

Exploring the Potential Use of *Bacillus* SMIA-2 as a Biological Indicator in Thermal Sterilization Processes

Larissa Pacheco Ferreira¹, **Erica Cruz**², Meire Lelis Leal Martins¹

¹. State University of North Fluminense, Food Technology Laboratory, Campos dos Goytacazes/RJ, Brazil

². University of São Paulo, Department of Food Science and Experimental Nutrition / Faculty of Pharmaceutical Sciences, São Paulo/SP, Brazil

Bacillus sp. SMIA-2 is a thermophilic, spore-forming bacterium, which was isolated from the soil of the municipality of Campos dos Goytacazes - RJ. The analysis of its genome classified it as *Bacillus licheniformes* Gibson 46T. In the sterilization process, some microorganisms are used as biological indicators to validate the effectiveness of these processes. These microorganisms are chosen according to their specific sensitivity to the sterilization method. In thermal sterilization processes, thermophilic microorganisms are widely used. Therefore, this study aimed to analyze specific characteristics of *Bacillus* sp. spores. SMIA-2 at different temperatures. To carry out thermal destruction tests on SMIA-2 spores, a sample with a concentration of 10^9 UFC.mL⁻¹ in saline solution (0.85%, pH 6.7) was used, aliquots of this sample with spore suspension were subjected to heat treatment at different temperatures (100°C, 105°C, 110°C and 120°C) and at the end of the incubation period, pre-determined for each sample, the tube was removed from the oil bath and quickly cooled in water. Until no viable spores could be obtained. Subsequently, serial decimal dilutions of the sample, using saline solution (0.85%, m/v), were used to determine the number of viable spores. From the prepared dilutions, sample aliquots were seeded in a TSY medium, the incubation temperature was at 50°C, and the counting results obtained were expressed as a number of spores/mL. The data obtained were evaluated following the Bigelow model, and the decimal reduction time (D value) was calculated for each of the temperatures studied using the equation $D = t / (\log N_0 - \log N)$. With the D values for each temperature, it was possible to obtain the Z value, using the equation $Z = (T_2 - T_1) / (\log D_1 - \log D_2)$. The D values found were: D_{100 °C} = 15.79 minutes, D_{105 °C} = 5.93 minutes and D_{110 °C} = 3.49 minutes. This value corresponds to the time required to reduce the microbial population by 90% in that specific condition. The Z value found was 15.15 °C, indicating the temperature required to reduce the microbial population by 90%. We found potential for use in validating the efficiency of sterilization processes, mainly for canned foods and dairy products, such as UHT milk, where they are subjected to higher temperatures for a short period. This is because the D and Z values obtained in the thermal resistance tests of SMIA-2 spores indicate greater heat resistance when compared to pathogenic microorganisms found in canned meat products (*Clostridium botulinum*, Z \cong 10°C) and in dairy products (*Listeria monocytogenes*, *Salmonella* spp, *Escherichia coli* O157, both with D 70°C \cong 2 minutes and Z \cong 7°C, *Campylobacter* spp. and *Mycobacterium tuberculosis*, both with D 60°C \cong 1 and Z \cong 7°C). Therefore, the use of spores of *Bacillus* sp. SMIA-2 in the process of validating thermal sterilization efficiency showed promise by offering an additional safety margin, however, it is necessary to adjust some tests to corroborate its efficiency.

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