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Activation of Milk Lactoperoxidase System for Controlling Pseudomonas in Cow's Milk

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Abstract: Microbial contaminations become critical for raw milk especially from the time between milking until it reaches to the consumers. *Pseudomonas* species are considered as the most important psychrotrophs that contaminate raw milk. Therefore, the incidence and identifications of *Pseudomonas* spp. in raw cow's milk and their surrounded environment, secondly to evaluate the activity of the raw milk Lactoperoxidase System (LPS) on controlling of *Ps.* spp. in raw milk at room temperatures were studied. A total of 90 samples, 30 each of raw cow's milk directly post-milking (250 mL), potable water (100 mL) and cow's manure (50 g) were aseptically collected from different dairy farms at Sharkia province, Egypt. In addition to, 100 swabs were collected from hands of milker, dairy equipments and surface of udder and teats (25 each). The minimum, maximum and mean values of total pseudomonas counts in raw milk samples were 2.14, 6.21 and 3.54 $\log_{-10}$ cfu mL^{-1}, respectively. There were a significant difference between control and activated samples regarding the pseudomonas counts up to 8 h under room temperature storage. From these results, it could be concluded that the activation of the LP-system can be used to improving the keeping quality of raw milk during transportation until suitable method of storage is applied by consumers or the factory.

Key words: Milk, lactoperoxidase, activity, pseudomonas, Egypt

INTRODUCTION

Milk is the nutrient-rich liquid contains significant amounts of lactose, unsaturated fat, protein and minerals. Raw milk may subject to microbial contamination during milking processes especially in hand milking system at most Sharkia Governorate dairy farms, Egypt. There are so many difficulties in cooling system, storage and transportation of raw milk because of shortage in educations, control and equipments. As a result the keeping quality of raw milk decrease before delivered to the consumers and may it leads to spoilage and un-safety of raw milk.

Pseudomonas species are among the most important bacterial contaminant found in the raw milk (Al-Ashmawy *et al*., 1992; Vela, 1997) They are considered as psychrotrophs growing well at common refrigerator temperature which is responsible for spoilage of dairy products and may also cause severe public health hazards to consumers (Jay, 2000). *Pseudomonas* spp. elaborate extracellular hydrolytic enzymes such as proteases and lipases during growth in the milk, these enzymes can survive pasteurization and causing organoleptic defects in milk and milk product (Champagne *et al*., 1994; Frank, 1997). *Ps. aeruginosa* and *Ps. fluorescence* are considered as the most important psychrotrophic strains contaminated the raw milk (Reincheimer *et al*., 1990). *Ps. aeruginosa* has been recognized as a potential human pathogen and constituted potential hazards to both human and animal health (Grover *et al*., 1990; Jay, 2000). It has been implicated in many types of infections and food poisoning outbreak (Grover and Srinivasan, 1988).

The lactoperoxidase-thiocyanate-hydrogen peroxide system is a natural antimicrobial system present in milk (Fonteh *et al*., 2002). Lactoperoxidase has been recognized as an effective antimicrobial agent for many years and has been used extensively as an antibacteriostatic agent in reducing microflora in milk (Garcia-Graells *et al*., 2000). Preservative action of the LP system in milk after addition of thiocyanate and hydrogen peroxide has been demonstrated previously (Erginkaya *et al*., 2001; Savci *et al*., 2002). Different workers (Bosch *et al*., 2000; Shin *et al*., 2001; Abd-El-Aal, 2004) confirmed its bactericidal activity against gram-negative bacteria such as *Campylobacter jejuni*, *Salmonella* spp., *Escherichia coli* and *Ps.* spp.

The purpose of the present work was firstly to monitor the incidence and identifications of *Pseudomonas* spp. in raw cow's milk and their surrounded environment, secondly to evaluate the activity of the raw milk LP system, on controlling of *Ps.* spp. in raw milk at room temperatures.

MATERIALS AND METHODS

Samples Collection

A total of 90 samples, 30 each of raw cows milk directly post-milking (250 mL), potable water (100 mL) and cow's manure (50 g) were aseptically collected from different dairy farms at Sharkia provinces, Egypt during winter season. In addition to, 100 swabs (2×5 cm) were collected from hands of milker, dairy equipments and surface of udder and teats (25 each). All Samples were put in sterile bag and kept in ice-bag whereas directly transferred to food hygiene laboratory, Suez Canal University for microbiological analysis.

Sample Preparation

Milk samples divided into two equal parts (125 mL each), one part used for activation of Lactoperoxidase System (LPS) and other kept as control. The samples were prepared according to the methods of by Gennari and Dragotto (1992) as follows: the original dilution 1:10 dilution from the collected samples was prepared by adding 25 mL of milk or water sample to 225 mL of 0.1% sterile buffer peptone water (Oxoid, UK) in sterile stomacher bags of approximately 500 mL capacity. Meanwhile, 5 g of manure samples was added to 45 mL of sterile solution of 0.85% NaCl and 1% tryptone (Difco, UK), then, samples were blended in a Seward Stomacher (400^R/UK) for 2 min. On the other hand, each swab was put in sterile test tube containing 9 mL of 0.1% sterile buffer peptone water (Oxoid, UK). One milliliter 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.

Activation of Raw Milk Lactoperoxidase System

Activation of LP was carried out within 2 h after milking according to International Dairy Federation (IDF, 1988) by adding sodium thiocyanate (14 mg L^{-1}) to the milk samples. Then, plunging milk for about one minute followed by sodium bicarbonate (30 mg L^{-1}). The milk sample was then stirred for another 2-3 min. The enzymatic reactions were completed in the milk within 5 min after addition of H_2O_2 donor. Another same volume of raw cow's milk samples were kept as control. Both samples were kept at room temperature 25±1°C and prepared for microbiological evaluation as former then examined after 0, 2, 4, 6, 8 and 10 h post-activation.

Enumeration of *Pseudomonas* spp.

0.1 mL from each serial dilution and was previously incubated swabs were plated on pseudomonas selective agar base plates containing CFC supplement (Oxide, cod. SR 102 E) then, incubated for 48 h of at 30°C ±1. All colonies growing on the plates were counted and the total numbers of pseudomonas count were expressed as cell forming unit (cfu) mL^{-1} for milk and water samples, gram for manure samples and cm^2 for swabs.

Identification and Characterization of Isolates

The different colonies were selected from each plate and after purification, they streaked into Tryptose Soya Agar (TSA) slant and incubated for 24 h at 30°C. Identification of isolates was carried out on bases of morphological, cultural and biochemical characteristics as described by Krieg and Holt (1984) and Kwan and Skura (1985).

RESULTS AND DISCUSSION

Incidence of *Pseudomonas* spp.

Microbial contaminations become critical for raw milk especially from the time between milking until reach to consumers. Pseudomonadaceae are among the most important spoilage bacteria in raw milk which constitutes for up 78% of psychrotrophic microflora (Muir *et al*., 1979; Vela, 1997). In this study, 114 (60%) out of 190 samples were positives for the presence of pseudomonas, whereas 20 (66.7%) out of 30 milk samples were recorded positive (Table 1). In concerning to contamination sources, higher recorded positive numbers for the prevalence of pseudomonas were obtained from water (100%), followed by dairy equipments (92%), then manure (83.3%) samples. Contamination of raw milk by Pseudomonas is mainly from soil and water (Zaki *et al*., 1996). Milker, udder surfaces and teat were reported the lower incidence for the presence of pseudomonas that may be due to their drying conditions and the predominance of other skin microflora. Many investigators in other study recorded various results for the incidence of *Pseudomonas* in raw milk and their pertinences (Eman, 1992; Zaki *et al*., 1996; Dinsmore *et al*., 2001).

Pseudomonas can gain access to milk via manure, polluted water, dairy equipments and dairy workers (Khalil, 1992). Such microbes as other psychrotrophes can multiply in milk stone deposits on equipment surfaces during shutdown periods and contaminate milk when started (Shah, 1994). The obtained results confirmed there are no controlled conditions during milk production in dairy farms at Sharkia province, Egypt.

Total Pseudomonas Counts in the Examined Samples

The mean values of total pseudomonas counts in the collected samples from dairy farms at Sharkia provinces, Egypt were shown in Table 2. It is not doubtful that manure samples had shown the highest pseudomonas counts ($\log_{-10}$ 6.22 cfu g^{-1}) among the evaluated samples. There were a considerable pseudomonas counts in the samples collected from water, udder and teat, hand's milker and equipments.

The major contamination sources of pseudomonas in raw milk are from improperly cleaned equipments and faeces, soil, water (Erskine *et al*., 1987). Consequently, the obtained results revealed that the minimum, maximum and mean values of total pseudomonas counts in raw milk samples were 2.14, 6.21 and 3.54 $\log_{-10}$ cfu mL^{-1}, respectively. The results obviously reflected the inadequate

Table 1: Numbers (%) of positive and negative samples for the incidence of *Pseudomonas* spp.

		Negative		Positive	
Samples	No.	No.	%	No	%
Raw milk	30	10	33.3	20	66.7
Water	30	0	0.0	30	100.0
Manure	30	5	16.7	25	83.3
Udder surface	25	23	92.0	2	8.0
Teat surface	25	22	88.0	3	12.0
Milker's hand	25	14	56.0	11	44.0
Dairy equipments	25	2	8.0	23	92.0
Total	190	76	40.0	114	60.0

Table 2: Log mean counts of pseudomonas in the examined samples from dairy farms

	Pseudomonas counts		
Samples	Minimum	Maximum	Mean±SE***
*Raw milk	2.14	6.21	3.54±0.64
*Water	3.12	5.62	4.24±1.01
**Manure	3.89	7.46	6.22±1.38
~Udder and teat	2.31	5.10	3.81±0.54
~Hand's milker	2.01	4.60	2.98±0.34
~Equipments	3.22	6.21	4.26±1.26

* Counts expressed as $\log_{-10}$ cfu mL^{-1}, ** Counts expressed as $\log_{-10}$ cfu g^{-1}, ~ Counts expressed as $\log_{-10}$ cfu cm^{-2}, *** SE Means Standard Error

Table 3: Total pseudomonas count ($\log_{-10}$ cfu mL^{-1}) of control and activated milk samples at storage room temperature (25°C)

Time (h)	Normal	Activated	Difference	%	SE*
0	3.56	3.60	0.04	-1.12	0.01^{a}
2	3.98	3.67	0.31	7.79	0.51^{b}
4	4.62	3.88	0.74	16.02	0.42^{c}
6	5.01	4.12	0.89	17.76	1.24^{d}
8	6.23	5.10	1.13	18.14	1.72^{e}

*SE with difference letter(s) in the column were significantly difference ($p>0.05$)

sanitary and hygienic measures applied during milking processes at those dairy farms. Many investigators at different institutes have recording various results concerning the total pseudomonas counts in raw milk depending on the hygienic statutes of the farms (Ahmed, 1995; Zaki *et al*., 1996; Desmasures *et al*., 1997).

Activation of Milk Lactoperoxidase System (LP)

The activation of milk lactoperoxidase system constitutes an alternative method of milk preservation in rural areas and its values where cooling facilities or chemical preservatives are unavailable. From the conducted study, the total pseudomonas count ($\log_{-10}$ cfu mL^{-1}) of control and activated milk samples at storage room temperature (25°C) were shown in Table 3. There were a significant difference between control and activated samples regarding the pseudomonas counts up to 8 h under room temperature storage. LP activation delayed or even caused complete inhibition of psychrotrophic microorganisms' growth in activated milk samples (Sondhi *et al*., 1992; Wolfson and Sumner, 1993). In addition, LP activation has a bactericidal effect against pseudomonas (Marks *et al*., 2001).

CONCLUSION

It is clear that manure water, dairy equipments and hand's milker were the most contaminated sites for pseudomonas. Enumeration of pseudomonas in raw milk gives a good evaluation of the farm hygiene. Activation of milk LP within 2-3 h after milking by using sodium thiocyanate (14 g L^{-1}) and sodium bicarbonate (30 mg L^{-1}) could be used for controlling the pseudomonas growth in the raw milk. Thus, improving the keeping quality of raw milk during transportation until suitable method of storage is applied by consumers or the factory.

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