *Styrax officinalis* and their antimicrobial activities.

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