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Identification and heat resistance evaluation of mesophilic spore-forming bacteria isolated from raw milk

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1 ABSTRACT

The aim of this study was to evaluate spore-forming species contamination levels in raw milk. The conditions of heat temperature and time were separated into the followed 4 methods; (1) 75℃, 15s, (2) 75℃, 10min, (3) 85℃, 10min, (4) 95℃, 10min. After heated the samples, the isolated strains were identified by 16S rDNA sequencing and characterized for their heat resistance. Aerobic and anaerobic spore counts were determined under 2log CFU/mL in the raw milk sample. The 16S rDNA sequencing enables the identification of a selection of 11 isolates; *Bacillus circulans* (two strain), *Bacillus cereus* (one strain), *Bacillus thuringiensis* (one strain), *Bacillus* sp. (four strain), *Geobacillus thermoleovorans* (one strain), *Paenibacillus timonensis* (one strain) and *Paenibacillus* sp. (one strain). The heat resistance (*D*-value, the decimal reduction time) and heat sensitivity (*z*-value, the temperature increase that lead to a ten-fold reduction of the *D* value) of spores of the 11 isolates were determined. The $D_{75℃}$-values were ranged 19.38–43.57 min, $D_{85℃}$-values and $D_{95℃}$-values, 20.13–52.08 min and 19.69–34.61 min, respectively, whereas the *z*-values 9.37-20.61℃.

2 INTRODUCTION

Heat treatments have long been established as one of the most important techniques to assure that microbial stability and safety of various food products including dairy products. Spore-forming bacteria such as *Bacillus* and related genera can survive pasteurization conditions and grow in pasteurized fluid milk during refrigerated storage, causing fluid milk spoilage and limiting the further extension of fluid milk's shelf life. In fresh raw and pasteurized milk and to an extent in cheeses, the predominant spore-forming species isolated using mesophilic incubation temperatures (~30℃) are *B. licheniformis*, *B. subtilis*, *B. pumilus* and *B. cereus* (Cook et al., 2000; Coorevits et al., 2008; Cosentino et al., 1997). Janstova et al (2001) reported that $D_{95℃}$ values of *B. cereus*, *B. subtilis*, *B. licheniformis* and *B. pumilus* isolated from raw milk were determined from 0.71-5.06 (min). Ziane et al (2016) showed the heat sensitive parameter z-value ranged 6.45-8.41 . The highest value was observed in *B. subtilis* and the lowest value in *B. cereus*.

The objective of this study is to evaluate the contamination level with spore-forming bacteria of raw milk by aerobic/anaerobic and to identify isolated strains by sequencing the 16S rDNA and to characterize their heat resistance.

3 METHOD & MATERIAL

❖ Collection of samples and Heating
- (1) 75℃, 15s, (2) 75℃, 10min, (3) 85℃, 10min, (4) 95℃, 10min

❖ Bacterial strain isolation and identification
- Inoculated on Brain Heart Infusion agar
- Aerobically and anaerobically incubated for 7 days at 30℃
- DNA extraction and 16S rDNA sequencing analysis

❖ Heat resistance evaluation
- Isolated spore preparation (spore concentration 10^{10} spores/mL)
- Spore suspension was heated from 75 to 95℃
- After heating, unit forming colony were counted

❖ Heat resistance and parameter determination
- *D* value (Gaillard et al., 1998) : ∗ Log N = Log N_0-(T/D)
- *z*-value (Bigelow et al., 1921) : ∗ Log D = Log D*-((T-T*)/Z_T)

4 RESULTS

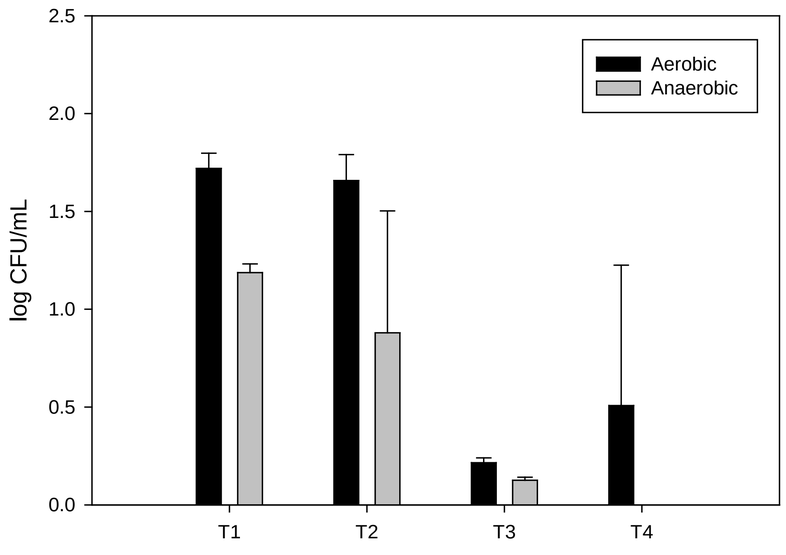

Figure 1. Presence of mesophilic spore-forming bacteria in raw milk according to different heating conditions.

Table 1. Estimated *D* values (min) and *z* values (℃) of spore forming bacteria strains isolated from raw milk; temperature range between 75 and 95℃

Strain identification	% homology 16S rDNA	*D* value (min) $D_{75℃}$	$D_{85℃}$	$D_{95℃}$	z-value (℃)
Bacillus circulans strain 05	100	25.12	21.19	21.68	19.09
Bacillus circulans strain HMF2507	100	33.59	33.69	/	10.10
Bacillus cereus strain GDUTAN3	100	34.40	30.10	31.19	19.45
Bacillus thuringiensis strain L-7601	100	19.38	21.68	19.69	20.11
Bacillus sp. FDAARGOS_235	100	36.31	38.50	33.29	19.52
Bacillus sp. GH5(2015)	100	41.89	44.75	34.61	18.98
Bacillus sp. strain TRB104	100	/	/	/	/
Bacillus sp. JCM 28848	100	24.62	20.13	/	9.37
Geobacillus thermoleovorans strain FJAT-2391	100	21.97	26.48	24.13	20.61
Paenibacillus timonensis strain PF4H_2.1	100	23.28	/	/	/
Paenibacillus sp. 7B-637	100	43.57	52.08	27.32	17.53

5 CONCLUSIONS

Eleven strains of *Bacillus* and related species were isolated from raw milk. Enumerations of mesophilic bacterial spores from raw milk present a contamination level close to < 2 log CFU/mL. The highest z_T-value (20.61℃) was observed for *Geobacillus thermoleovorans* strain FJAT-2391 whereas the lowest z_T-value (9.37℃) was obtained for *Bacillus sp.* JCM 28848. These *Bacillus* and related species were found to possess a sufficient heat resistance to survive in camel's milk during pasteurization temperature at 95℃ for few minutes.