Bioconversion of raw glycerol into lactic acid by endophytic bacteria from mangrove plants

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In the production of biodiesel, glycerin is formed, the disposal of which could harm the environment. Microorganisms can bioconvert glycerin into lactic acid, which is an essential in the polymer industry.To add value to the microbial biodiversity preserved in the Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries (CMMAABio) at Embrapa Agroenergia, the research objectives were: (i) evaluate the viability and purity of a subcollection of endophytic bacteria isolated from mangrove plants; (ii) investigate the ability of these bacteria to use raw glycerin as a source of nutrients for the production of lactic acid. The present work evaluated 96 bacteria isolated from Avicennia schaeuriana (27 strains), Laguncularia racemosa (24 strains), and Rhizophora mangle (45 strains) from a mangrove area impacted by an oil spill. The 96 bacteria were preserved by deep freezing (20% glycerol).They were reactivated from the stock in Tryptone Soy Agar and incubated at 28°C for 7 days. The purified strains were preserved by deep freezing in individual cryotubes in 20% glycerol (-80°C). Their ability of producing lactic acid was evaluated in two stages. In the primary screening, the bacteria were cultivated in M9 broth containing 2% pure glycerin as a carbon source in miniaturized cultivation in 96-well microplates. Incubation occurred at 28°C, 200 rpm for 72 h. In the secondary screening, selected bacteria were cultivated in 250 mL Erlenmeyer flasks containing 50 mL of three culture media: (1) M9 with pure glycerin (2%); (2) M9 with tryptone (0.5%) and pure glycerin (2%); and (3) M9 with tryptone (0.5%) and raw glycerin (81.85% purity; 2% glycerin). The initial optical density was adjusted to 0.15 (OD600nm), and incubation occurred at 28°C, 180 rpm for 72 h. Quantitative analysis of lactic acid was performed by liquid chromatography. It was found that all 96 bacteria were viable. As the way they were originally preserved was not ideal (96-well microplates susceptible to cross-contamination), before their characterization, it was necessary to evaluate the purity of the strains. In this case, 54 strains required successive purifications until pure cultures were obtained. Thus, from the initial 96 strains, 180 viable and pure strains were recovered. The detection of lactic acid was observed in the supernatant of eight strains, obtained in the second screening. The Enterobacter hormaechei strain MBIA1.18 H3 (isolated from A. schaeuriana) was selected to continue the research, as it produces the highest concentration of lactic acid (1.147 g/L) from crude glycerin.

Key words: Endophytic Bacteria; Mangrove; Bioprospecting; Lactic Acid

Bioconversão de glicerina bruta em ácido lático por bactérias endofíticas de plantas de manguezais
Uma coleção de 180 linhagens de bactérias endofíticas de plantas de manguezal foi caracterizada quanto à capacidade de realizar a bioconversão de glicerina em ácido lático. A linhagem *Enterobacter hormaechei* MBIA1.18 H3 foi selecionada para continuidade do projeto de pesquisa, pois produziu a maior concentração de ácido lático (1,147 g/L) a partir de glicerina bruta residual da produção de biodiesel. As bactérias identificadas representam novas linhagens da biodiversidade brasileira capazes de converter glicerina em ácido lático.

Palavras-chave: Bactérias endofíticas; Manguezal; Bioprospecção; Ácido Lático