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Optimization of NaCl Concentration in a General Purpose Medium to Inhibit the Swarming Motility of *Proteus mirabilis*

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Background: *Proteus mirabilis* (*P. mirabilis*) causes infections in humans and is often found in polymicrobial communities in clinical samples and interferes with the isolation of other bacteria due to swarming mortality. Swarming behavior is affected by Sodium chloride (NaCl) concentration. Although commercially available selective media including MacConkey and Cystine-Lactose-Electrolyte-Deficient (CLED) agar inhibit *P. mirabilis* swarming, their NaCl concentration is different [0.034%, 0.056% (w/v) respectively]. Thus, it is important to determine the optimal concentration of NaCl for a general-purpose medium like nutrient agar, which can promote the growth of a broad spectrum of microorganisms while effectively preventing swarming in *P. mirabilis.*

Objective: To optimize NaCl concentration in nutrient agar to inhibit the swarming motility of *P. mirabilis*

Methods: Nutrient agar plates were prepared with a range of NaCl concentrations (5 g/L, 4 g/L, 3 g/L, 2 g/L, 1.5 g/L, 1 g/L, 0.5 g/L, 0.4 g/L, 0.3 g/L, 0.1 g/L). Twenty *P. mirabilis* isolates were separately inoculated in 5 mL nutrient broths and incubated at 37 ^{0}C for 16-20h. A 5 µL droplet from each broth was then cultured on to the center of the agar plates, incubated and the diameter of the colony was recorded. Biochemical assays were carried out in the *P. mirabilis* colonies cultured on modified media with the NaCl concentration found to inhibit its swarming motility. As a quality control, ATCC Strains of *K. pneumonia, E. coli, P. aeruginosa, S. aureus, E. faecalis* and *P. mirabilis* were cultured on modified media with NaCl concentration found to inhibit its swarming motility.

Results: *P. mirabilis* did not swarm on modified nutrient agar with ≤0.66 g/L NaCl concentration, giving 4 mm diameter isolated colonies. Biochemical assays ensured that no biochemical characteristics had been changed while growth of selected ATCC strains ensured that no effect on growth and metabolic activities by the presence of ≤0.66 g/L of NaCl in the medium.

Conclusions: The modified nutrient agar with ≤0.66 g/L of NaCl concentration inhibits the swarming motility of *P. mirabilis* without interfering with its growth or altering its physiology. Therefore, it would be an alternative medium that supports the growth of a variety of bacteria including *P. mirabilis*, particularly in samples with polymicrobial nature.

Keywords: *NaCl, Nutrient agar, Proteus mirabilis, Polymicrobial, Swarming*