

Interactions encountered inside dual-species biofilms formed by *Salmonella* Typhimurium and autochthonous microbiota recovered from leafy salads on stainless steel

Agapi I Doulgeraki¹, Anastasios Vorgias¹, Maria Lazarou², Efstathios Giaouris², George John E. Nychas¹

¹Department of Food Science and Human Nutrition, Laboratory of Microbiology and Biotechnology of Foods, Agricultural University of Athens (AUA), Iera Odos 75, Athens, 11855, Greece

²Department of Food Science and Nutrition, University of the Aegean, Mitropolitou Ioakeim 2, Myrina, 81400, Lemnos Island, Greece

Last years, leafy salads have been increasingly associated with foodborne diseases caused by pathogenic bacteria, such as *Salmonella enterica*. Fresh produce can be contaminated with microorganisms at all steps of the “farm to fork” chain, when the hygienic conditions are not the appropriate. Various factors are believed to affect attachment and survival of pathogens on salads, with the microbial interactions encountered between pathogenic bacteria and indigenous plant microorganisms to play a significant role. In the present study, the ability of bacteria isolated from leafy salads to affect biofilm formation by *Salmonella* Typhimurium (ST), when all these were cultured together on stainless steel (SS) coupons, was investigated. To achieve this, isolates recovered from either rocket or spinach salads were left to form mixed culture dual-species biofilms with ST on SS coupons immersed in: (i) LB medium, (ii) rocket sterile extract, and (iii) spinach sterile extract, at 20°C. Results revealed a reduction in *Salmonella* biofilm cells higher than 1 log cfu/cm² in 10% and 8,4% of cases of dual-species conditions with rocket and spinach isolates, respectively, compared to ST biofilm cells under mono-species conditions, in LB broth. Similarly, a reduction in *Salmonella* biofilm cells ranging from 0.5 to 0.99 log cfu/cm² was observed in 23,3% and 15,7% of cases of dual-species conditions with rocket and spinach isolates, respectively, in LB broth. However, the use of either sterile rocket or spinach extracts as immersed media, led to less significant differences in *Salmonella* biofilm cells between mono- and dual-species conditions. This observation could be attributed to the different nutritional conditions inside these plant extracts compared to LB broth, which may favor attachment and/or biofilm growth of ST on the SS surface. These results contribute to our knowledge related to dual-species biofilms formed by food relevant microorganisms under food related conditions and may be helpful in our efforts to control *Salmonella* diseases connected with the consumption of contaminated fresh salads.

Acknowledgments

This work was found by the action THALIS: “*Biological Investigation Of the Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles; BIOFILMS*”, falls under the Operational Programme (OP) "Education and Lifelong Learning (EdLL)" and is co-financed by the European Social Fund (ESF) and National Resources