JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2021, *27*(3), 323-325

Journal of Agroalimentary Processes and Technologies

Evaluating soil spore-forming bacteria to prevent food contamination

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Abstract

Assessing the microbial load of endospore-forming bacteria on the soil surface of the "Aurel Vlaicu" University's campus was performed to evaluate the risk of contamination with heat-resistant bacteria, for food processed at technology laboratories and cafeterias. Endospore-forming bacteria ubiquitous in soil, conveyed by air currents, are able to withstand food processing, becoming a major problem for food quality and consumer safety. Extracts obtained from samples of surface soil were subjected to thermal treatment at 80ºC for 20 minutes. Two different methods, the standard plate count (SPC) and the quick turbidimetric measurement, were used for the evaluation of endospore-forming bacteria. SPC results (3 log CFU/g) were much lower than turbidimetric method results (5 log CFU/g), because SPC method being more specific, measures only viable cells able to grow on aerobic conditions, whereas turbidimetric method takes into account the whole bacterial biomass (both viable and nonviable). The obtained results are in line with those published in other studies, suggesting that endospore-forming bacteria should be monitored and prevented to contaminate the processing line or to generate food quality and safety issues in the final product.

Keywords: soil endospore-forming bacteria, heat-resistant bacteria evaluation, food contamination risk.

1. Introduction

Spore-forming bacteria, ubiquitously residents in soil, are able to withstand physical and chemical treatments, consequently being recognized as important contaminants in food processing [1, 2]. This group of spore-forming bacteria, implying important issues regarding food safety and/or quality, enumerates approximately 200 species of the *Bacillaceae* family, including both obligate anaerobes such as the genus *Clostridium* and, aerobes/ facultative anaerobes like *Bacillus* and related genera [3].

Some spores have adhesive properties that facilitate their attachment to processing equipment, playing an important role in biofilm's resistance and spread [4,5] and, are able to germinate and grow in conditions occurring in food [6, 7]. Supplementary concerns are raised by some species with increased tolerance to high or low temperature, or low pH. Foods subjected to thermal treatments at 65–95ºC enable spores survival, and during refrigeration storage represent opportunities for psychrotolerant species of spore formers.

Therefore, products such as milk and dairy desserts, chilled ready-to-eat meals, vegetable purees, salads and fruit juices raise concerns regarding food borne diseases and food spoilage produced by Bacillus cereus, for example [1].

Soil, and consequently air, is considered to be the initial contamination source for food by spore-formers through the direct route of contamination - soil, raw materials and ingredients - or indirect, via the processing environment [8, 9].

For this reason, evaluation of the endospore-forming microbiota of the soil is important and provides elements to prevent food contamination and outbreaks. The aim of this study was to determine the microbial load of endospore-forming bacteria on the soil surface of "*Aurel Vlaicu*" University's campus in order to assess the risk of contamination with heat-resistant bacteria, for food processed on the premises of the technology laboratories, snack bar and cafeteria.

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2.Materials and methods

Samples of superficial soil hay with from the surroundings of the university campus located in Micalaca, Arad were collected (March and July, 2021) and brought to microbiology laboratory. Sterile saline was added as diluent to produce a 1/10 initial suspension which was well homogenized afterwards. The suspension was heated using a water bath at 80±1ºC for 20 minutes, in order to select the endospore forming microbiota.

Standard plate count (SPC) and turbidimetric method were used for the quantification of endospore formers. Serial dilutions of the initial suspension were prepared, and then were mixed with molten tryptone soybean agar (TSA), poured into Petri dishes, and allowed to solidify. The work was carried out under a laminar flow hood to ensure sterility. The plates were aerobically incubated at 30ºC for 48 hours to allow microbial reproduction and colonies development [10]. Assuming that each bacterial colony resulted from an individual cell that has divided, by counting the number of colonies and accounting for the dilution factor, the number of spores or colony forming units (CFU) in the original sample was determined.

For the turbidimetric measurement we used a turbidimeter DEN-1 (Grant Instruments Ltd) to compare the turbidity of the sample with the turbidity of a McFarland standard scale. The method is designed to estimate bacterial concentrations by means of a turbidity scale (absorbance) which consists of a series of previously calibrated tubes with an optical density produced by the precipitation of $BaSO_4$. This absorbance (at 550 nm) is compared to the one of a known bacterial populations (cfu/mL). E.g.: the most used absorbance is the one corresponding to standard 0.5 on that scale, which assumes a population of 1.5×10^8 cfu/mL.

The work was carried out in triplicates both for Standard plate count (SPC) and turbidimetric method. Statistical analyses were performed with MedCalc statistical software [11]. Coefficients of variation (CVs) of the SPC and turbidimetric method were compared using likelihood ratio test. Data sets with a P value ≤0.05 were considered significantly different.

3.Results and discussions

For the interpretation of the results read on the densitometer display, the correspondence table between Mc Farland scale and bacterial concentration was used (Table 1).

Table 1. Correspondence McFarland scale/Bacterial concentration/Optical density [12]

Standard McFarland scale	Bacterial concentration (x10^{8}cells/ml)	Theoretical optical density at 550nm
0.5	1.5	0.125
1	3	0.25
2	6	0.50
3	9	0.75
4	12	1.00
5	15	1.25
6	18	1.50

Table 2. Aerobic spore counts in soil samples (g^{-1})

Samples	(mean cfu/g)		Coefficient of variation CV (%)		P value
	Turbidimetric method	SPC	Turbidimetric method	SPC	
I (colected in march)	0.6 x 10^6	0.8 x 10^3	50	6.25	0.0357
II (colected in july)	0.7 x 10^6	1 x 10^3	42.86	6	0.0429

Experimental results (Table 2) showed significantly lower concentrations (P≤0.05) of endospore-forming bacteria for SPC(3 log cfu/g) than turbidimetric method (5 log cfu/g), with much more narrower CV values for the SPC.

This significant difference observed between the two methods for the concentrations of endospore-forming bacteria, can be explained by several reasons. Firstly, the turbidimetric method counts not only endospore forming bacteria, but also other bacterial cells that died during heat treatment. Secondly, due to the way the light is detected with the densitometer, the size of the microorganism influences significantly the accuracy of the determination. The values in table 1, allowing the correspondence between McFarland scale and bacterial concentration represent a mean, valid for the bacteria. As other study confirmed, differences in size and shape of different species of bacteria imply more uncertainty with other organisms as McFarland scale was initially designed to estimate concentrations of the Gram-negative rod-shape bacterium Escherichia coli [13].

On the other hand, the SPC used proves to be a more precise method, counting only viable endospore-forming bacteria capable to grow in aerobic conditions at 30ºC.

Also, as previously showen in other studies, counts of spore-forming bacteria depends and vary significantly with the type of soil, and the culturing conditions (temperature, culture media) [10].

4.Conclusions

SPC and the turbidimetric measurement used for the evaluation of endospore-forming bacteria in soil lead to significantly different results, because SPC method being more specific, measures only viable cells able to grow on aerobic conditions, whereas turbidimetric method takes into account the whole bacterial biomass (both viable and nonviable). SPC proved to be the more reliable method, justifing the fact that, even if it is not as quick as the turbidimetric method, it is widespread used to monitor and prevent the contamination of food processing and food quality and safety issues.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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