



Spoilage Potential of Biofilms and Planktonic Cells of *Bacillus subtilis* and *Bacillus velezensis* in Extended Shelf-Life Milk

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Abstract

Thermophilic bacilli pose a threat in the dairy industry because of their inherent ability to survive pasteurisation either as planktonic cells or process biofilms. This contamination results in the spoilage of extended shelf-life (ESL) milk due to the production of proteolytic and lipolytic enzymes by the bacteria. In this study, we aimed to quantify and compare biofilm-induced proteolysis and lipolysis of *B. subtilis* and *B. velezensis* with that of the planktonic cells. To grow the cells, 0.5 mL and 0.1 mL of pre-incubated bacterial inocula were added to a centrifuge tube containing 4.5 mL of UHT for the biofilms and planktonic cells, respectively. A stainless-steel coupon was placed in each of the centrifuge tube containing the inoculated UHT milk as a substratum for biofilm formation except for planktonic cells. All the tubes were incubated for 24 h at 30°C. Both planktonic and biofilm cells were stained and enumerated in a flow cytometer after incubation. The concentrations of proteolytic and lipolytic enzymes produced by the submerged biofilm and planktonic cells of the isolates were quantified using azocasein and p-nitrophenol palmitate (p-NPP) assays, respectively. All experiments were done in triplicate. In the planktonic cells, sample B48 (*B. subtilis* subsp. *subtilis* NCIB 3610) has the highest proteolysis with 1033.6 $\mu\text{L}/\text{CFU}$ while B50 (*B. subtilis* ATCC 11774) has the highest lipolysis of 34.5 $\mu\text{L}/\text{CFU}$. For the biofilms, B168 (*B. subtilis* ATCC 168- negative control) has the highest proteolysis and lipolysis per cell with a mean of 3706 μL and 179.9 μL . The result of this study indicated that the spoilage potential (proteolysis and lipolysis) both of biofilms and planktonic culture are strain-dependent and that there seems to be a relationship between the strength or complexity of the biofilms and spoilage potential of the isolates. The implication to the industry is that weak biofilm formers have better spoilage potential than strong biofilm formers in spore-forming bacilli.

Biography: James Elegbeleye

James Elegbeleye is a graduate of Microbiology from the University of Lagos (Nigeria) and PhD from the Department of Consumer and Food Sciences, University of Pretoria. He is a recipient of MOBILE UNLIMITED Project International Credit Mobility Universidade do Porto and Erasmus+ International Credit Mobility Universitat Autònoma de Barcelona. He is an experienced educator with a tenacity for helping young people.