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IMPACT OF PROBIOTICS ON *STAPHYLOCOCCUS AUREUS* GROWTH IN SOME DAIRY PRODUCTS

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ABSTRACT

A total of 180 samples of yoghurt (small and large scale), soft white cheese (Tallaga and Feta) and rayeb milk (small and large scale) 30 of each were arbitrarily collected from different localities as dairy shopes and markets in Beni-Suef city, Egypt. The results revealed that the mean values of *S.aureus* were $5.7X10^3 \pm 3.2X10^3$, $9.3X10^2 \pm 3.9X10^2$, $4.3X10^4 \pm 2X10^4$, $5.1X10^3 \pm 1.7X10^3$, $3.6X10^4 \pm 1.3X10^4$ and $5.5X10^2 \pm 2.7X10^2$, respectively. Also, the result regarding that 60% of examined *S. aureus* strains were enterotoxigenic by multiplex PCR technique as carried one or two se- genes. The present work evaluated the influence of probiotic as live organisms on the survival of *S.aureus* in some dairy product as Tallaga cheese and rayeb milk during their production and refrigerator storage.

Key words: Yoghurt, Soft cheese, Rayeb milk, *S.aureus*, enterotoxigen, PCR, probiotic, *L.acidophilus* and *B.bifidum*.

INTRODUCTION

Milk and dairy products play an essential role in human nutrition therefore, it is preferred that 25% of the daily nutrition intake contains dairy products (Hoven, 1987).

Fermented milk precognitive as a dairy product obtained by the fermentation of milk through the action of Lactic Acid Bacteria "LAB" which resulted in lowering of pH with or without coagulation. These bacteria must be viable, active and abundant in the product. The popular fermented milk products are Yoghurt, Labneh, Acidophius milk, Rayeb and Butter milk, etc. (Ahmed *et al*., 2014).

Yoghurt is a classical fermented milk product which consumed all over the world mainly in northern European countries, Balkans, Middle- East and Indian sub-continents (Tamine, 2002).

Yoghurt is manufactured by the addition of healthy bacteria and live cultures to milk as Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (Steinkraus, 1997 and Tamine and Robinson, 2007). The main reason pointed out for yoghurt and other fermented dairy products (Guarner *et al*., 2005; McKinley, 2005; Álvarez-León *et al*., 2006 and Cueva and Aryana, 2008).

consumption is the cultural, as improves lactose digestion and eliminates the symptoms of lactose intolerance, high in protein, calcium, phosphorus, magnesium, potassium, riboflavin and vitamin A Soft white cheese is processed from cow's or buffalo's milk or a mixture of them according to the Egyptian cheese-making technology. Production may be artisanal or industrial, depending on whether the cheeses are manufactured with raw thermized (heated below pasteurization level) or pasteurized milk (Robinson and Tamime, 2002).

Laban Rayeb is one of the fermented milks product consumed by different ages in Egypt and other countries, for its highly nutritive value and therapeutic properties (Sayed, 2012).

The genus Staphylococci includes over 30 species, with 18 of these species and subspecies are of potential hazard in food poisoning as they produce either coagulase, heat stable nuclease or enterotoxins. S. aureus subsp. aureus is coagulase positive and the most are common enterotoxigenic species (Le Loir *et al*., 2003 and Loncarevic *et al*., 2005). *S. aureus* is a common pathogen associated with serious community and hospital acquired diseases and has been considered for long as the important food- borne pathogen and made a major problem of public health (Pesavento *et al*., 2007 and Morandi *et al*., 2009).

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S. aureus is a desiccation tolerant organism as it has the ability to survive in dry and stressful environments and can grow in wide range of temperatures (7° to 48.5° C; optimum 30 to 37°C), pH (4.2 to 9.3; optimum 7 to 7.5), and sodium chloride concentration up to 15% NaCl. It is existing on human nose, skin and inanimate surfaces such as clothing and surfaces (Chaibenjawong and Foster, 2011).

S. aureus produces 15 enterotoxins (Atichou *et al*., 2004). The five classical enterotoxins (SE, type A, B, C, D and E) which queried for 95% of staphylococcal food poisoning (SFP) cases and the new types of SEs (SEG- SEO) responsible for other cases (Jay *et al*., 2005).

SEs are highly resistant to environmental conditions such as heat, freezing and drying (Le Loir *et al*., 2003). They are also resistant to lowered pH and proteolytic enzymes such as pepsin or trypsin that rendering them completely functional in the gastrointestinal tract after ingestion. They also belong to superantigens family, which subverted the immune system of the host by targeting the innate and adaptive responses (Argudín *et al*., 2010).

The invention of a large number of *S. aureus* in the food ($>10^6$ cfu/g or ml) is verificated that the isolated *S. aureus* strain is able to produce the enterotoxin (Najera-Sanchez *et al*., 2003). Dairy products with a level of enterotoxin as low as 0.5 ng /g or ml are frequently involved in SFP cases (Bergdoll, 1991 and Vernozy-Rozand *et al*., 2004).

SFP symptoms as nausea, abdominal cramps, diarrhea and a characteristic projectile vomiting are appeared within 1-6 h from ingestion of contaminated food, depending on individual susceptibility and ingested toxic dose and the clinical signs disappear within 24-48 h. Deaths occur rarely and specifically in the very young or elderly (Jay *et al*., 2005).

A concentration of 10^5 bacteria/g in foods is sufficient for toxin production and induction of disease (Kluytmans and Wertheim 2005, Paciorek *et al*., 2007 and Fooladi *et al*., 2010).

SEA and SEB consider the most FP agents (> 60%) in USA and England (Kluytmans and Wertheim 2005). As associated with human contamination (mainly food manipulators) and SEC and SED are associated with animal contamination (mainly cows and pigs) (Un Lee *et al*.,1998).

Enterotoxins have been detected by various methods but the PCR technique has the ability of detecting specific gene sequences by DNA amplification (Mclauchlin *et al*., 2000; Mehrotra *et al*., 2000; Sharma *et al*., 2000 and Omoe *et al*., 2002). PCR is much faster and can be applied to detect SEs in most kinds of food such as milk and cheese products irrespective of the available conventional techniques (compared with animal tests) (Shijia *et al*., 2016).

New SEs (SEG, SEH, SEI and SEJ) and its genes were reported through the 1990s, (Zschock *et al*., 2005). More recent data resulting from partial or complete genome sequence analyses have led to the description of further ‘‘new’’ se genes: sek, sel, sem, sen, seo, sep, seq, ser and seu. (Omoe *et al*., 2002 and Letetre *et al*., 2003). The role of these new SEs has not yet been explained in FPcases (Vernozy-Rozand *et al*., 2004 and Boerema *et al*., 2006).

Probiotics are known as live microorganisms that have alternative effect to antibiotics in the treatment of foodborne diseases as it provide a health benefit to the host when administered in adequate amounts. (FAO/WHO, 2001 and Sanders, 2003). It's important to analyze probiotics stability in their characteristics during manufacture and storage and to ensure that they are retained in different types of foods (Lee and Salminen, 1995 and Huis In’t Veld and Shortt, 1996).

Probiotics have a public health significance against many diseases such as pathological allergic including atopic eczema and rhinitis, diarrhoea, necrotizing enterocolitis inflammatory bowel disease and viral infection (Robles Alonso and Guarner, 2013). Also they have benefit effect in lactose digestion, control intestinal infections and balance the intestinal mucosal barrier, immunogenic effects, shortening the duration of diarrhea and other health effects (Salminen *et al*., 1998 and Pal and Jadhav, 2013).

Dairy products consider as probiotic carrier foods especially fermented milk products (Bergamini *et al*., 2005). Lactobacillus and Bifidobacterium genera are the most known probiotic microorganisms (Prasad *et al*., 2000).

Lactobacilli and *bifidobacteria* are recognized as a good example for health-promoting constituents of the microflora. *Lactobacilli* has a health importance in gastrointestinal disturbance as reduce constipation, infantile diarrhea, traveller's diarrhea, irritable bowel syndrome (IBS), lactose-intolerant individuals and resist infections such as Salmonellae. Bifidobacteria also has benefit effect as stimulate the immune system, inhibit pathogen growth, help to restore the normal flora after antibiotic therapy, produce B vitamins, reduce blood ammonia and blood cholesterol levels (Gibson, 2002).

PH is an important factor which can dramatically affect bacterial growth, Lactobacillus spp. as a

probiotic can tolerate a wide range of pH (1-9) and grow well at acidic pH 1-5 (Chowdhury *et al.*, 2012).

Food containing such LAB should contain at least 10^7 live microorganisms per g or ml at the time of consumption, in order to benefit the consumer (Ishibashi and Shimamura, 1993 and Hathout and Aly, 2010).

Probiotic bacteria possess antagonistic activity against numerous food-borne disease organisms such as S. aureus, Salmonella spp., E. coli, L. monocytogenes and Cl. perfringens (Millette *et al*., 2007). Lactobacillus acidophilus consider as one of the body's primary defense mechanisms against Candida as it has protection effect against pathogenic yeast infections (Mercenier *et al*., 2003). As well as, many researches indicated that L. acidophilus is the most popular species of probiotic bacteria produces substances that slow or prevents the growth of Candida (Yang, 2000 and Mohamed *et al*., 2010).

Lactic acid bacteria have antagonistic effect due to producing some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins (Quwehand and Vesterlund, 2004).

Therefore, this study was planned on the impact of probiotics on *S.aureus* growth in some dairy products.

MATERIALS AND METHODS

I-1- Collection and handling of the samples:
A total of 180 samples of yoghurt (small and large scale), soft white cheese (Tallaga and Feta) and rayeb milk (small and large scale) 30 of each were arbitrarily collected from different localities as dairy shopes and markets in Beni-Suef city, Egypt. The collected samples were delivered as soon as possible to the laboratory in an insulated ice-box and examined in the same day.

I-2-a- Enumeration and isolation of Staphylococcus aureus.
It was performed on Baird parker agar plate according to APHA, (1992).

1-2-b- Identification of the isolated Staphylococcus organisms.
Microscopical and biochemical examination were done according to Quinn *et al*. (2002).

1-3- Detection of enterotoxins genes of Staphylococcus aureus.
Nine pairs of primers have specific sequence were supplied for detection five enterotoxins genes of S.aureus as *Sea, Seb Sec Sed* and *See,* according to Mehrotra *et al*. (2000).

1-4- Impact of probiotic bacteria on the growth and survival of Staphylococcus aureus in vitro:

1-4-a- Bacterial strains:
The bacterial strains used in this study were *S.aureus* which obtained from this work after the identification by multiplex PCR, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* which were obtained from The Animal Health Research Institute (Dokii, Egypt).

1-4-b- Media used for growth of the bacteria:
Probiotic strains were propagated in de Man Rogosa and Sharpe (MRS) broth supplemented with 0.05% L-cysteine hydrochloride (Sigma, Buchs, Switzerland) at 37^0C for 24 h under an atmosphere of 5% CO_2 for L. acidophilus and anaerobically for *B.bifibum*. *S.aureus* strain was propagated in 10 ml of Brain–Heart Infusion broth (CM1135B, Oxoid) at 37^0C for 24 h.

1-4-c- Suspension inoculations for bacterial strains:
S.aureus counts were adjusted at concentration of 10^8cfu/ml, while probiotic strains were adjusted at concentration of 10^9cfu/ml.

1-4-d- Tallaga cheese manufacturing:
Cheese was prepared according to the method outlined by Abou-Donia (1986) as the sodium chloride was added at levels of 6% w/v and the samples were refrigerator storage and examined each two days**.**

1-4-e- Rayeb-milk manufacturing:
Rayeb-milk was prepared according to the method outlined by Sayed (2012) and Ramesh and Arun (2013) and the samples were refrigerator storage and examined each day.

1-4-f-Chemical analysis of manufactured products:
The pH of all cheese and rayeb samples was measured using (AD 111 digital pH meter 609) at 25±1 ^{o}C APHA (1974).

The salt percentage (sodium chloride content) in cheese samples was measured by the standered method described by APHA (1992).

1-4-g-Enumeration of probiotic strains was performed according to Souza and Saad (2009).

1-4-h- Enumeration of *S.aureus* was done according to Roberts and Greenwood (2003).

RESULT

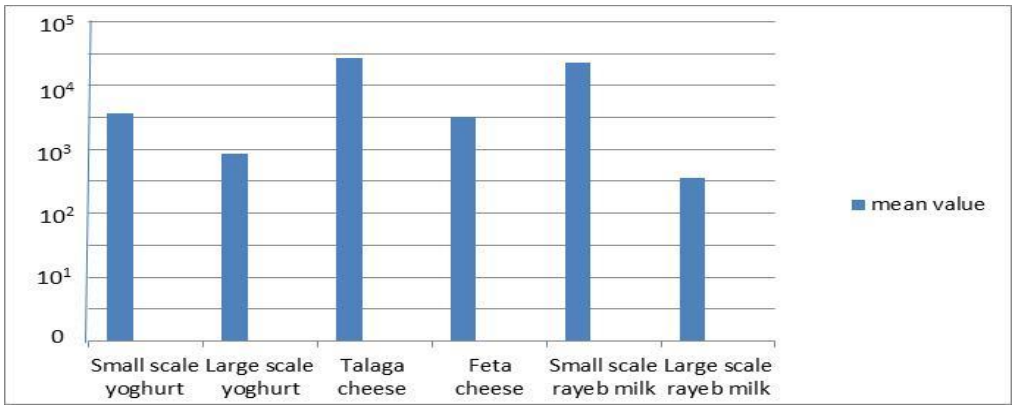

Figure 1: Statistical analytical results of the examined samples based on their S.aureus count /ml or g.

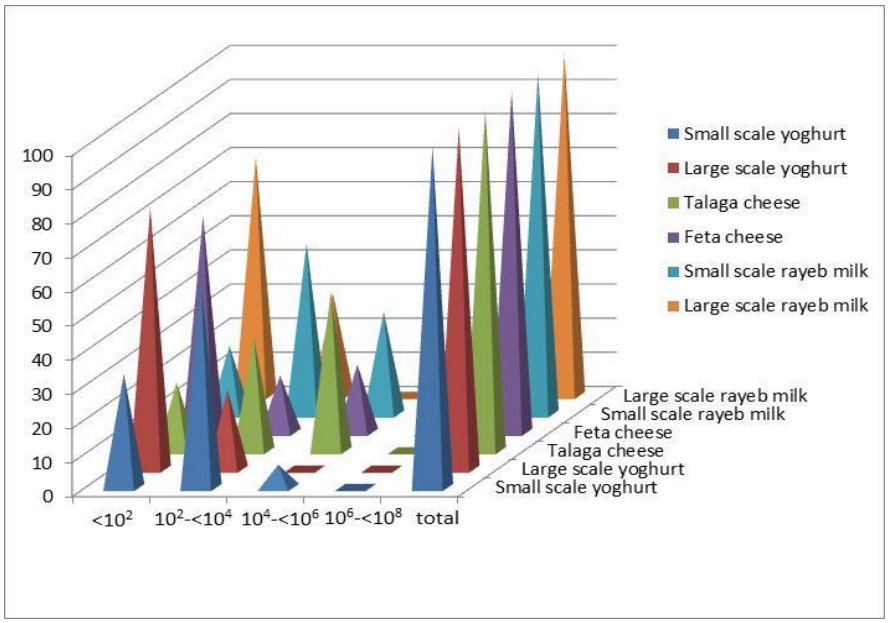

Figure 2: Frequency distribution of the examined samples based on their S.aureus count /ml or g.

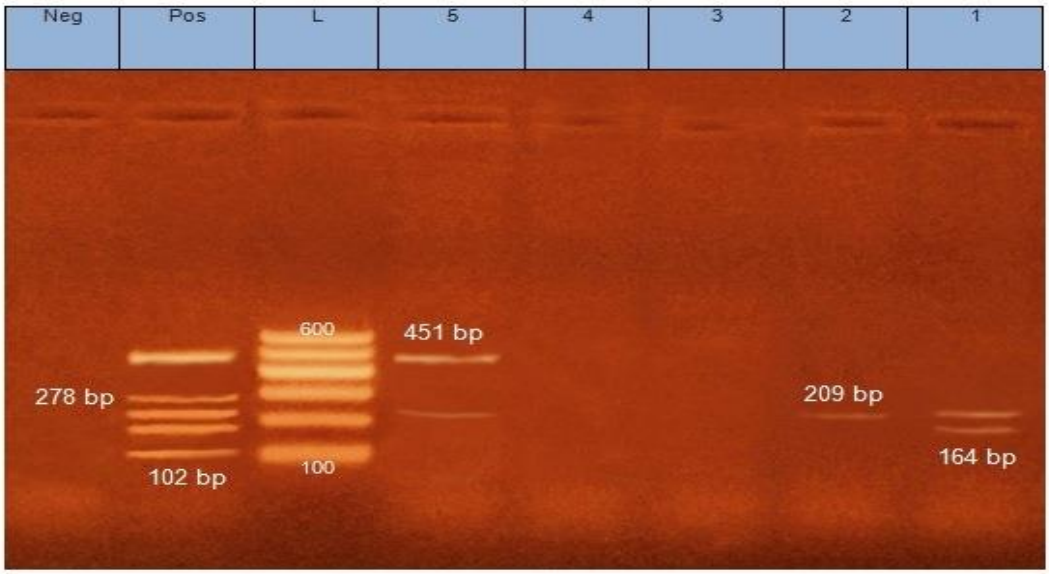

Figure 3: Incidence of enterotoxins genes of Staphylococcus aureus.

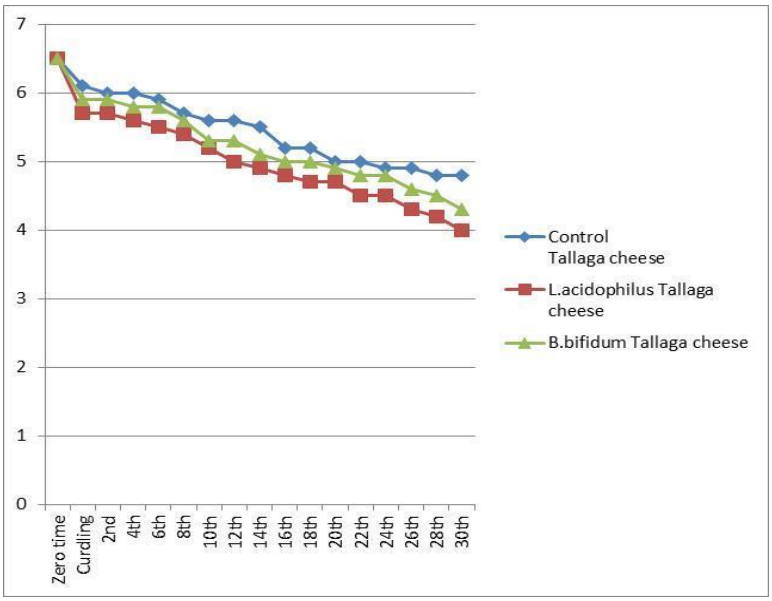

Figure 4: pH of control and probiotic Tallaga cheese during production and refrigerator storage.

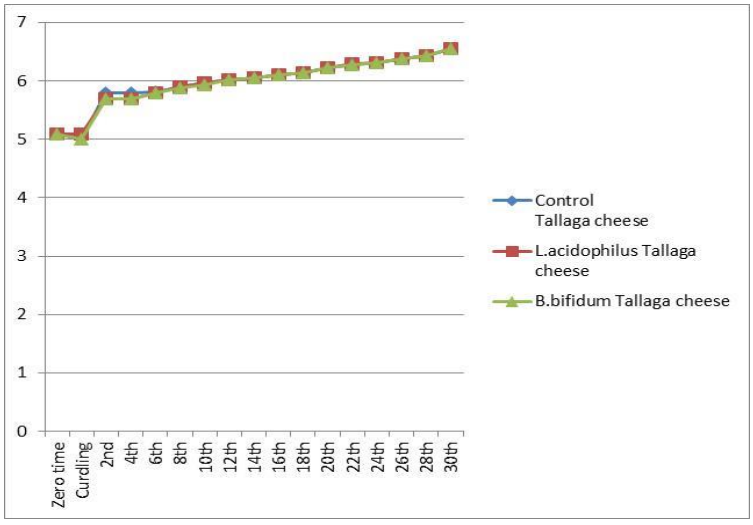

Figure 5: Salt content (NaCl %) of control and probiotic Tallaga cheese during production and refrigerator storage.

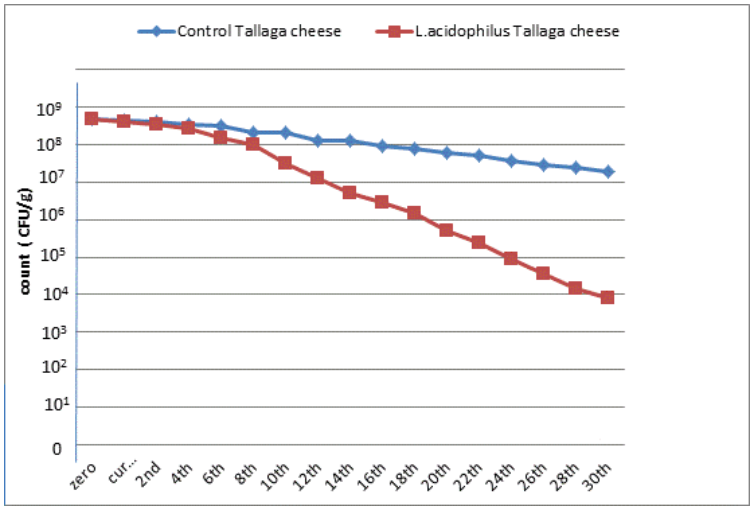

Figure 6: Impact of *L.acidophilus* on *S.aureus* organism in Tallaga cheese during production and refrigerator storage.

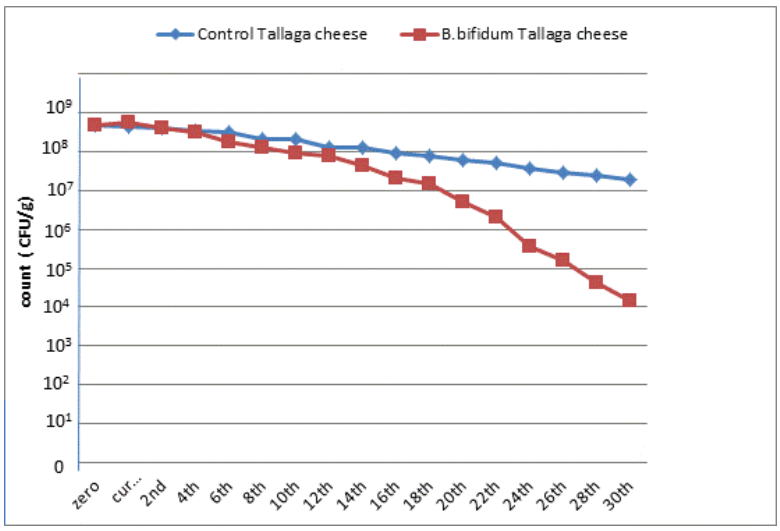

Figure 7: Impact of *B.bifidum* on *S.aureus* organism in Tallaga cheese during production and refrigerator storage.

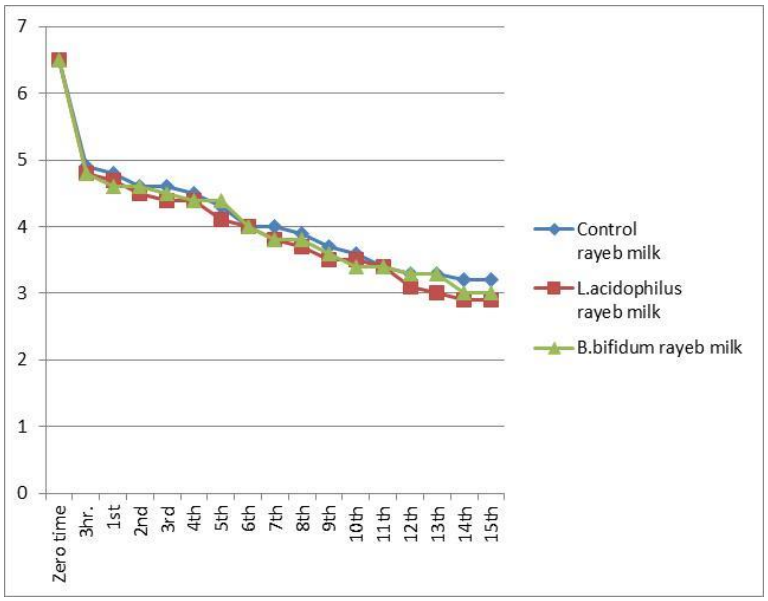

Figure 8: PH of control and probiotic rayeb milk during production.

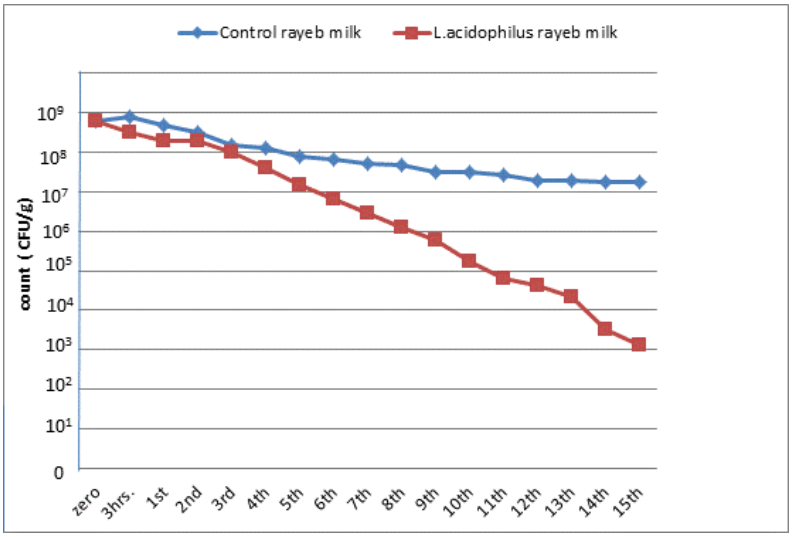

Figure 9: Impact of *L.acidophilus* on *S.aureus* count (growth) in rayeb milk during production and refrigerator storage.

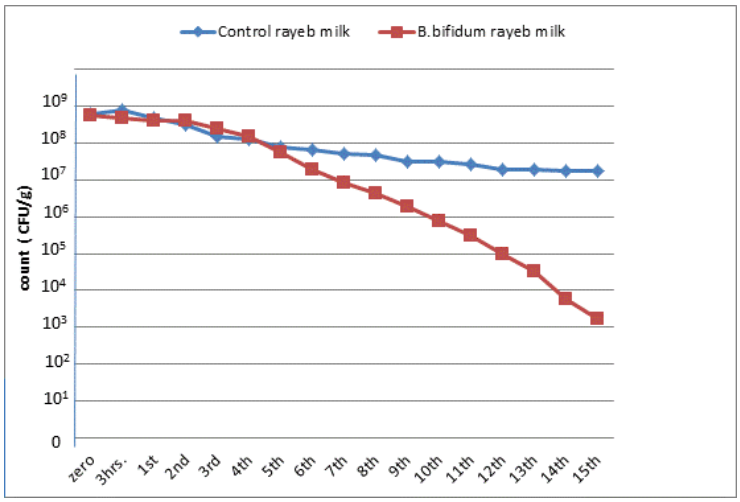

Figure 10: Impact of *B.bifidum* on *S.aureus* count (growth) in rayeb milk during production and refrigerator storage.

DISCUSSION

The high contamination rate of dairy products with S. aureus is mainly due to use of poor quality milk, environmental contamination as unclean hands of worker either suffering from diseased or apparent healthy carriers, unsanitary production and marketing practices (Araujo *et al.*, 2002).

In this study, it is obvious in Figure (1) that the occurrence of *S.aureus* organism was 66.7, 23.3, 80, 36.7, 80 and 30 % of the examined yoghurt (small and large scale), soft white cheese (Tallaga and Feta) and rayeb milk (small and large scale) samples with mean values of $5.7X10^3 \pm 3.2X10^3$, $9.3X10^2 \pm 3.9X10^2$, $4.3X10^4 \pm 2X10^4$, $5.1X10^3 \pm 1.7X10^3$, $3.6X10^4 \pm 1.3X10^4$ and $5.5X10^2 \pm 2.7X10^2$, cfu/ml or g., respectively. The highest frequency distribution 60, 76.7, 80, 63.3, 50 and 70 % lies within the ranges of 10^2-$<10^4$, $<10^2$, 10^2 - $<10^6$, $<10^2$, 10^2- $<10^4$ and $<10^2$, respectively Figure (2).

Several investigators had higher results of *S.aureus* in the small scale yoghurt samples as Oksuztepe *et al*. (2007) and Abou El-Makarem (2013).

Nearly similar counts of *S.aureus* in the large scale yoghurt samples were postulated by Abdel-Fattah (2007), while higher values were observed by Abou El-Makarem (2013) and Ahmed *et al*. (2014), but lower results were obtained by El-Bessery (2001).

Similarly, the counts of *S.aureus* in the Tallaga cheese samples were previously found by Aiad (2013), however lower results were reported by Ghada *et al*. (2004) and Amer *et al*. (2005).

The dropping of *S.aureus* counts in the Feta cheese samples were detected by Al-Tahiri (2005) and El Sayed *et al.* (2011).

The lower rate of contamination in the small scale rayeb milk samples were observed by Shawer (2013) and Tarekgne *et al*. (2015), but in the large scale rayeb milk lower count were demonstrated by El-Bessery (2001) and Shawer (2013).

In spite of pasteurization destroy *S. aureus* pathogen, thermostable SEs can resist heat treatment and still spirit with their biological activity which may cause severe health hazard (Balaban and Rasooly 2000). Also, the presence of less than 10^6 staphylococcal counts in the products doesn't help in enterotoxin production (Tatini, 1973 and Robinson, 2002).

The obtained results in Figure (3) regarded that 60% of the examined *S. aureus* strains were enterotoxigenic by PCR technique as carried one or two se- genes and *See* gene consider the highest frequency incidence 3 (60%) then Seb and Sec 1(20% of each) but the *Sea* and *Sed* could not be detected.

Higher values were recorded by Morandi *et al*. (2007) and Mathenge *et al*. (2015), but lower results were indicated by Arcuri *et al*. (2010) and Kav *et al*. (2011).

Probiotics are live organisms that produce benefits to health of the consumer or host when ingested through their ability to implantation on the intestinal microflora, resist gastric acidity and bile digestion (Sfakianakis and Tzia, 2014). Also, probiotics are added with concentrations of 10^7–10^8 cfu/gm or ml. as adjunct cultures to food and products, if no longer participating or participating in the fermentation, they able to reach a concentration of10^8-10^9 cfu/g or ml after the fermentation happened (Vinderola *et al.*, 2011).

The mechanism of lactic acid bacteria "LAB" in the controlling of microbial and pathogens growth is their production of lactic acid in addition to other antimicrobial compounds which exerted inhibition action on the growth of pathogens (Tadesse *et al.*, 2005).

Lactobacillus acidophilus had antagonistic actions against *Staphylococcus aureus* as it secretes hydrogen peroxides which partially responsible for antimicrobial interaction rather than the produced amounts of acid (Gilliand and Speck, 1977).

The result obtained in Figure (4) showed that after 30 days refrigerator storage control Tallaga cheese had pH value "4.8", *L.acidophilus* Tallaga had "4.0" and *B.bifidum* Tallaga cheese "4.3".

From the data presented in Figure (5) it is evident that the salt content (NaCl%) nearly similar in *L.acidophilus* Tallaga cheese, *B.bifidum* Tallaga cheese and control one during production and after 30 days refrigerator storage.

The results given in Figure (6) showed that *L.acidophilus* strain had inhibitory effect on *S.aureus* organism viability in Tallaga cheese as decreased its count from 6.7 $x10^8$ to $9.0x10^3$ cfu/g through a month storage in the refrigerator than control one that decreased from 6.7 $x10^8$ to $3.0x10^7$cfu/g and the results were in harmony with the results obtained by Arques *et al.* (2005) , Charlier *et al.* (2009) Amer (2011) and Abou El-Makarem (2013)., but did not agree with that postulated by Abd El-Gawad *et al.* (2014).

In Figure (7) it was found that *B.bifidum* strains had antimicrobial effect against *S.aureus* pathogen growth in Tallaga cheese as lowering its count from 6.8 $x10^8$to $1.6x10^4$ cfu/g through a month storage in the refrigerator than control one that decreased from 6.7 $x10^8$ to $3.0x10^7$ cfu/g and this agree with Arques *et al.* (2005), Charlier *et al.* (2009) and Abou El-Makarem (2013) .

The antimicrobial properties of yoghurt samples were mostly higher than these of the cell free and these activities may due to lactic acid production, decreasing in pH and also other antimicrobial compound that may be present in the yoghurt (Hassan *et al*., 2013).

The data illustrated in Figure (8) showed that after 15 days refrigerator storage control rayeb milk had PH values "3.2", *L.acidophilus* "2.9" and *B.bifidum* rayeb milk "3.0" and these result agree with that revealed Abd El-Gawad *et al.* (2014).

It is evident from the results which presented in Figure (9) that *L.acidophilus* strains had antibacterial effect on *S.aureus* organism viability in the rayeb milk as lowered its count from 7.8 $x10^8$to $1.0x10^3$cfu/ml through 15days storage in the refrigerator than control one that decreased from 8.0 $x10^8$to $2.5x10^7$cfu/ml and these data was nearly similar to that obtained by Arques *et al.* (2005) , Charlier *et al.* (2009), Kaboosi (2011) and Abou El-Makarem (2013), but did not agree with that revealed by Abd El-Gawad *et al.* (2014).

It is clear from the data showed in Figure (10) that *B.bifidum* strains had inhibitory effect against *S.aureus* growth in the rayeb milk as decreased its count from 7.7 $x10^8$to $2.1x10^3$cfu/ml. through 15days storage in the refrigerator than control one that decreased from 8.0 $x10^8$to $2.5x10^7$cfu/ml and the results were nearly similar to those recorded by Arques *et al.* (2005), Charlier *et al.* (2009), Kaboosi (2011) and Abou El-Makarem (2013).

Finally, it is clear the importance of probiotics to health of the consumers when ingested in the dairy products and we advise that all dairy products must be produced with addition of probiotics at a certain number to produce a good quality and safe guard dairy product.

CONCLUSION

It was concluded from this work that some of dairy products as yoghurt, soft cheese and rayeb milk sold in Beni-Suef city, Egypt were of bad quality as heavy microbial contaminated with S.aureus that rendering them threating to public health and may causes many diseases .

Also, the information which given in this work demonstrated that for improvement the microbiological quality of dairy products probiotics strains as *L.acidophilus* and *B.bifidum* may be used as it's have antagonistic affect against many species like *S.aureus* strain so, these confirm the health benefits derived from the human consumption of fermented dairy products.

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تأثير المعززات الحيوية على نمو المكور العنقودى الذهبى فى بعض المنتجات اللبنية

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تعتبرالألبان ومنتجاتها من أكثر الأغذية أهمية وذلك لما تحتويه من العناصر الغذائية الضرورية لبناء جسم الإنسان ووقايتة من كثير من الأمراض.

فى هذه الدراسه تم تسليط الضوء على موضوعين هامين :-

الجزء الاول: قد اشتمل على جمع ١٨٠ عينة من الزبادي (صغير وكبيرالانتاج) والجبن الطري (تلاجة وفيتا) واللبن الرايب (صغير وكبيرالانتاج) ٣٠ من كل منهما بشكل عشوائى من أماكن مختلفة مثل محلات الألبان والأسواق فى مدينة بني سويف وقراها ، مصر.

اظهرت النتائج ان متوسط قيمة الميكروب العنقودى الذهبي فى المنتجات على التوالى كانت ٧،٥ × ١٠^{٣} ±٣,٢× ١٠^{٣} و٩,٣×١٠^{٢} _+٣,٩× ١٠^{٢} و٤,٣×١٠^{٤} _+٢× ١٠^{٤}و ٥،١ × ١٠^{٣} _+١,٧× ١٠^{٣} و٣,٦× ١٠^{٤} _+١,٣×١٠^{٤}و٥,٥×١٠^{٢} _+٢,٧×١٠^{٢}/مللى او جرام.

كذلك ، بينت النتائج بان ٦٠٪ من سلالات المكورات العنقودية الذهبية التى تم فحصهاعن طريق تقنية انزيم البلمرة حملت واحد أو اثنين من جينات السموم المعوية.

الجزء الثانى: تم تقييم تأثير المعززات الحيوية ككائنات حية على نمو المكورالعنقودى الذهبى فى بعض منتجات الألبان مثل جبن التلاجة واللبن الرايب ضد الميكروب العنقودى الذهبى من خلال تقدير التغيرات الكيميائية والميكروبية اثناء تخزين المنتجات فى الثلاجة .

هذا وقد تم مناقشة النتائج منا .

الهدف من البحث : وقد لخص من هذا العمل إلى أن بعض منتجات الألبان مثل اللبن الزبادي والجبن الطري وحليب الرايب التي تباع في مدينة بني سويف بمصر كانت ذات جودة سيئة لانها كانت شديدة التلوث بالميكروبات مثل الميكروب العنقودى الذهبى مما يجعلها تهدد الصحة العامة وقد تتسبب في العديد من الأمراض. أيضا ، بينت النتائج التي وردت في هذا العمل أنه لتحسين جودة الميكروبيولوجية لمنتجات الألبان يمكن أن تستخدم بعض العترات من المعززات الحيوية مثل الاكتوبسليس اسيدوفلس والبيفيدوبكتريم بيفديم حيث انها لها تأثير عدائي ضد العديد من الأنواع مثل الميكروب العنقودى الذهبى ، وهذا يؤكد الفوائد الصحية المستمدة من الاستهلاك البشري لمنتجات الألبان المخمرة بالمعززات الحيوية.