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Quantitative, Qualitative and Enzymatic Activities of Aerobic Sporforming Bacteria in Foods of Animal Origin

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Abstract. The incidence of aerobic sporeforming bacteria in some foods of animal origin obtained from Ismailia city markets was investigated. A total of 150 food samples, 25 each of frozen beef, frozen minced beef, frozen sausage, cow milk, soft Dommietta cheese and full cream milk powder were subjected to microbiological analyzed. Aerobic sporeforming organisms were detected with 100% in all examined frozen meat samples, while 23 (92%), 14 (56%) and 10 (40%) of cow milk, soft dommietta cheese and milk powder samples were positive for the incidence of aerobic sporeformer bacteria. The mean values of total aerobic sporeforming counts for frozen meat, minced beef and frozen sausage were 6 x 10^3, 2.2 x 10^6 and 4 x 10^5 10^2 CFU/g respectively, while for cow milk, soft Dommietta cheese and milk powder samples were 3.5 x 10^4, 5.1 x 10^3 and 2 x 10^2 cfu/ml or g respectively. Nine Bacillus strains represented by 203 isolates were identified from the food samples. Genus of *B. cereus, B. flexus* and *B. pumilus* had both proteolytic and lipolytic activities.

Keywords: Sporeforming bacteria, beef, sausage, milk, cheese

1. Introduction

Foods of animal origin have exerted a crucial role in human evolution and are an important component of a healthy and well balanced diet due to its nutritional richness. Meat, milk and their products are an excellent source of protein, good sources of vitamin B-complex and minerals especially calcium, iron and phosphorus [1], [2]. Food contaminations by aerobic sporeformer during various stages of production and processing are inevitable. Air, water, soil, intestine, lymph nodes, processing equipment, utensils and human being, in addition to further processing and handling techniques share the responsibility of microbial contamination of the meat during slaughtering processes [3]. On the other hand, milk may be contaminated via fecal contamination of the udder. Sporeformer bacteria present in milk not only survive in their end products but may also activate causing products spoilage and/or constitute pathogens as *Bacillus cereus*, which has raised considerable health concern [4].

Aerobic sporeforming bacteria, genus Bacillus, are ubiquitous and widely distributed in nature. Genus Bacillus is described as gram positive, aerobic, mostly catalase positive rods forming endospores very resistant to many adverse conditions [5]. The Bacillus spores are resistant to heat, desiccation, disinfectants, ionizing radiation and ultraviolet light [6]. *Bacillus spp.* is among the main spoilage organisms in food due to their versatile metabolism and heat-resistant spores. Except for *B. cereus*, no specific identification method is available, although information about *B. spp.* would be useful in monitoring good manufacturing practice [7]. Thus, the objective of this work was to evaluate the incidence and enzymatic activity of aerobic sporeforming bacteria in the meat and milk samples randomly collected from the retail markets at the Ismailia city, Egypt.

2. Materials and Methods

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2.1. Samples Collection

A total of 150 samples of meat, milk and their products were microbiological analyzed in this study allover of 8 months. Twenty five samples (200 ±15g) each of frozen beef, frozen minced beef, frozen sausage, raw cow milk, soft dommietta cheese and full cream milk powder were randomly purchased from more than fifteen-retail foods markets located in various regions of Ismailia city, Egypt. Sterile techniques were used during samples collection, packaging and microbiological analysis. Samples were transferred in ice box to the laboratory where they were subjected to microbiological analysis for detection of the total sporeforming bacterial counts, and identification of the most isolates and their proteolytic and lipolytic activities.

2.2. Samples Preparation

The technique recommended by APHA [8] was used to prepare the food samples. Frozen beef and their products were kept over night in refrigerator at 8-12°C in its original case for thawing. Beef, minced beef, sausage and soft Dommietta cheese samples were minced in a separate sterile food processor (Ts/12/Omas/UK). 25g or ml of each sample was added to 225 ml of 0.1% sterile buffer peptone water (Oxoid) in sterile stomacher bags of approximately 500-mL capacity. Samples were blended in a Seward stomacher (400R/UK) for 2 min to prepare a 1:10 dilution. One ml of the original dilution was transferred serially into sterile test tubes containing 9 ml of 0.1% sterile peptone water to obtain a final dilution of 10^7.

2.3. Determination of Sporeforming Counts

The technique recommended by Austin [9] was used for enumeration of aerobic sporeforming counts. A loopful from each dilution was streaked duplicate onto trypticase soy agar plates (Oxoid), and incubated at 35 ±0.5°C for 48 ± 2hr. The average number of colonies per countable plates was determined and the total number of colonies per gram was then expressed as cfu/g.

2.4. Identification of Isolates

Five colonies were randomly picked from each plate. Each colony was isolated for further purification, identification and determinations the proteolytic and Lipolytic activities according to MacFadyean [10].

3. Results and Discussion

3.1. Total Aerobic Sporeforming Counts

The obtained results in the present study revealed that all frozen meat samples from the Ismailia markets (100%) were positive for aerobic sporeforming organisms, while 23 (92%), 14 (56%) and 10 (40%) of cow milk, soft dommietta cheese and full cream milk powder respectively were positive for the presence of aerobic sporeformer bacteria (Table 1).

Table 1: Incidence of Aerobic Sporeforming Bacteria in Food Samples

	Frozen Beef and Beef Products			Milk and Milk Products		
	Raw	*Minced*	*Sausage*	*Raw*	**S.D. Cheese*	*Powder*
Positive No. (%)	25 (100)	25 (100)	25 (100)	23 (92)	14 (56)	10 (40)
Negative No. (%)	0 (0)	0 (0)	0 (0)	2 (8)	11 (44)	15 (60)
Total	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)

**S.D. Soft dommietta Cheese*

The mean values of total aerobic sporeformer counts for meat, minced meat and sausage were 6 x 10^3, 2.2 x 10^6 and 4 x 10^5 CFU/g respectively. For raw milk, soft domiati cheese and full cream milk powder samples were 3.5 x 10^4, 5.1 x 10^3 and 2 x 10^2 CFU/ml or g respectively (Table 1 and 2). In 1999, Nassar [11] showed that the counts of the *B. spp.* in the locally manufactured meat products ranged between 10^4 to 10^5 CFU/g. Other investigators recorded variable data concerning the incidence of aerobic sporeformer bacteria in locally manufactured meat, milk and their products from Egypt [12], [13]. The variable values of total aerobic sporeformer counts in this study reflected the level of hygienic and sanitary measures applied during the production and processing of meat and milk in Egyptian markets. Minced meat usually made from the less desirable cuts that may contain higher surface bacterial counts and consequently distributed inside the meat during mincing.

Khalafalla *et al.* [14] confirmed that minced meat manufactured from the frozen meat had a higher microbial load when compared with that produced from fresh one; they confirmed that the additional contaminations were related to technique and operation used for mincing of meat. Aerobic sporeforming bacteria form spores during unfavorable growth conditions. These spores are heat-resistant and can survive processing, pasteurization and cooking [15]. Generally, if foods are cooled slowly or kept warm before serving for suitable time and under room temperature, sporeformer may germinate and multiply rapidly at such temperatures and produce their exotoxin which is heat stable and may cause a severe public health hazard [16].

Table 2: Statistical Analysis of Aerobic Sporeformer Counts in Food Samples

		Aerobic Sporeforming Count (CFU/g)			
		Minimum	Maximum	Mean	*± S.E.
Frozen Beef	*Raw*	$< 10^2$	1.4×10^6	6×10^3	2×10^3
	Minced	2×10^2	6.7×10^8	2.2×10^6	2.1×10^4
	Sausage	$< 10^2$	2×10^6	4×10^5	5×10^4
Cow Milk	*Raw*	2×10^2	4.2×10^6	3.5×10^4	3×10^3
	S.D. Cheese	$< 10^2$	1.6×10^5	5.1×10^3	2×10^2
	Powder	$< 10^2$	3.8×10^4	2×10^2	3×10^2

#Sample No. = 25
**± S.E. means ± Standard Error*

The Egyptian Organization for Standardization and Quality Control [17] dose not establish a microbial standard limit for aerobic sporeformer bacteria in frozen meat, minced meat and sausage, meanwhile Food Safety Authority of Irelan [18] established that the acceptable limit should not be more than 10^5 CFU/g for *B. spp*. in meat and meat products. Accordingly the presence of *B. spp*. counts of 2(8%), 7(28%) and 5(20%) meat, minced meat and sausage are considered unfit for human consumption and constitute a public health hazard. Minced meat seem to be the most hazard meat products on consumer's health in the retail markets, which may be due to their locally manufacture under inadequate hygienic measures from low quality meat. On the other hand it is worthy to mention that, there is no clear microbial standard limit for the presence of the *B. spp*. in raw milk and their products. Whatever, the poor microbiological quality of milk and their products may associate with the preparation on the premises, premises type and little or no food hygiene control.

The frequency distributions of aerobic sporeforming strains isolated from food samples were shown in Table 3. Nine Bacillus strains out of 203 isolates were identified from meat and milk samples. Sporeformer bacteria commonly isolated from frozen minced meat (46 strains) followed by frozen sausage (42), cow milk (35) and soft dommietta cheese (28) then frozen meat and full cream milk powder (26 strains). Sporeformers in frozen meat and minced meat samples mainly consisted of *B. subtilis* (30.8%, 23.9%) and *B. brevis* (19.2%, 10.9%), followed by *B. flexus* (15.4%, 13%) respectively, while in frozen sausage samples mainly consisted of *B. brevis* (23.8%) followed by *B. cereus* and *B. subtilis* (21.4%). On the other side, sporeformers in cow milk, soft dommietta cheese full cream milk powder samples consisted mainly of *B. cereus* (28.5%, 39.3% and 42.4%) followed by *B. subtilis* (14.3% and 34.6%) respectively.

The identification with classical methods based on morphological and biochemical criteria showed *B. cereus* to be the most frequently occurring *Bacillus spp*. which isolated from examined food samples (Fig. 1). It is assumed that raw meat or milk directly obtained from healthy animals is free of microorganisms. Microbial contamination of foods during processing is undesirable but unavoidable. The initial microflora on the surfaces of the raw foods of animal origins mainly constitutes of aerobic sporeforming organisms [19]. The non hygienic methods of milk production and high ambient temperatures with the lack of prompt cooling after milking are the main reason affecting microbial contamination of raw milk [20]. *B. spp*. is the most predominated foods contaminant which capable of existing in two forms: active vegetative cells and dormant spores. These two forms often differ in their resistance properties to heat, chemicals, irradiation and other processing stresses. Similarly, spores are typically more resistant than vegetative cells to the alternative food processing technologies which interpret the presence of these organisms in the meat and milk products.

Aerobic sporeformer are considered either considered food spoilage microorganisms as genera of *B. flexus* and *B. pumilus* or food-borne pathogens as *B. cereus* and *B. subtilis* [21]. Of those most frequently isolated from the food of animal origin, *B. brevis*, *B. cereus* and *B. subtilis* are important for food hygiene because of their hydrolytic activities on food components and the ability of some strains to produce toxins or to grow at refrigerated temperatures.

Table 3: Frequency Distribution of Aerobic Sporeforming Species in Food of Animal Origin

Sporeformer Strains	Meat			Cow Milk			Isolates No.
	Raw	*Minced*	*Sausage*	*Raw*	*D. Cheese*	*Powder*	
	*F(%)	F(%)	F(%)	F(%)	F(%)	F(%)	
B. brevis	4(19.2)	5(10.9)	10(23.8)	3(8.6)	2(7.1)	0(0)	24
B. cereus	2(3.8)	7(15.2)	9(21.4)	10(28.5)	11(39.3)	11(42.4)	50
B. coagulans	0(0)	2(4.4)	0(0)	5(14.3)	1(3.6)	1(3.9)	9
B. flexus	4(15.4)	6(13)	2(4.8)	1(2.8)	3(10.7)	0(0)	16
B. megaterium	3(11.6)	4(8.7)	2(4.8)	3(8.6)	1(3.6)	2(7.6)	15
B. polymyxa	4(15.4)	3(6.5)	4(9.5)	2(5.7)	1(3.6)	0(0)	14
B. mycoides	0(0)	8(17.4)	6(14.3)	3(8.6)	2(7.1)	1(3.9)	20
B. pumilus	1(3.8)	0(0)	0(0)	3(8.6)	3(10.7)	2(7.6)	9
B. subtilis	8(30.8)	11(23.9)	9(21.4)	5(14.3)	4(14.3)	9(34.6)	46
Total	**26(100)	46(100)	42(100)	35(100)	28(100)	26(100)	203

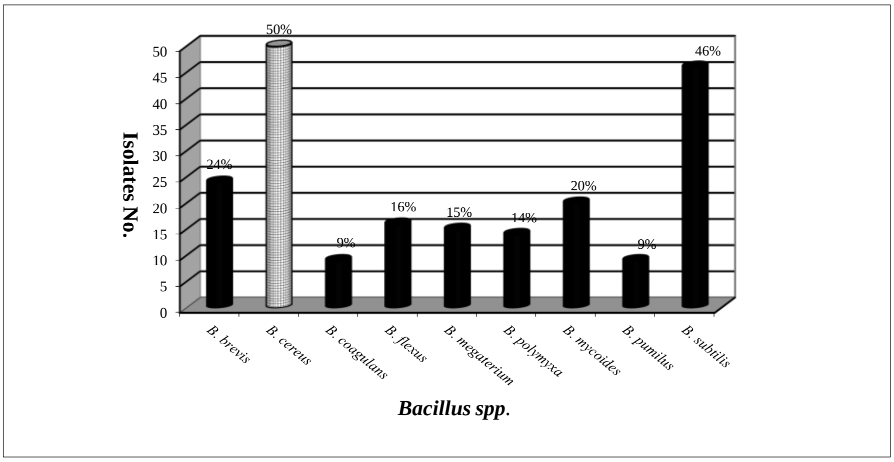

Fig. 1: Incidence of *B. cereus* in compared with other *B. spp.* in foods of animal origin

The high frequency of *B. cereus* (62.5%) was recorded in the meat products [22]. It is clear from the obtained results that a high incidence of *B. cereus* was observed in the frozen sausage samples. Asplund *et al*. [23] showed that the growth of B. cereus is a problem in the sausage especially when are stored at high temperatures. A dried milk product is known to be frequently contaminated with *B. cereus* [24]. *B. cereus* is known as producer of emetic and enterotoxin, it is described as causative agent in food-borne illness, most of *B. spp*. produces emetic toxins and cytotoxins, but none of those cytotoxic strains produced enterotoxin like *B. cereus* [25].

The proteolytic and lipolytic activities of *B. spp*. isolated were shown in Table 4. Genus of *B. cereus*, *B. flexus* and *B. pumilus* had both proteolytic and lipolytic activities. Proteolytic bacillus strains had variable degree of proteolysis on the examined plates. On the other hand, genus of B. *cereus*, *B. flexus*, *B. coagulans*, *B. pumilus* and *B. subtilis* had lipolytic activity, meanwhile genus of *B. megaterium*, *B. polymyxa* and *B. mycoides* not shown lipolytic activity in this study. *B. cereus* and *B. subtilis* had strong lipolytic activities by 31.2% and 28.6% respectively.

Food spoilage required contamination of foods by proteolytic and lipolytic bacterial strains have the ability to produce extracellular protease and lipases enzymes that can decomposed the protein and fat to low molecular weight substances. The most Bacillus strains which had proteolytic activity were *B. subtilis* (23.9%) followed by *B. cereus* (17.9%) and *B. brevis* (14.9%), while *B. coagulans* had the lowest proteolytic activity by 1.5%. Aerobic sporeformer organisms have the ability to produce thermostable protease, especially from *B. cereus* and *B. polymyxa* that can survive ultra-heat treatment of foods [26]. Protease enzymes can attack the nitrogen molecules naturally occur in meat causing, severe deteriorative changes in the colour and odour of foods even under refrigerated or frozen storage [27]. Proteolysis is the most important process recovered during cheese storage. It contributes cheese off-flavor, off odor and abnormal texture through the breakdown of the released proteolytic products of amino acids and peptides into amines and acids [13].

Table 4: Proteolytic and Lipolytic Activities for *B. spp.* in Foods of Animal Origin

Sporeformer Strains	Isolates	Proteolytic Activity		Lipolytic Activity	
	(No.)	+ve	%	+ve	%
B. brevis	24	20	14.9	7	9.1
B. cereus	50	24	17.9	24	31.2
B. coagulans	9	2	1.5	1	1.3
B. flexus	16	11	8.2	11	14.3
B. megaterium	15	9	6.7	0	0
B. polymyxa	14	7	5.3	0	0
B. mycoides	20	17	12.7	0	0
B. pumilus	9	12	8.9	12	15.5
B. subtilis	46	32	23.9	22	28.6
Total	203	134	100	77	100

Meat contains significant amount of fat that is susceptible to hydrolytic and oxidative rancidity. The growths of lipolytic bacteria in meat and their products play a role in quality losses and render the foods unwholesomeness [28]. Growth of aerobic sporeformer bacteria in raw milk produces extracellular lipase enzyme which adsorbed on milk fat globules and concentrated in the manufactured cheese. During storage, the enzyme causes bitter flavor by hydrolysis of fats into fatty acids and glycerides [29].

Conclusion

It could be concluded that the presence of aerobic sporeforming bacteria in the meat, milk and their products at the retail level in Ismailia city confirmed the original microbial contamination of raw meat and milk as well as the additional contamination occured during foods processing. Application of Hazard Analysis and Critical Control Point (HACCP) systems in foods production established aids in identifying and evaluating the food safety hazards. HACCP provides a quality control tools designed to replace or supplement traditional food inspection.

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