

## STUDIES ON STAPHYLOCOCCUS AUREUS IN MILK AND YOGHURT TREATED BY BIFIDOBACTERIA

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### SUMMARY

Milk samples and lab-made yoghurt were used to investigate the antibacterial effect of *bifidobacteria bifidum* on *Staphylococcus aureus* pathogen. All samples were analyzed microbiologically for presence of *S. aureus* when fresh and after 2 and 5 days of storage at refrigeration temperature for milk and yoghurt respectively proven to be contaminated with *S. aureus* from infected, non treated dairy cows and expermintally milk inoculated with *S. aureus* ( $5 \times 10^4$  cfu/ml). The obtained results indicated that there were adequate difference in *S.aureus* count in the examined samples treated with *Bifidobacteria bifidum* from zero time till the 2<sup>nd</sup> day of storage in bifidus milk. The reduction of *S.aureus* were 100% at 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> days of storage in bifidus yoghurt manufactured from inoculated milk with *S.aureus*. The current study indicates that Bifido-

bacteria may restrict and /or inhibit *S. aureus* in milk and yoghurt.

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### INTRODUCTION

In recent years probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts for many reasons: Inhibition of pathogenic species; anticarcinogenic activity; control of serum cholesterol levels and increasing the immune response (Shin, et al. 2000). Milk is extremely perishable and many means have been developed to preserve it, the earliest one which has been used for many thousands of years is fermentation. Milk can be fermented by inoculating fresh milk with the appropriate bacteria, as the bacteria grow, it convert milk sugar (lactose) to lactic acid which preserve the milk by lowering the pH which prevents the growth of putrefactive and/or pathogenic bacteria who do not grow well in acid

conditions. Casein consider the predominant protein in milk soluble at a neutral pH but insoluble in acid thus when milk sours, casein precipitates and thickens of the product occur.

The high prevalence of *S. aureus* may be attributed to the wide distribution of the organism inside the mammary gland and skin of the teats and udder. *S.aureus* establishe a mild subclinical infection over a long duration and they usually shed in the milk, which serve as a source of infection for milk consumer, also *Staphylococci* are present as major mastitis pathogens in the dairy industry worldwide (Leslie & Schukken, 1993 and Rados-tits. et al., 2000). The uses of milk and milk products with *Bifidobacteria* inhibit *S. aureus* pathogens besides its production of lactic acid and acetic acid, these organisms have become popular in various fermented dairy products because of their potential health benefits. Dahi (1986); Robenson & Tamime, (1990); Hughes & Hoover (1995); Nayra & hosney (2003) and Barkema, et al.(2006).So the purpose of this work to find a solve for protection of the consumer by Bifidobacteria against *S.aureus* to inhibit this pathogen in milk and yoghurt during refrigeration.

## MATERIALS AND METHODS

- Group I, raw milk collected from non treated dairy cows proved to be free from *S. aureus* sample was sterilized in the autoclave at 121°C for 5 minutes, after cooling it divided into two

portions. The 1<sup>st</sup> used as a control bifidus and non bifudus milk each into 100 ml sterile glass bottles, while the 2<sup>nd</sup> one used as a control bifidus and non bifudus yoghurt.

- Group II, raw milk sample collected from non treated dairy cows proved to be free from *S. aureus* was sterilized in the autoclave at 121°C for 5 minutes, after cooling and expermintally inoculated with *S. aureus* at a rate of  $5 \times 10^4$  cfu/ml (the same *S. aureus* isolates and count in proven contaminated milk), the inoculated milk were divided into two portions. The 1<sup>st</sup> used in bifidus and non bifudus milk each into 100 ml sterile glass bottles. While the 2<sup>nd</sup> one used in manufactured bifidus and non bifudus yoghurt.
- Group III, raw milk collected from non treated dairy cows and proven to be contaminated with *S. aureus* were divided into 1<sup>st</sup> part as non bifudus milk and bifidus milk each into 100 ml sterile glass bottles, the 2<sup>nd</sup> part for manufacturing yoghurt which in turn subdivided into bifidus and non bifudus yoghurt.
- Lyophilized *Bifidobactereria bifidum* (obtained kindly from Fac.of Sci. Zagazig Univ.) was suspended and incubated at 45°C for 6 hours to give a viable count of  $1 \times 10^7$  cfu/ml (Robenson and Tamime, 1990).
- Purified strains of *Strept. thermophilus* were suspended and incubated at 36°C for 8 hours to be used in manufacturing of yoghurt.
- pH measurement by using electric pH meter (3310 pH meter, UK) were done daily all over the study period.

- All milk and yoghurt samples were incubated at 40°C for 4 hrs followed by storage at refrigerator temperature, milk only 2 days and 5 days for yoghurt each experiment was replicated three times.
- Microbiological enumeration of *S.aureus* according to ICMSF(1986) while isolation and

identification were carried out according to Buchanan and Gibbons (1975).

- Statistical analysis: the obtained data from the present study were analysed statistically with SPSS for windows version 9.0 software.

## RESULTS

**Table (1): Boiled milk and manufactured yoghurt from the same boiled milk free from *S. aureus* ( negative control) .**

Storage Time (days)	Control negative of milk						Control negative of yoghurt					
	Non bifidus milk			Bifidus milk			Non bifidus yoghurt			Bifidus yoghurt		
	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	reduction %	pH	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	reduction%	pH
0 time	0	0	6.5	0	0	5.0	0	0	4.6	0	0	4.5
1 <sup>st</sup> day	0	0	6.5	0	0	4.7	0	0	4.5	0	0	4.5
2 <sup>nd</sup> day	0	0	6.4	0	0	4.5	0	0	4.5	0	0	4.2
3 <sup>rd</sup> day	-	-	-	-	-	-	0	0	4.5	0	0	4.2
4 <sup>th</sup> day	-	-	-	-	-	-	0	0	4.4	0	0	4.0
5 <sup>th</sup> day	-	-	-	-	-	-	0	0	4.4	0	0	3.8

**Table (2): Survival and mean values of *S. aureus* log 10 CFU / ml in milk samples.**

Storage Time (days)	Milk proven to be contaminated with <i>S. aureus</i>						Milk experimentally inoculated with <i>S. aureus</i>					
	Non bifidus milk			Bifidus milk			Non bifidus milk			Bifidus milk		
	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	Reduction %	pH	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	Reduction %	pH
0 time	4.69	0.00	6.5	4.69	0.00	5.0	4.69	0.00	6.5	4.69	0.00	5.0
1 <sup>st</sup> day	4.60	1.92	6.5	2.69	42.64	4.7	4.60	1.92	6.5	2.47	47.33	4.8
2 <sup>nd</sup> day	4.47	4.69	6.4	2.47	47.33	4.5	4.40	6.18	6.4	2	57.36	4.5

**Table (3) : Survival and mean values of *S. aureus* log 10 CFU / g in yoghurt samples.**

Storage Time (days)	Yoghurt manufactured from milk proven to be contaminated with <i>S. aureus</i>						Yoghurt experimentally inoculated with <i>S. aureus</i>					
	Non bifidus yoghurt			Bifidus yoghurt			Non bifidus yoghurt			Bifidus yoghurt		
	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	reduction%	pH	<i>S.aureus</i> count	reduction%	pH
0 time	4.69	0.00	4.6	4.60	1.92	4.5	4.69	0.00	4.6	4.60	1.92	4.5
1 <sup>st</sup> day	3.84	18.12	4.5	2	57.36	4.5	3.77	19.62	4.5	2	57.36	4.5
2 <sup>nd</sup> day	3.30	29.64	4.5	0	100.00	4.3	3.30	29.64	4.5	0	100.00	4.2
3 <sup>rd</sup> day	2.69	42.64	4.4	0	100.00	4.2	2.69	42.64	4.4	0	100.00	4.2
4 <sup>th</sup> day	2	57.36	4.4	0	100.00	4.0	2	57.36	4.4	0	100.00	4.0
5 <sup>th</sup> day	2	57.36	4.4	0	100.00	3.9	2	57.36	4.4	0	100.00	3.8

## DISCUSSION

Milk and milk products may be produced by individual owners with lack of sanitary conditions, thus milk and dairy products harbor a natural microbial flora and/ or other microorganisms. So, consumption of milk and yoghurt constitutes a major source of food borne diseases (Montasser & Abdel-wahab 2003). Presence of *S.aureus* in milk and its products is usually taken as an index for contamination especially in cases of mastitis. The proportion of *S.aureus* isolated from milk with an isolation rate of 39.40% to 48.7%. as reported by Kayihura, et al. (1987); Vandal & Biasizzo (2000) and Kirkan, et al. (2005).

As shown in table (1), pH value of bifidus milk and bifidus yoghurt neither contaminated nor ex-

perimentally inoculated with *S. aureus* reach to 4.5 in bifidus milk and 3.8 in bifidus yoghurt during storage. this could be due to the production of more acetic acid by *Bifidobacteria* during storage. Similar results were obtained by Lim, et al.(1995); Samona, et al. (1996), and Shin , et al. (2000) Montasser & Abdel-wahab (2003) who evaluated milk and yoghurt containing *Bifidobacteria* for viability of *Bifidobacteria* during refrigerated storage and found that viability of *Bifidobacteria* and lactic acid bacteria in milk and yoghurt remained high.

Table (2) reveals the survival of *S. aureus* could survive. The whole storage time in contamination and experimental inoculated bifidus milk at pH of 4.5 with reduction rate of 57.36% and 47.33% respectively. This is an important consideration,

because *Bifidobactereria* produce appreciable quantities of acetic acid from the fermentation of lactose besides their pH- reducing effect on either *S.aureus* or other organisms which may present in milk. Some degree of similarity was observed between the results of our study and previous ones as reported by Kurman and Rasic (1991) and Baron & Vuillemand (2000). Also Lankaputhra, et al. (1996) and Nremark, et al., (2002) concluded that fermented milk with probiotic organisms inhibit the growth of *S. aureus*.

Table (3) showed that in bifidus yoghurt manufactured from milk proven to be contaminated and experimentally inoculated milk with *S. aureus* showed a noticeable reduction 100% and *S.aureus* disappeared at 2<sup>nd</sup>, 3<sup>th</sup>, 4<sup>th</sup> and 5<sup>th</sup> days. Generally, these results agree with those given by Rybka (1994) and Ashutosh, et al. (2002) who observed that *Shigella dysentriae* showed maximum inhibition followed by *S. aureus*, it may be attributed to the *Bifidobacterium bifidum* numbers in bifidus milk and bifidus yoghurt were nearly constant, also its acetic acid production has the highest inhibitory effect against pathogenic organisms than another acids.

Murad, et al. (2000) stated that the bactericidal action of *Bifidobacteria* may be attributed to the production of some kind of antimicrobial agent (bacteriocins) which was relatively stable at acid conditions in addition to their pH- reducing effect which inhibit the food borne pathogens such

*S.aureus*, *E.coli*, *Bacillus cereus*, and *Pseudomonas flouresences*.

As *Bifidobacteria* are probiotic organisms that improve the microbial balance in the human gut it can be incorporated as a live cultures in fermented dairy foods, *Bifidobacteria* have the ability to survive as a normal inhabitant microorganism in the upper gastrointestinal tract of man and animal as human fecal flora playing a role in colon carcinogenesis prevention; Bartram, et al.(1994); Lankaputhra & Shah (1995); Adhikari, et al.(2000); Amer and El-ham (2000); Xiao, et al. (2003) and Pochart, et al.(2004).

The results of the current study indicate that the use of *Bifidobacteria* in milk and yoghurt restricts and /or inhibits the growth of the pathogen examined in this study. Therefore, it can be concluded from the observations presented here that *Bifidobacteria* have a bactericidal effect against *S. aureus* in milk and yoghurt.

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## دراسة مدى التواجد الميكروبي فى اللبن والزبادى والمعاملة بالبفيدوباكتر

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مركز البحوث الزراعية - معهد بحوث صحة الحيوان - الدقى - مصر

تم أخذ عينات من ألبان الأبقار الحلابة سواء بها الميكروب العنقودى الذهبى والخالية من هذا الميكروب وتم تصنيع الزبادى بالمعمل من هذه الألبان لدراسة تأثير البوفيدوباكتر على الميكروب العنقودى الذهبى فى كل من هذه الألبان والزبادى المصنع منه. كل نوع من العينات المختبرة تم تقسيمها إلى مجموعات إحداها تم إعتبارها كمجموعة ضابطة وبقى المجموعات تم معاملتها بالبوفيدوباكتر ثم حفظها لمدة يومين بالثلاجة بالنسبة للألبان وخمسة أيام بالنسبة للزبادى لدراسة أثر البوفيدوباكتر على نمو البكتيريا العنقودية.

وقد أوضحت عينات اللبن والمعاملة بالبوفيدوباكتر إنخفاضاً ملحوظاً فى قيم الميكروب العنقودى الذهبى ، بينما عينات الزبادى التى عوملت بالبوفيدوباكتر أظهرت إختفاء هذا الميكروب فى الأيام ٣ ، ٤ ، ٥ على التوالى وتم مناقشة أهمية ميكروب البوفيدوباكتر وكذلك الأهمية الصحية لميكروب العنقودى الذهبى بهذه الألبان ومنتجاتها.