TITLE: PRODUCTION AND CHARACTERIZATION OF RHAMNOLIPIDS USING GUAVA SEED OIL AS SUBSTRATE

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ABSTRACT:
Biosurfactants are substances synthesized by microorganisms. These molecules have the ability to reduce surface and interfacial tension, and can be applied in various sectors of industry. Rhamnolipids (RLs) are biosurfactants of the glycolipids class that have high surface activity and higher production yield. RLs produced by *Pseudomonas aeruginosa* are constituted by a mixture of homologous species, where mono-RL, RL1 ($RhaC_{10}C_{10}$) and di-RL, RL3 ($RhaRhaC_{10}C_{10}$) are the predominant ones. The ratio between these homologues in the rhamnolipid results in different properties and applicability for the compound, the carbon sources used for the synthesis of these molecules may have influence on this ratio. This research aimed to study the production of RLs from an alternative substrate from the fructorefinery. The aim was to obtain a higher yield of this metabolite and to verify the composition of homologous species present. The experiments were carried out in the laboratory from flask cultivations using *P. aeruginosa* LBI 2A1, evaluating three different concentrations of guava seed oil (80, 100 and 120 g/L). Microbial growth was evaluated from dry mass. The quantification and characterization of RL and its homologues were performed using HPLC and LC-MS. The concentration of guava oil that showed the best outcome was 100 g/L, it showed maximum growth of the microorganism after 96 h of cultivation with a dry biomass of 3.89 ± 1.12 g/L. Regarding RL synthesis, the highest production occurred after 48 h of fermentation in all evaluated conditions. The highest yield was obtained in the cultivation with 100 g/L of guava oil (15.08 ± 2.52 g/L). In general, the production profile was very similar for the media with 80 and 120 g/L, which reached the values of 4.98 ± 2.46 and 5.71 ± 2.41 g/L of RL, respectively. LC-MS analysis indicated the presence of eight different homologues, 4 mono- and 4 di-RLs. The components found demonstrated *m/z* 475.6 ($RhaC_8C_{10}$/ $RhaC_{10}C_8$); 503.4 ($RhaC_{10}C_{10}$); 529.2 ($RhaC_{12:1}C_{10}$/ $RhaC_{10}C_{12:1}$); 530.9 ($RhaC_{12}C_{10}$); 621.4 ($RhaRhaC_{10}C_8$/ $RhaRhaC_8C_{10}$); 649.1 ($RhaRhaC_{10}C_{10}$); 675.6 ($RhaRhaC_{10}C_{12:1}$/ $RhaRhaC_{12:1}C_{10}$) and 677.6 ($RhaRhaC_{10}C_{12}$/ $RhaRhaC_{12}C_{10}$). The carbon source used shows viability for the cultivation of the microorganism and RL production. These results showed that guava seed oil is an interesting carbon source for the production of a variety of homologues, which can be incorporated in future applications in several industrial areas.

Keywords: Biosurfactants, alternative substrates, fructorefinery oil, homologues.

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