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**Influence of nutritional level on mono- and dual-species biofilm formation by *Escherichia coli*, *Salmonella* and *Proteus***

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**Abstract**

Biofilms are microbial community adhered to biotic or abiotic surfaces and embedded within an extracellular Polymeric matrix. They have potential to resist antibiotics and disinfectants, causing health concerns in medical and food industries. Biofilm formation is affected by nutrient availability, temperature and other environmental factors. The present study was conducted to study the mono- and dual-species biofilm formation of bacteria, using *Escherichia coli*, *Salmonella* and *Proteus*, and their combinations, in two different nutritional levels of the growth medium (undiluted Luria-Bertani Broth - LBB and 1:100 diluted LBB). Biofilm forming ability was assessed by crystal violet microtiter plate assays, incubated at 28 °C. The optical density was measured at 600nm wavelength. Undiluted LBB was more inductive in biofilm formation compared to 1:100 diluted LBB. *E. coli* formed a stronger biofilm ( $1.31 \pm 0.32$ ) in undiluted LBB than in diluted LBB ( $0.41 \pm 0.15$ ). Similar patterns were observed for *Salmonella*, *Proteus* and their combinations tested. *Proteus* also exhibited greater biofilm formation ( $2.0894 \pm 0.32$ ) at undiluted media and lower in diluted media ( $1.1174 \pm 0.37$ ). *Salmonella* showed optical density of  $1.8602 \pm 0.24$  at undiluted media and  $1.0312 \pm 0.33$  at diluted media. Combinations of *Salmonella-Proteus*, *Salmonella-E. coli* and *Proteus-E. coli* also demonstrated greater biofilm formation in undiluted LBB ( $2.08 \pm 0.35$ ,  $0.92 \pm 0.32$  and  $1.67 \pm 0.41$ ) compared to that in diluted LBB ( $1.25 \pm 0.41$ ,  $0.72 \pm 0.15$  and  $1.12 \pm 0.34$ ). Results also revealed that the biofilm formation by dual species was greater than that of mono-species. The combination of *Salmonella* with *Proteus* showed significantly higher biofilm formation than the combination of *Salmonella* with *E. coli*, and *E. coli* with *Proteus*, in both the diluted and undiluted LBB. The study indicated the effect of nutritional levels on biofilm formation of *E. coli*, *Salmonella* *Proteus* and their combinations, with greater biofilm formation at higher nutritional levels.

**Keywords:** Biofilm, Mono-dual-species, Nutritional level

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