**Title: ASSESSMENT OF *Bacillus thuringiensis* GROWTH ON MILK USING PREDICTIVE MICROBIOLOGY.**

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**Abstract:**

*Bacillus spp*. are sporulating bacteria often involved in food outbreaks. *B. thuringiensis* is similar to *B. cereus* as morphological, biochemical and enterotoxin production aspects. The major difference between them is the presence of intracellular crystals in *B. thuringiensis*. Due to the resistance to heat treatments of *B. thuringiensis*, this study aimed to model the growth prediction of these bacteria on UHT (Ultra High Temperature) processed milk, using predictive microbiology tools to determine the kinetic growth parameters. *B. thuringiensis* strain was isolated from a UHT milk sample donated by a dairy. The strain was identified by sequencing analysis in MegaBACE$^{TM}$500 (APBiotech, Japan), followed by *in silico* analysis in Basic Local Alignment Search Tool and Ribosomal Database Project. For the inoculum, the strain was grown in BHI (Brain Heart Infusion) at 37°C for 24 hours. After centrifugation and bacterial pellet washing in 0.1% peptone water was performed for twice. Then the pellet was resuspended in 0.1% peptone water to obtain a final cell concentration of $10^8$ CFU/ml. Decimal serial dilutions in 0.1% peptone water were prepared and *B. thuringiensis* was inoculated to reach a final concentration on UHT milk (purchased from supermarkets) of nearly 100 CFU/ml. This milk was stored at 26°C and sampling was carried out in 0; 2.5; 5; 6; 7; 8.5; 10; 11.5; 13; 14; 15; 16; 24 and 26 h. At each time point, were performed decimal dilutions and aliquots were plated onto Mossel and BHI agar, following incubation at 37°C for 24 h. All bacterial counts were carried out in duplicate. The experiments were repeated twice. Growing curves were built by fitting data to the Baranyi's DMFit, generating $R^2$ of 0.98 for primary model. Experimental data showed that at 26°C, no growth was observed until approximately 5 h (lag phase = 5.4 h). However, the maximum population number of the bacteria was attained after approximately 15 h ($10^7$ CFU/ml) and the growth rate was 0.6. Experimental data demonstrated similar *lag* phase and growth rate comparing those generated by ComBase Predictive Models, suggesting that the essays were performed well. In conclusion, the developed model can be used to predict the growth of *B. thuringiensis* on milk at 26°C. Besides this, it is very important to avoid the milk contamination with spores, because if *B. thuringiensis* spores can survive to UHT process they will germinate and reach the maximum population density in about 15 h at room temperature.

**Key-words:** *Bacillus thuringiensis*, milk, predictive microbiology.