INFLUENCE OF INOCULUM CONCENTRATION AND CULTIVATION TIME ON EXOPOLISACCHARIDES PRODUCTION BY ENDOPHYTIC FUNGI *Neofusicoccum parvum* AND *Fusarium* sp

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Abstract

Endophytic fungi have been described as producers of important bioactive compounds. They could be exploited as sources of exopolysaccharides (EPS) which can be applied in food industry, medicine, and cosmetics. To obtain the high yield of EPS the submerged cultivation parameters should be established. The goal of this work was to determine the inoculum concentration and cultivation time to obtain the EPS from *Neofusicoccum parvum* and *Fusarium* sp isolated from *Maytenus aquifolia* leaves. The microorganisms have been cultivated in minimal salts medium with glucose as carbon source (50 g/L) and yeast extract (2 g/L) as nitrogen source at 180 rpm and 28 ºC for 72 hours. The tested inoculum concentration was 1.5; 2.0; 2.5; 3.0; 3.5 and 4.0 mL/ 25 mL of medium. The best inoculum concentration was used to study the time cultivation parameter: 24, 48, 72, 96, 120, 168, 240, 360 hours. After mycelium removal, the cell-free extracellular fluid was dialyzed against distilled water (24 h), concentrated and precipitated with ethanol (3 volumes) for recovering the polysaccharides and aliquots were used to determine total and reducing sugars and proteins. The polysaccharide's homogeneity and molecular weight were estimated by HPSEC/RID. The monosaccharide composition was obtained after acid hydrolysis (TFA 2M, 3h, 100 °C) and HPAEC/PAD analysis. To *N. parvum* the 2.5 mL of inoculum/25 mL of medium and 72 hours were the best conditions for producing EPS (367 mg/L) and the polysaccharide composition was glucose (79%), galactose (13%) and mannose (8%) with MW 84 kDa. To *Fusarium* sp the 2.0 mL of inoculum/25mL of medium and 96 hours of cultivation were the best conditions for producing EPS (256 mg/L) and the polysaccharide composition was mannose (69%), galactose (18%) and glucose (13%) with MW 45 kDa. HPSEC/RID analysis showed -for both polysaccharides- single peaks with high polydispersivity degree, characteristic of secreted polysaccharides.

Keywords: EPS, submerged cultivation, filamentous fungi