

**TITLE:** MODELING THE VARIABILITY ON ADHESION AND BIOFILM FORMATION BY THREE DIFFERENT *Bacillus cereus* ISOLATES AS TO THE PRESENCE OF ENTEROTOXIN-PRODUCING GENES ON STAINLESS STEEL SURFACE

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**ABSTRACT:** *Bacillus cereus* is one of the microorganisms of major concern in the food industry, as it has a high capacity to adhere and form biofilms on the surfaces of the equipment and can contaminate the food that comes into contact with these surfaces. Control strategies should be sought in order to eliminate this possibility of occurrence of microorganisms in the food chain. For this, it is necessary to know the conditions that favor its adhesion. Mathematical models can be used to describe microbial behavior under different environmental conditions. Thus, this study aimed to establish a mathematical model capable of predicting the adhesion and biofilm formation by three different *Bacillus cereus* isolates as to the presence of enterotoxin-producing genes such as toxin not hemolytic (*nhe*), hemolysin BL (*hbl*) or cytotoxin k (*cytk*) on stainless steel surface under different environmental conditions. Experiments were conducted in different combinations of pH (4.5, 6.0, 7.5, and 9.0) and temperature (5, 15, 25, and 32 °C). Adhesion and biofilm formation was evaluated using 24-well plates containing stainless steel coupons and Brain Heart Infusion broth. Colonies were counted each 24 h up to 120 h of incubation for determining adhesion and biofilm formation. *B. cereus* counts  $\geq 2$  and  $<5$  log CFU/cm<sup>2</sup> were assumed as adherence, whereas counts  $\geq 5$  log CFU/cm<sup>2</sup> with EPS formation on the coupons observed in scanning electron microscope were considered biofilm formation. The logistic regression models were developed to predict the probability of adhesion or biofilm formation on the surface by the pathogen. The factors temperature and pH have influenced adhesion and biofilm formation responses, where higher temperatures (25 and 32 °C) and pH values (6.0, 7.5, and 9.0) led to more robust biofilm formation than lower temperatures (5 and 15 °C) and the lowest pH value (4.5). Isolate 3 showed greater adhesion capacity compared to the other two isolates at 15 °C. Only after 96 h, the isolates conditioned at 15 °C and pH values of 6.0, 7.5, and 9.0 formed biofilms. The combination of pH 4.5 and temperature of 5 °C avoided bacterial adhesion and biofilm formation during the incubation period. The probabilistic models obtained were effective to describe the interface region of adhesion/non-adhesion and biofilm formation/non-biofilm formation. Thus, these mathematical models can be used to define combinations of pH, temperature, and time that can affect the adhesion and biofilm formation by *B. cereus* on a food contact surface.

**Keywords:** foodborne pathogens; food industry surfaces; growth no-growth models; probabilistic models.

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