Enhanced Surfactin Production by Genetically Modified *Bacillus subtilis* LBBMA RI4914 IsrfA through Culture Condition Optimization

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Abstract:

Microbial enhanced oil recovery (MEOR) is a sustainable strategy to improve oil extraction through biosurfactant-mediated interfacial tension reduction, but its application is limited by production costs and low yields. This study optimized surfactin production by the genetically modified strain Bacillus subtilis LBBMA RI4914 IsrfA, carrying the surfactin operon under the IPTG-inducible Pgrac promoter. Production was optimized in IPTG-supplemented mineral medium using central composite rotatable design (CCRD) in orbital shaker. Carbon source concentration (20–80 $g.L^{-1}$; glucose, refined sugar, or sugarcane molasses) and temperature (20–42 °C) were first evaluated. Then, agitation (100–300 rpm), nitrogen source concentration (5.6–94 mM; ammonium chloride, sodium nitrate, or urea), and initial OD600 (0.05–1.00) were optimized. Cultures were conducted for 120 h, and surfactin was quantified by HPLC-UV. The best conditions of carbon source and temperature were tested in a 2 L bioreactor, and purified surfactin was quantified by UPLC-MS/MS. Glucose at 28.72 $g.L^{-1}$ and 42 °C resulted in the highest surfactin production among the carbon sources tested, reaching 7.4 $g.L^{-1}$. Under these conditions, agitation at 200 rpm provided the same result previously observed, yielding 6.9 $g.L^{-1}$ (no statistical difference). Urea was the most effective nitrogen source, with 18 mM nitrogen and an initial OD600 of 0.188 resulting in 8.3 $g.L^{-1}$ of surfactin. This production was about twofold higher than preliminary assays with this bacterium. In bioreactor cultivation, using the pO_2 of 80%, surfactin production reached 2.0 $g.L^{-1}$ with approximately 81% purity, but scale-up and purification may have affected bioreactor performance.

Keywords:

CRISPR-Cas9, Bioprocess optimization, Surfactin, Microbial enhanced oil recovery (MEOR).