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Improved rhamnolipid biosurfactant production by *Burkholderia thailandensis* E264 using agro-industrial wastes

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Biosurfactants are amphiphilic surface-active compounds, produced by various microorganisms, that reduce surface and interfacial tension. These compounds are attracting increasing interest over their chemical counterparts due to their advantages, such as biodegradability, high stability in extreme environments, low toxicity, low critical micelle concentrations (CMC) and the fact that they can be effectively produced from agro-industrial wastes and renewable resources. Furthermore, their diversity allows for a significant number of uses, including microbial enhanced oil recovery (MEOR), bioremediation and biomedical applications. However, the high operational costs, mainly regarding the use of expensive raw materials in the fermentation and the complex downstream processing due to low production yields restricts their industrial-scale applications. Several attempts to solve these limitations by reducing production costs have been conducted and include the use of low-cost agro-industrial wastes and by-products as substrates. One of these low-cost substrates, that has been successfully used to produce biosurfactants by *Bacillus subtilis* and *Pseudomonas aeruginosa*, is corn steep liquor (CSL). In this work, CSL is evaluated for rhamnolipid biosurfactant production by *Burkholderia thailandensis* E264. When grown in a culture medium containing CSL (7.5% v/v) as sole substrate, this strain produced 1.8 g rhamnolipid/L, about 2.6 times the amount of rhamnolipid produced in the standard synthetic medium. By supplementing the culture medium with olive oil mill wastewater (OMW, 10% v/v), a residue originating from the olive oil extraction industry, rhamnolipid production is increased up to 2.6 g/L, most likely due to the inductive effect of long-chain fatty acids present in OMW (mainly oleic, palmitic, linoleic and stearic acids) on rhamnolipid production. Rhamnolipids purification was also done and the biosurfactant produced in the low-cost medium (CSL+OMW) exhibited better surface-active properties when compared with those produced in the synthetic medium, reducing the surface tension of water to 26.8 ± 0.1 mN/m, with a CMC of 280 mg/L (28.8 ± 0.2 mN/m and 460 mg/L, respectively, in the synthetic medium). These results demonstrate that rhamnolipid production is more efficient in the low-cost medium. Furthermore, to the best of the authors' knowledge, this is the first experimental research that describes the utilization of CSL and OMW as substrates for the production of rhamnolipids by *B. thailandensis,* with very optimistic results in terms of cost and production levels.

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