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Abstract		The widespread use of Pseudomonas aeruginosa for RL production is hampered by its pathogenicity. Burkholderia thailandensis E264 presents a safer and potentially more cost-effective alternative, yet research on its RL production, particularly regarding the influence of carbon source type and chain length, remains limited. This study addresses this gap by investigating the effects of different carbon sources on the physicochemical properties of RLs produced by B. thailandensis E264. B. thailandensis E264 was cultured in Mineral Salt Medium (MSM) supplemented with glycerol (miscible) and oleic acid (immiscible) at concentrations of 2%, 4%, and 6% (w/v). These concentrations were chosen based on considerations of solubility, growth inhibition, and cost-effectiveness. Bacterial growth was monitored using optical density (OD600) and cell dry weight measurements. RL production was assessed using Fourier-Transform Infrared Spectroscopy (FTIR) analysis, oil displacement tests, and emulsification index (E124) measurements. Maximum cell dry weight was achieved at 4% (w/v) for both glycerol (0.2290 g/L) and oleic acid (0.1590 g/L). FTIR analysis confirmed RL production, evidenced by characteristic C-O stretching bands that show the existence of bonds between carbon atoms and hydroxyl groups in the chemical structure of the rhamnose ring (e.g. 1045.10 cm-1 for glycerol, 1044.9 cm-1 for oleic acid). Oil displacement was highest at 4% (w/v) glycerol (1.70±0.99 cm) and 6% (w/v) oleic acid (3.60±1.22 cm). The emulsification index (E124) peaked at 50% with 6% (w/v) glycerol and 30% with 2% (w/v) oleic acid. Statistical analysis (ANOVA) confirmed significant differences (p<0.05) between carbon sources except there is no significant difference between various concentrations of glycerol (p-value = 0.55) and oleic acid for the oil displacement test (p-value = 0.27).