TITLE: PRODUCTION OF EXOCELLULAR $(1\rightarrow6)\beta$-D-GLUCAN BY *Lasiodiplodia theobromae* MMPI BY FERMENTATION ON SOYBEAN MOLASSES AS AN ALTERNATIVE SUBSTRATE

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ABSTRACT:
Lasiodiplodan, an exopolysaccharide (EPS) of the $(1\rightarrow6)\beta$-D-glucan type produced by the ascomyceteous fungus, *Lasiodiplodia theobromae* MMPI, is a carbohydrate biopolymer demonstrated to possess several biological functions, including immunomodulation, antioxidant and antiproliferative activity. Glucose and sucrose have been demonstrated to be efficient carbon sources to produce lasiodiplodan when cultured by this fungus. Production costs could be reduced with the use of agro-industrial by-products rich in carbohydrates as raw material. In this context, the present study evaluated the use of soybean molasses as a potential substrate to produce lasidiplodan by submerged fermentation (SmF). Soybean molasses is co-generated in the industrial process of extracting soy proteins, and is a rich source of sugars such as glucose, fructose, sucrose, raffinose and stachyose. SmF was performed in Erlenmeyer flasks (72 h, 28°C and 150 rpm) containing nutrient medium based on clarified soybean molasses (3.5 °Brix), with and without supplementation with yeast extract (2 g/L), KH_2PO4 (2 g/L) and $MgSO_4.7H_2O$ (2 g/L). Lasiodiplodan and mycelial biomass were quantified by gravimetry after drying at 60ºC. Sugars were determined by High Performance Liquid Chromatography (HPLC) using a Refractive Index detector and a HPX-87H column (Bio-Rad, Hercules, CA) at 45°C. Sulfuric acid solution (0.005 mol/L) was used as eluent, at a flow rate of 0.6 mL/min. The injection volume was 20 µL. *L. theobromae* was able to assimilate all of the sugars present in soybean molasses. Complete assimilation of stachyose, and consumption of >90% of raffinose, glucose and sucrose was verified by HPLC at the end of the fermentation run, with and without nutrient supplementation. Nutritional supplementation of the culture medium promoted higher production of mycelial biomass (9.15 g/L) and lasiodiplodan (2.6 g/L) and volumetric productivity (0.04 g/Lh). However, nutrient supplementation did not promote an increase in EPS yield ($Y_{P/S}$: 0.22 g/g under both conditions), nor an effective increase in the global sugars assimilation. The results suggest that soyben molasses could be an interesting alternative to reduce processing costs in the production of EPS's such as lasiodiplodan. Nutritional supplementation of soybean molasses was shown not to be necessary for lasiodiplodan production by *L. theobromae* MMPI, as it did not lead to performance improvements of the process.

Keywords: fungal exopolysaccharide, submerged fermentation, experimental design.
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