



J. Dairy Sci. 100:1–10
<https://doi.org/10.3168/jds.2016-11616>
 © American Dairy Science Association®, 2017.

Seasonal and regional occurrence of heat-resistant spore-forming bacteria in the course of ultra-high temperature milk production in Tunisia

S. Kmiha,* C. Aouadhi,*¹ A. Klibi,* A. Jouini,* A. Béjaoui,* S. Mejri,† and A. Maaroufi*

*Laboratory of Epidemiology and Veterinary Microbiology, Group of Bacteriology and Biotechnology, Pasteur Institute of Tunisia (IPT), University of Tunis El Manar (UTM), 2092 Tunis, Tunisia

†Laboratory of Animal Resources and Food, National Institute of Agronomy, University of Carthage, Tunis (INAT) Tunisia, 43, Rue Charles Nicole, Cité Mahrajène, Le Belvédère, 1082 Tunis, Tunisia

ABSTRACT

Spore-forming bacteria, principally *Bacillus* species, are important contaminants of milk. Because of their high heat resistance, *Bacillus* species spores are capable of surviving the heat treatment process of milk and lead to spoilage of the final product. To determine the factors influencing the contamination of milk, spore-forming bacteria occurrence throughout the UHT milk production line during winter, spring, and summer was studied. The obtained results confirm that the total viable rate decreases rapidly throughout the production line of UHT milk showing the efficiency of thermal treatments used. However, the persistent high rate of spore-forming bacteria indicates their high heat resistance, especially in spring and summer. In addition, a significant variation of the quality of raw milk according to the location of the collecting centers was revealed. The molecular identification showed a high degree of diversity of heat-resistant *Bacillus* species, which are isolated from different milk samples. The distribution of *Bacillus* species in raw milk, stored milk, bacto-fused milk, pasteurized milk, and UHT milk were 28, 10, 16, 13, and 33%, respectively. Six *Bacillus* spp. including *Bacillus licheniformis* (52.38%), *Bacillus pumilus* (9.52%), *Bacillus* sp. (4.76%), *Bacillus sporothermodurans* (4.76%), *Terribacillus aitingensis* (4.76%), and *Paenibacillus* sp. (4.76%) were identified in different milk samples.

Key words: spore-forming bacteria, milk, seasonal, geographical area, *Bacillus*

INTRODUCTION

Bacillus and *Paenibacillus* spp. are important spoilage bacteria in various sectors of the food industry,

including dairy processing (Fromm and Boor, 2004; Scheldeman, 2004). These bacteria are of particular concern because they are capable of forming endospores and can thus survive pasteurization and other heat treatments commonly used to process raw food materials (Collins, 1981; Crielly et al., 1994). In addition, spores of *Bacillus* species are survival forms that are extremely resistant to most environmental stress factors (Andersson et al., 1995). The heat resistance of aerobic spore-formers isolated from dairy products was examined to give an overview of occurring highly heat-resistant spores (**HRS**). These spores have a special position among total microflora of milk with regard to their ability to survive thermal treatment of milk and subsequently to propagate in final products (Abouelnaga et al., 2002).

The *Bacillus* group has been identified as the prominent genera of gram-positive spore-formers in raw and pasteurized milk (Huck et al., 2007). The highly heterogeneous genus *Bacillus* comprises the largest species group of endospore-forming bacteria. Because of their ubiquitous nature, *Bacillus* spores can penetrate food production at several stages, resulting in significant economic losses and posing a potential risk to consumers due to the capacity of some *Bacillus* strains for toxin production (Ehling-Schulz and Messelhauser, 2013). The major spore-forming bacilli have contaminated and spoiled treated-UHT or sterilized milk, especially *Bacillus licheniformis*, *Bacillus cereus*, *Geobacillus stearothermophilus*, *Bacillus coagulans*, *Bacillus sporothermodurans*, *Brevisbacillus brevis*, *Paenibacillus lactis*, and *Bacillus sphaericus* (Pettersson et al., 1996; Cosentino and Palmas, 1997; Rombaut et al., 2002; Scheldeman, 2004; Aouadhi et al., 2014). Moreover, Huck et al. (2007) demonstrated that the principal contamination source of dairy products by spore-forming bacteria is the raw milk (Huck et al., 2007a). In addition, several entry points of these microorganisms have been identified at the farm level including concentrate feeds, silage, bedding, manure, soil, wash water, clusters, teat cups, and filter cloths (Vaerewijck et al., 2001; te

Received June 6, 2016.

Accepted September 12, 2016.

¹Corresponding author: chediaaouadhi@yahoo.fr

Table 1. Sampling plan of milk during 3 seasons and from different manufacturing steps of UHT Tunisian milk

Sampling	Collection season	Date	Production step	Sample origin
1	Winter	Jan. 6, 2014	Raw milk	Tankers from 2 farms
			Raw milk	Storage tank
			Bactofuged milk	Bactofuge unit
			Standardized milk	The cream separator
			Pasteurized milk	Pasteurizer output
			UHT milk	Package (production Jan. 7, 2014)
2	Winter	Jan. 22, 2014	Raw milk	Tankers from 7 farms
			Raw milk	Storage tank
			Bactofuged milk	Bactofuge unit
			Standardized milk	The cream separator
			Pasteurized milk	Pasteurizer output
			UHT milk	Package (production Jan. 23, 2014)
3	Spring	Mar. 19, 2014	Raw milk	Tankers from 6 farms
			Raw milk	Storage tank
			Bactofuged milk	Bactofuge unit
			Standardized milk	The cream separator
			Pasteurized milk	Pasteurizer output
			UHT milk	Package (production Mar. 20, 2014)
4	Summer	Jun. 13, 2014	Raw milk	Tankers from 6 farms
			Raw milk	Storage tank
			Bactofuged milk	Bactofuge unit
			Standardized milk	The cream separator
			Pasteurized milk	Pasteurizer output
			UHT milk	Package (production Jun. 14, 2014)

Giffel et al., 2002; Scheldeman et al., 2005; Huck et al., 2008). The processing plant has also been identified as a source of spore-forming bacteria, and there might be potential for milk contamination due to the presence and persistence of *Bacillus* and *Paenibacillus* spp. in processing environments (Lin et al., 1998).

An improved understanding of the sources of potentially HRS throughout the production line of UHT milk is necessary to prevent or reduce their presence in final product and to increase product shelf life (Meer et al., 1991). To achieve this, the nature and origin of spores and in particular of spores in raw milk must be better understood.

According to the literature reports, variations in the spore-forming bacterial community within regions (Ranieri and Boor, 2009), seasons (Phillips et Griffiths, 1986; Sutherland and Murdoch, 1994), production runs (Scott et al., 2007), pasteurization conditions (Ranieri and Boor, 2009; Monsallier et al., 2012), and processing facilities (Huck et al., 2007) were evaluated. Although the incidence of *B. sporothermodurans* and other heat-resistant bacteria in Tunisian milk (raw milk, pasteurized milk, and UHT milk) and the characterization of their phenotype and genotype properties have been evaluated by Aouadhi et al. (2014), the factors influencing their incidence and their origin have not previously been studied. The present study aims to evaluate the quality of different types of milk. In addition, the factors influencing the presence of *Bacillus* and related

genera in a milk chain in Tunisia and the source of these bacteria in packaged fluid milk were determined.

MATERIALS AND METHODS

Milk Sampling

Forty-one samples were taken at different stages during the UHT milk manufacturing. Twenty-one samples of raw milk have been provided from different collected centers situated in northern and northwest of Tunisia during winter, spring, and summer periods.

Milk collection was achieved in a dairy plant in Tunisia and the different sampling points are tankers of raw milk originated from different collecting centers, raw milk storage tank, bactofuged milk, standardized milk, pasteurized milk, and UHT milk (Table 1).

Determination of Milk Quality and Isolation of Bacteria. The quality of different types of milk samples was evaluated using 2 parameters: total and spore-forming bacteria counts. In fact, the total flora presented in different samples of milk was determined by plating on plate count agar after serial dilutions prepared in peptone water (0.01%). The determination of spore-forming bacteria from UHT milk was obtained by spreading 0.1 mL of product on brain-heart infusion agar supplemented with 1 mg/L of vitamin B₁₂. After incubation for 48 or 72 h at 37°C, the plates were examined and the counts of individual microbial groups were

Download English Version:

<https://daneshyari.com/en/article/5541893>

Download Persian Version:

<https://daneshyari.com/article/5541893>

[Daneshyari.com](https://daneshyari.com)