# Controling Biofilms with *Serratia plymuthica* secondary metabolites: the permanent search for a great natural antimicrobial product

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One of the main steps in biofilm development is the primary colonization and attachment. Several chemical products are used nowadays to control this important step. As bacteria are permanently acquiring resistance to chemical commercial products it is of enormous interest the development of antimicrobials based on natural formulations.

Biofilms are functional consortia of cells bound within exopolymer matrices and organized at interfaces. It is well known that bacteria in biofilms are more resistant to treatment with antimicrobial compounds than corresponding planktonic cells. Intentional and unintentional biofilms concern a broad range of areas, comprising special attention in the industrial/environmental and biomedical areas. The attachment of the bacteria to the food product or the product contact surfaces leads to serious hygienic problems and economic losses due to food spoilage.

The main goal of this work was to investigate the ability of secondary metabolites produced by *Serratia plymuthica* to control *Staphylococcus aureus* bacterial attachment and biofilm formation.

The bacteria *S. plymuthica* was isolated in a heat-exchanger in a milk dairy industry. The bacteria ability to produce metabolites with surfactant properties and/or antimicrobial character against planktonic *S. aureus* growth was studied by means of interfacial tensiometry method and by measurement of the S. *aureus* inhibition halos. The role of the temperature on the metabolite production was also studied.

The potential antimicrobial effect of those by-products against *S. aureus* biofilms were assessed using the microtiter plate technique, by means of CV and XTT to assess, respectively, attached biomass and respiratory activity. The influence of temperature on *S. aureus* biofilm formation and on its response to the antimicrobial attack with bacterial by-product was also tested. For comparison purposes the toxic effect of the chemical commercial disinfectant was also evaluated.

The by-products released by *S. plymuthica* didn't show any significant surface tension properties but revealed antimicrobial activity at 20 ºC. The overall results showed that the preconditioning of the adhesion surfaces with the metabolites reduced notably the total mass and activity of the biofilms, being this reduction more marked again at 20ºC. The natural antimicrobial product *S. plymuthica* sub-products revealed to have similar action in biofilm control as the commercial disinfectant tested when used in the preconditioning of the adhesion surfaces. The use of both products together increased their efficacy in the control of biofilm adhesion. Significant biofilm detachment and respiratory inactivation also occurred when the biofilms were submitted to the aggression with the S. *plymuthica* by-products.

This study represents a step ahead in the possible use of natural by-products to control undesirable biofilms, in terms of biofilm development in the initial adhesion step and control of total biomass and biofilm activity in mature biofilm.

**Keywords** *Serratia Plymuthica*; *Staphylococcus aureus*; Secondary metabolites;natural antimicrobial product; biofilm control.