

## Prevalence of Spore-Forming Bacteria Associated with the Processing of Milk and Dairy Products Processing in Mansoura City

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### ABSTRACT

Spore forming bacteria are heat resistance organisms able to survive and germinating in milk and heat treated dairy products . They could deteriorate the quality of dairy products by producing proteolytic and lipolytic enzymes under refrigeration temperature in fluid milk . The aim of this study was to evaluate the prevalence of spore forming bacteria (mesophilic, thermophilic and psychrotrophic strains) associated with raw, UHT milk and dairy products in Mansoura city. The total viable bacterial count (TVB), aerobic mesophilic sporeformers counts (MSC), thermophilic sporeformers counts (TMC) and the average of psychrotrophic sporeformers counts (PSC) in raw milk samples were ranged from 10.06 to 10.66, 3.70 to 4.30, 2.00 to 2.88 and 0.15 log cfu/mL, respectively. verages of TVB , MSC, TMC and PSC of Ras cheese samples were 9.1, 3.5, 1.40 and 0.14 log cfu/ml, respectively, while means of TVB , MSC, TMC and PSC in Domiati cheese samples were 9.47, 3.72, 1.40 and 0.23 log cfu/ml. However, TVB, MSC, TSC and PSC of UHT milk samples in the present study was nil. Lastly, means of TVB, MSC and TSC of skim milk powder were 4.44, 1.7 and 0.45 log cfu/mL, respectively. All skim milk powder samples were free of PSC.

**Keywords:** Spore forming bacteria, UHT milk, Raw milk, Ras cheese, Domiati cheese, Skim milk powder

### INTRODUCTION

A spore-forming bacteria are special interest in the food industries for many reasons (Andersson *et al.* 1995; Heyndrickx and Scheldeman 2002). Firstly, spore former strains are basically impossible to eliminate their existence in raw food and ingredients due to their ubiquitous nature. When insufficiency take place in the sterilization of the packaging material or filling machine, cross contamination can easily happen. Secondly, the spore forming bacteria can survive pasteurization temperature or heating treatment in food processing, although these decontamination methods adequate in inhibiting vegetative cells. Thirdly, the several spore former strains were able to attach processing equipment and pipelines surfaces, leading to the biofilms formation (Andersson and Ronner 1998; Burgess *et al.*, 2010). And lastly, there is a special concern about the high tolerance of spores or vegetative cells to adverse condition or treatments generally used to inhibit growth (e.g. low temperature and low pH).

Aerobic spore forming bacteria are able to present in milk and different dairy products, due to their capability to survive different industrial stress conditions including heat treatments (Huck *et al.*, 2008; Ranieria *et al.*, 2009). These specialized communities of spore-forming bacteria are vastly distributed in environments of dairy farm and easily transferred into raw milk and at following steps during milk and dairy processing (Magnusson *et al.*, 2007; Watterson *et al.*, 2014). More substantially, the prevalence of bacillus and related spore forming bacteria in milk and dairy products may cause several human diseases and can result low quality dairy products. For the production of fluid milk, psychrotrophic spore forming bacteria can cause final products spoilage during refrigerated storage by the capability of these species to result several lipolytic or proteolytic enzymes at low temperatures (Meer *et al.*, 1991). Among those bacterial communities are *Bacillus* spp., *paenibacillus* spp. and *Viridibacillus* spp. (Fromm and Boor, 2004; Ivy *et al.*, 2012; Estrada, 2014).

At the first regarding the milk powder production, high counts of spore-forming bacteria (< 500 spores/mL) in the finished product restricts their markets size, resulting in opportunities loss when quality standards of more remunerative markets may not be met (Bienvenue, 2014).

The optimum growth temperature for special concern thermophilic spore former isolates ranged from 45-60 °C.

To reduce cross contamination rate of milk and dairy products in Egypt with aerobic spore former strains, it is important to ensure that quality system procedure should be used in Egyptian dairy farm to provide high quality raw milk production. To fulfill this, the origin and nature of spores former strains associated with raw milk have to be better understood (Montanari *et al.*, 2004; Scheldeman *et al.*, 2005). Therefore the objective of our study was to evaluate the prevalence aerobic mesophilic, thermophilic and psychrotrophic spore forming bacteria in milk and dairy products.

### MATERIALS AND METHODS

#### Samples collection:

Thirty raw milk samples , Ras cheese samples (15), Domiati cheese samples (15), UHT milk (15) samples and skim milk powder (15) were collected from the local market in Mansoura city, Egypt. All samples were kept under low temperature, and immediately transferred to dairy microbiology laboratory in the dairy department.

#### Preparation of sample

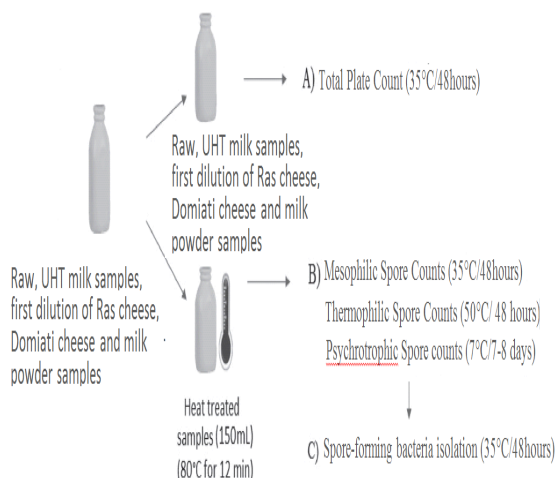
Using aseptic procedure, one mL of each raw milk and UHT milk samples were put into test tubes with 9 ml buffered peptone water, and blended at 1000 rpm for 90 seconds using a vortex. Samples of skim milk powder were dissolved by adding 10 g samples to 90 ml of sterile buffer peptone water in screw blue cap bottles, and stirred at 200 rpm for 10 min at 27°C according to the Standard Methods for the Examination dairy products Examination (1978). Regarding the preparation of Ras cheese and Domiati cheese samples, aliquots of 10 g of these samples were suspended in the 90 ml BPW, and mixed at 250 rpm for 10 min at room temperature using shaker (Standard Methods for the Examination of Dairy Products, 1978).

#### Enumeration of total aerobic viable in milk and dairy samples

Total viable aerobic counts of milk and dairy samples were enumerated using tryptone soya agar medium. The plates were incubated upside down in plastic bags at 35°C for 48 h (Difco, 1984). TVCs were expressed as cfu/ml for raw milk and UHT milk samples or cfu/g for Ras cheese, Domiati cheese and skim milk powder. Figure

1 presents the steps used for microbial analysis of raw, UHT milk samples, Ras cheese, Domiati cheese samples and skim milk powder samples.

For counting of spore formers in raw, UHT, skim milk powder. The first dilution of each Ras cheese or Domiati cheese samples, were homogenized in the lab and aliquots (200 mL) were put in sterile 500 mL screw capped bottles. The bottles were heated at 80°C for 15 min, using water bath in order to kill vegetative cells (Fig. 1). This protocol has been widely performed for the isolation of spore-forming bacteria isolation by other food microbiology research groups ( Ivy *et al.*, 20112; Estrada, 2014).



**Fig. 1. Microbial analysis used in the examination of raw, UHT , skim milk powder , Ras cheese and Domiati cheese.**

## RESULTS AND DISCUSSION

Raw milk and dairy products from local market in Mansoura city were collected for analysis of microbial quality during the year 2016. Analysis of samples were carried out independently. Microbial profile for raw milk are presented in Table 1. Ras cheese, Domiati cheese, skim milk powder and UHT milk are shown Tables 2 to 5, respectively. The total viable count of raw milk samples ranged from 10.06 to 10.66 log cfu/ml. Regarding the quality of raw milk, it could be seen that total viable count of raw milk samples were extremely higher than the limits of regulation for grade A milk in which the average of total viable counts do not exceed 100.000 cfu/ml or 5 log cfu/ml) according to the PMO for TPC ( FDA, 2015) , suggesting bad quality of raw milk before manufacture (Table 1). The total viable count was higher than 10 log cfu/ml in the local market . Poor hygienic practices were performed during initial stage of manufacture. For raw milk the PMO and ESS regulation does not appoint limits for aerobic mesophilic sporeformers, aerobic thermophilic sporeformers and aerobic psychrotrophic sporeformers. Therefore, these counts should be taken in consideration as part of milk quality characterization. Regarding aerobic mesophilic sporeformers counts (MSC), They varied from 3.70 to 4.30 log cfu/ ml, which were in disagreement with the previous researches being carried out in the Midwest region (South Dakota; Buehner *et al.*, 2014) and the New York (Miller *et al.*, 2015). However, the counts of thermophilic sporeformers of raw milk ranged from 2.00 to 2.88 log cfu/ml (Table 1&6), whereas the average of psychrotrophic sporeformers in raw milk samples was 0.15 log cfu/ml. The low counts of psychrotrophic might be due

to preservation and transportation of raw milk under low temperature.

The total viable counts of Ras cheese and Domiati cheese varied from 8.78 to 9.35 and 9.28 to 9.65 log cfu/ml, respectively (Tables 2 and 3). Total viable count is essential role for indicating the hygiene conditions under which the dairy products were the processed in some dairy plants.

**Table 1. Microbial analysis of raw milk samples collected from local market in Mansoura city**

Samples	TVC (Log CFU/ml)	MSC (Log CFU/ml)	TSC (Log CFU/ml)	PSC (Log CFU/ml)
Rm1	10.40	4.00	2.18	0.85
Rm2	10.41	4.04	2.40	0.78
Rm3	10.18	3.95	2.48	0.30
Rm4	10.30	3.98	2.00	0.48
Rm5	10.54	4.18	2.40	0.60
Rm6	10.60	3.81	2.48	0.00
Rm7	10.57	3.98	2.30	0.30
Rm8	10.58	4.08	2.54	0.30
Rm9	10.34	4.13	2.40	0.48
Rm10	10.28	4.19	2.78	ND
Rm11	10.49	4.22	2.88	ND
Rm12	10.66	4.30	2.48	ND
Rm13	10.18	3.85	2.85	ND
Rm14	10.55	3.78	2.40	ND
Rm15	10.61	3.93	2.30	ND
Rm16	10.63	4.04	2.00	ND
Rm17	10.64	4.08	2.74	ND
Rm18	10.65	4.11	2.70	ND
Rm19	10.59	4.15	2.30	ND
Rm20	10.57	3.85	2.85	ND
Rm21	10.45	3.70	2.70	ND
Rm22	10.41	3.93	2.60	ND
Rm23	10.43	3.98	2.54	ND
Rm24	10.20	4.19	2.54	ND
Rm25	10.28	4.18	2.48	ND
Rm26	10.57	4.24	2.40	ND
Rm27	10.51	4.27	2.18	0.48
Rm28	10.59	4.15	2.00	0.30
Rm29	10.15	3.81	2.81	ND
Rm30	10.06	3.85	2.85	0.48

TVC= total Viable counts; MSC = mesophilic spore counts;

TSC= thermophilic spore counts PSC = psychrotrophic spore counts; ND = not detected (below the detection limit <1 cfu/mL).

A high of total count indicates poor quality of raw material, growth contamination during manufacture and unsuitable temperature or time within storage (Mossel, 1983). Regarding the average of MSC, TSC and PSC in Ras cheese, they were 3.5, 1.40 and 0.14 log cfu/ml (Table 5), respectively. However the means of MSC, TSC and PSC in Domiati cheese were 3.72, 1.40 and 0.23 log cfu/ml (Table 5), respectively. The samples of Ras cheese and Domiati cheese revealed slight decline in total viable count , MSC, TSC and PSC, compared with the raw milk due to salting, low pH and low moisture content (El-Leboudy *et al.*, 2014). An increase in the counts of mesophilic spore bacteria in raw milk, Ras cheese and Domiati cheese compared with the thermophilic spore bacteria and psychrotrophic spore bacteria. The present results are consistent with Khater & Abdella (2017), who found the

average of TVC and MSC in raw milk were  $2.7 \times 10^9$  and  $1.5 \times 10^4$ , respectively, whereas total viable count and total aerobic spore forming counts in Ras cheese  $1.8 \times 10^8$  and  $1.8 \times 10^4$ , respectively. The counts of Total viable bacteria and sporeforming bacteria in Domiati cheese were  $3 \times 10^8$  and  $1.2 \times 10^4$ .

**Table 2. Microbial analysis of Ras cheese samples collected from local market in Mansoura city.**

Samples	TVC (Log CFU/ml)	MSC (Log CFU/ml)	TSC (Log CFU/ml)	PSC (Log CFU/ml)
Rc1	9.30	3.40	1.54	1.04
Rc2	9.00	3.18	1.30	ND
Rc3	8.93	3.30	1.23	ND
Rc4	8.98	3.48	1.28	ND
Rc5	8.81	3.54	1.11	ND
Rc6	8.78	2.70	1.08	ND
Rc7	8.90	2.70	1.20	ND
Rc8	9.18	3.60	1.48	ND
Rc9	9.08	3.40	1.38	ND
Rc10	9.16	3.54	1.46	ND
Rc11	9.30	3.18	1.60	ND
Rc 12	9.35	3.00	1.65	ND
Rc 13	9.29	3.00	1.59	0.60
Rc 14	9.31	3.30	1.61	ND
Rc 15	9.13	3.48	1.43	0.48

TVC= total Viable counts; MSC = mesophilic spore counts; TSC= thermophilic spore counts PSC = psychrotrophic spore counts; ND = not detected (below the detection limit <1 cfu/mL).

**Table 3. Microbial analysis of Domiati cheese samples collected from local market in Mansoura city.**

Samples	TVC (Log CFU/ml)	MSC (Log CFU/ml)	TSC (Log CFU/ml)	PSC (Log CFU/ml)
Dc1	9.49	3.88	1.65	ND
Dc2	9.55	3.85	1.41	ND
Dc3	9.53	3.78	1.45	0.60
Dc4	9.60	3.93	1.46	ND
Dc5	9.64	3.95	1.36	0.90
Dc6	9.47	3.30	1.34	ND
Dc7	9.49	3.40	1.41	ND
Dc8	9.38	3.30	1.58	ND
Dc9	9.28	3.54	1.51	0.78
Dc10	9.47	3.78	1.57	ND
Dc11	9.62	3.81	1.65	ND
Dc 12	9.65	3.65	1.70	ND
Dc 13	9.61	3.85	1.62	0.90
Dc 14	9.58	3.81	1.68	ND
Dc 15	9.50	3.90	1.59	0.30

TVC= total Viable counts; MSC = mesophilic spore counts; TSC= thermophilic spore counts PSC = psychrotrophic spore counts; ND = not detected (below the detection limit <1 cfu/mL).

Depending on the regulatory limits of UHT milk process, the presence of the total viable count in UHT milk have to be minimal or completely absent (Hassan *et al.*, 2009). As foreseen, total viable count, MSC, TSC and PSC of UHT milk samples in the present study was nil (Tables 4 & 6). The current study provides indication that UHT milk samples were within the regulatory limits for UHT milk (> 10 cfu/mL) according to PMO for TPC (FDA, 2015), showing that good manufacture practices and good hygienic practices were implemented during each of processing stages. The essential reasons for the existence of bacteria UHT milk may be due to dairy processing plant sanitation, quality of packaging material,

quality of raw milk and also the handling process (Tekinsen *et al.*, 2007). UHT milk free of bacteria indicates the certainty that the UHT milk plant applied high total quality system. The above result is consistent with Banik *et al.*, (2014) in Bangladesh.

**Table 4. Microbial analysis of UHT milk samples collected from local market in Mansoura city.**

Samples	Total viable count (LogCFU/ml)	Aerobic Mesophilic Sporeformers (LogCFU/ml)	Aerobic thermophilic Sporeformers (LogCFU/ml)	Aerobic Psychrotrophic Sporeformers (Log CFU/ml)
UHT1	ND	ND	ND	ND
UHT2	ND	ND	ND	ND
UHT3	ND	ND	ND	ND
UHT4	ND	ND	ND	ND
UHT5	ND	ND	ND	ND
UHT6	ND	ND	ND	ND
UHT7	ND	ND	ND	ND
UHT8	ND	ND	ND	ND
UHT9	ND	ND	ND	ND
UHT10	ND	ND	ND	ND
UHT11	ND	ND	ND	ND
UHT12	ND	ND	ND	ND
UHT13	ND	ND	ND	ND
UHT14	ND	ND	ND	ND
UHT15	ND	ND	ND	ND

TVC= total Viable counts; MSC = mesophilic spore counts; TSC= thermophilic spore counts PSC = psychrotrophic spore counts; ND = not detected (below the detection limit <1 cfu/mL).

The present research has been aimed to evaluate microbial profile of skim milk powder (SMP). However, the count of bacteria in SMP owing to their low percentage of moisture may not survive, and thus do not play any essential role in their spoilage. The total viable count ranged from 4.00 to 4.87 log cfu/ml (Tables 5 & 6), whereas MSC and TSC varied from 1.38 to 2.02 and ND to 0.90 (Tables 5 & 6), respectively. All skim milk powder samples were free of PSC (Table 5 & 6).

**Table 5. Microbial analysis of skim milk powder samples collected from local market in Mansoura city.**

Samples	TVC (Log CFU/ml)	MSC (Log CFU/ml)	TSC (Log CFU/ml)	PSC (Log CFU/ml)
Dm1	4.21	2.02	0.90	ND
Dm2	4.87	1.78	ND	ND
Dm3	4.12	1.56	ND	ND
Dm4	4.62	1.46	ND	ND
Dm5	4.03	1.78	0.78	ND
Dm6	4.22	1.63	0.78	ND
Dm7	4.76	2.12	0.48	ND
Dm8	4.30	1.50	ND	ND
Dm9	4.08	1.38	ND	ND
Dm10	4.19	1.83	ND	ND
Dm11	4.00	1.88	ND	ND
Dm 12	4.37	2.00	ND	ND
Dm 13	4.47	2.00	0.90	ND
Dm 14	4.53	1.46	0.90	ND
Dm 15	4.69	1.82	0.90	ND

TVC= total Viable counts; MSC = mesophilic spore counts; TSC= thermophilic spore counts PSC = psychrotrophic spore counts; ND = not detected (below the detection limit <1 cfu/mL).

**Table 6. Maximum, Minimum and average of TVB, TSC, TSC and PSC of milk and dairy product samples**

Samples	Total viable count (Log CFU/ml)			Aerobic Mesophilic Sporeformers (Log CFU/ml)			Aerobic thermophilic Sporeformers (Log CFU/ml)			Aerobic Psychrotrophic Sporeformers (Log CFU/ml)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
	Raw milk	10.06	10.66	10.50	3.70	4.30	4.04	2.00	2.88	2.48	ND	1.15
Ras cheese	8.78	9.35	9.10	2.70	3.60	3.25	1.08	1.65	1.40	ND	1.04	0.14
Domiat cheese	9.28	9.65	9.52	3.30	3.95	3.72	1.34	1.70	1.40	ND	0.90	0.23
UHT milk	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skim milk powder	4.00	4.87	4.44	1.46	2.12	1.79	ND	0.90	0.45	ND	ND	ND

The above results clearly indicate that TVC of skim milk powder samples within a regulatory limits for skim milk powder ( $> 4 \log \text{cfu/ml}$ ) established by Egyptian standard, Depending on the Codex Alimentarius Commission (CAC) and ICMSF (Codex Alimentarius, 1999; ICMSF,1986),the skim milk powder include lower than  $5 \times 10^4 \text{ cfu g}^{-1}$  and  $3.0 \times 10^4$ , respectively. The skim milk powder samples are generally considered high quality products. For skim milk powder, the CAC, ICMSF and Egyptian standards do not appoint limits for MSC, TSC and PSC, the spore forming bacteria counts should be taken into consideration only as crucial part of the skim milk powder characterization, which was found to be consistent with previous studies carried out in Mansoura city during the period from June 2016 to October 2016 (El-Etriby, 2017).

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### تواجد البكتريا المكونه للجراثيم المرتبطه بالعمليات التصنيعية للبن ومنتجات الالبان في مدينة المنصورة طه عبدالحليم نصيب ، محمد سمير درويش ، ابراهيم عبدالباقى و دعاء مطيع قسم الالبان - كلية الزراعة - جامعة المنصورة

تعتبر البكتريا المكونه للجراثيم كائنات مقاومة للحرارة لها القدرة علي المقاومة والتكاثر في اللبن ومنتجات الالبان المعاملة بالحرارة كما انها تستطيع أن تتلف منتجات الالبان عن طريق إنتاج الإنزيمات المحللة للبروتين والدهون تحت ظروف التبريد في اللبن السائل. والهدف من هذه الدراسة هو تقدير تواجد البكتريا المكونة من جراثيم) المزارع المتوسطة والمحببة للحرارة العالية والمحببة لدرجات الحرارة المنخفضة (المرتبطه مع اللبن الخام واللبن المعامل بالحرارة العالية ومنتجات الالبان في مدينة المنصورة. وجد أن إجمالي عدد البكتريا القابلة للنمو (TVB) من البكتريا المحببة للحرارة المتوسطة الهوائية (MSC) و البكتريا المكونة للجراثيم المحببة للحرارة العالية (TMC) والبكتريا المكونة للجراثيم والمحببة للحرارة المنخفضة (PSC) في عينات اللبن الخام تتراوح من 10.06 إلي 10.66 ومن 3.70 إلي 4.30 ومن 2.00 إلي 2.88 و 0.15 لوغاريتم وحدة تكوين المستعمرة / مل علي التوالي. يتراوح كل من TVB و MSC و TMC و PSC في عينات الجبن الراس تكون 9.1 و 3.5 و 0.14 و 0.14 لوغاريتم وحدة تكوين المستعمرة / مل علي التوالي بينما أعداد كل من TVB و MSC و TMC و PSC في عينات الجبن الدماطي تكون 9.47 و 3.72 و 1.40 و 0.23 لوغاريتم وحدة تكوين المستعمرة / مل بينما أثبتت الدراسة الحالية عدم تواجد TVB و MSC و TMC و PSC في عينات اللبن المعامل بدرجات الحرارة العالية بينما أعداد كل من TVB و MSC و TMC في اللبن الفرز المجفف تكون 4.44 و 1.7 و 0.45 لوغاريتم وحدة تكوين المستعمرة / مل علي التوالي. وجد أن جميع عينات اللبن الفرز المجفف تكون خالية من PSC