

Sulfation and Anticoagulant Activity of Exopolysaccharide Produced by the *Botryosphaeria rhodina* MAMB-05 Grown on Fructose.

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Botryosphaeran, a β -(1 \rightarrow 3, 1 \rightarrow 6)-D-glucan with 31% branching at C-6, produced by the fungus *Botryosphaeria rhodina* MAMB-05 when grown on fructose as carbon source (EPS_{FRU}), belongs to an important class of polysaccharides exhibiting biological and pharmacological activities. Botryosphaeran was derivatized by sulfation (2x) to induce anticoagulant activity, and to render the polysaccharide more water-soluble. The effectiveness of the sulfation reaction was monitored by the degree of substitution, and FT-IR analyses of the sulfated EPS_{FRU} (EPS_{FRU} SULF and EPS_{FRU} RESULF). Activated Partial Thromboplastin Time (APTT) and Thrombin Time (TT) tests of EPS_{FRU} RESULF indicated significant *in vitro* anticoagulant activity that was dose-dependent. The results can be explained by the high density of negative sulfate groups presented by the sulfated EPS molecules. The prolongation of APTT in the presence of EPS_{FRU} RESULF suggested inhibition of the intrinsic pathway of coagulation, while the extension of the TT probably indicated inhibition of the conversion of fibrinogen into fibrin. The results of inhibition of thrombin by antithrombin suggested that the action of anticoagulant EPS_{FRU} RESULF was related to the activation of antithrombin, thus prolonging the time of clot formation. EPS_{FRU} didn't inhibit any of the coagulation tests.

Keywords: *Botryosphaeria rhodina* MAMB-05, exopolysaccharide, β -(1 \rightarrow 3, 1 \rightarrow 6)-D-glucan, sulfation, anticoagulant activity.

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