Effect of Temperature on Mono and Dual Species Biofilms Formed by *Salmonella*, *E. coli* and *Proteus* spp.

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Biofilms are microbial communities encased with self-producing extracellular matrix, composed with polysaccharide, DNA and protein. Persistent survival capability of these biofilms in food contact surfaces cause contamination of food batches and pose health risk among the public. The biofilm formation is affected by several environmental factors such as temperature and humidity etc. This study was conducted to investigate the effect of temperature on biofilms formed by *Salmonella*, *E. coli* and *Proteus* spp. when they are present as mono species and in combinations. Organisms were incubated at two different temperatures (28^0C and 37^0C) for 120 hr and biofilm formation was quantified using microtiter plate method at different time points as 24 hr, 48 hr, 72 hr, 96 hr and 120 hr. Biofilm formation at 24 hr by *Proteus* spp. with *Salmonella* and *E. coli* (i.e. *Proteus* with *E. coli* and *Proteus* with *Salmonella*) were higher in the temperature of 28^0C compared to 37^0C. There was no significant effect of temperature on mono species biofilm at 24hr incubation period. At 48 hr, biofilm formation by *Salmonella* together with *Proteus* and *E. coli* (*Salmonella* with *E. coli* and *Proteu*s with *Salmonella*) and by *Salmonella* alone was higher at 28^0C than that of at 37^0C. At 48 hr, *Proteus* and *E. coli* alone did not exhibit significant ($p > 0.05$) difference in biofilm formation at 28^0C and 37^0C. At 72 and 96 hr, the biofilm formation by *Proteus* and *Salmonella* alone and *Salmonella* together with *Proteus* (*Proteus* with *Salmonella)* showed higher biofilm formation at 28^0C than at 37^0C. But *Proteus* with *E. coli*, *Salmonella* with *E. coli* or *E. coli* alone did not exhibit any significant ($p > 0.05$) effect of temperature. At 120 hr, all the organisms alone and their combinations showed significantly higher biofilm formation at 28^0C than at 37^0C. This study concluded that temperature and time significantly ($p < 0.05$) affect on biofilm formation by *Salmonella*, *E. coli* and *Proteus* spp. individually and as combinations. This study revealed that biofilm formation at 28^0C is higher than their optimal growth temperature (37^0C).

Keywords: Biofilm; *E. coli; Proteus; Salmonella*; Temperature