

Isolation and growth capacity of bacteria in fat residues from dairy wastewater

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The dairy industry faces the challenge of ensuring food safety while minimizing environmental impact. By identifying and controlling contamination risks, the industry can produce high-quality products while promoting environmental sustainability. Analyzing the microbiota of dairy effluents can aid in the development of more effective treatment procedures, potentially leading to the production of valuable industrial compounds. The study aimed to isolate and to evaluate the growth capacity of bacteria in fat residues from dairy wastewater. Fat residues were collected in a sterile bottle from the grease trap at the wastewater treatment station of the “Laticínio Escola – Produtos Viçosa” dairy factory from the Fundação Arthur Bernardes (FUNARBE) in Viçosa, MG, Brazil. A portion of the collected fat residue was heated to 100 °C, centrifuged at 5000 rpm for 5 min at room temperature in a 50 mL tube, and the fat separated at the top of the tube was collected and stored in a sterile bottle at -20 °C protected from light. Both fat residue and separated fat used for culture media. For isolation, a non-sterile aliquot of 100% (w/v) fat residue was added aseptically to minimum salt medium (MSM) with 1.5% (w/v) agar, poured onto Petri plates, and incubated at 25, 30, and 37 °C under both aerobic and anaerobic conditions for five days. Different colonies were streaked on Plate Count Agar (PCA) and Cetrimide Agar with incubation at 37 °C for 24 h; Gram-stained; and stored in Luria Bertani (LB) broth with 20% (v/v) glycerol at -20 °C. For growth capacity assessment, MSM with 22% (w/v) fat residues and 1.5% (w/v) agar was incubated at 25, 30, and 37 °C for five days; MSM with 0.5 and 2% (w/v) fat and 1.5% agar was incubated at 30 °C for five days; LB broth with 0.5 and 2% fat and 1.5% agar was incubated at 30 °C for five days. For this experiment, bacteria isolated from the fat residue, along with *Pseudomonas aeruginosa* ATCC 29853, *Pseudomonas aeruginosa* PAO1, and *Pseudomonas fluorescens* 07A, were used. In all isolation conditions, both brown and white colonies were observed, with brown colonies predominating at higher temperatures, as well as under anaerobic conditions. Ten colonies were isolated and named LIP 1 to LIP 10. On PCA, LIP 1, 2, 5, 7, and *P. aeruginosa* ATCC 29853 presented white streaks with motility, while LIP 3, 4, 6, 8, 9, 10, and *P. aeruginosa* PAO1 presented translucent streaks with motility. On cetrimide agar, LIP 1, 2, 5, 7, *P. aeruginosa* ATCC 29853, and *P. fluorescens* 07A presented few white colonies, while LIP 3, 4, 6, 8, 9, 10, and *P. aeruginosa* PAO1 presented green streaks with motility and fluorescence under ultraviolet (UV) light, indicating these isolates can be *P. aeruginosa*. All isolated bacteria from the fat residue were Gram-negative short bacilli. No bacteria grew in MSM with 22% fat residues at 25 °C, but LIP 4, 8, 9, 10, and *P. aeruginosa* PAO1 grew at 30 °C, and LIP 8 and 9 at 37 °C. All bacteria grew in MSM and LB with 0.5 and 2% fat at 30 °C, except *P. aeruginosa* ATCC 29853. LIP 3, 4, 6, 8, 9, 10, and *P. aeruginosa* PAO1 showed browner streaks with increased fat content in the medium. This study highlights the importance of understanding bacterial growth in fat residues from the dairy industry, emphasizing the exploration of opportunities for more



efficient treatment processes and the development of new industrial products and procedures. These efforts contribute to a safer and more environmentally responsible sector.

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