

Biofilms of *Salmonella*, *E. coli*, *Proteus* and Their Combinations; A Time Scale Investigation

SU Pathirana¹, KVDM Hasintha¹, HC Nadishani¹, DNN Madushanka¹, JLPC Randika², GCP Fernando¹, TSP Jayaweera¹ and HAD Ruwandeepika^{1*}

¹Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Sri Lanka

²Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Sri Lanka

*ruwandeepika@yahoo.co.uk

Bacterial biofilms are self-produced matrix of extracellular polymeric substances which provides protection against unfavourable conditions. Biofilm formation is affected by several factors such as time, temperature and humidity etc. This study investigated the biofilm forming ability of *Salmonella*, *E. coli* and *Proteus* spp. isolated from chicken meat when they are present as mono species and in combinations as a time series analysis. Fifty broiler chicken meat samples collected from retailer shops for a period of 6months from December 2019 were used for the isolation of organisms, which were allowed to grow for different time durations (24hr, 48hr, 72hr, 96hr and 120hr). Biofilm forming ability was determined by microtiter plate assay and they were classified in to different groups as weak, moderate, strong biofilm formers. This study revealed that majority of the organisms developed strong biofilms: *Salmonella* alone, *Proteus* together with *E. coli* and *Proteus* together with *Salmonella* and *Salmonella* together with *E. coli* at 48 hours. *Salmonella* and *E. coli* together showed moderate biofilm forming ability at 24, 72, 96 and 120hrs. *Salmonella* and *Proteus* together form moderate biofilm at 24hrs and strong biofilm at 48hrs and remained for 72hrs. *Proteus* together with *E. coli* showed moderate biofilm forming ability at 24, 72 and 96hrs whereas at 48 and 120hrs they were strong biofilm formers. When *Salmonella* was alone as a mono species at 24hrs it showed weak biofilm forming ability whereas they reached to strong biofilm levels at 48hrs. *E. coli* started the biofilm formation little later than the other organisms checked, it reached to the weak biofilm states at 72hrs and it remains as it is at 96hrs as well, at 120hrs it produces moderate biofilm. *Proteus* showed moderate biofilm from the beginning of quantification and it showed the capacity of forming biofilm even before 24hrs and it remained at 48hrs also as moderate. Strong biofilm by *Proteus* was at 72hrs and at 96hrs it was moderate. All the mono and dual species biofilms remained as strong at 120hrs except for *Salmonella*, *E.coli* combination and *E. coli* alone, where they remained as moderate biofilms. This study concluded that biofilm forming ability of *E. coli*, *Proteus*, *Salmonella* and their combinations varied with time. Majority of them have the capability of having strong biofilms at 48hrs, whereas some at 72hrs. Some organisms have delayed response in forming biofilms. Findings of this study will pave a way towards developing some biofilm eliminating methods in the future based on the characteristics of different biofilms.

Keywords: Biofilm, *E. coli*, *Proteus*, *Salmonella*, Time Scale