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# Bio-control of *Pseudomonas fluorescens* in Domiati Cheese

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**Abstract**

Concerning the antimicrobial activities of some probiotics bacteria *Lactobacillus acidophilus* P109, *Lactobacillus plantarum* P164, *E. durans* P174 and *B. longum* CHRS using an agar well-diffusion as In Vitro assay against selected isolates of *Pseudomonas fluorescens*, *Bacillus cereus*, *Enterococcus fecalis* and *Staphylococcus aureus*, indicated that *B. longum* CHRS appeared to have antimicrobial activity against these isolates. *B. longum* CHRS was injected with and without *Ps. fluorescens* in Domiati cheese during manufacturing as In Vivo experiment, results revealed that this strain of probiotic bacteria was reduced the count of *Ps. fluorescens*, while the chemical composition showed reduce production of soluble nitrogen, which has relation with the decomposition of protein as well as led to reduced volatile fatty acids, which refers to the decomposition of fat as a result of antimicrobial activity of *B. longum* against *Ps. fluorescens*.

**Keywords:** *Ps. fluorescens*; Probiotic; Domiati cheese; Bio control

## Introduction

Lactic acid bacteria show broad spectrum antimicrobial activity against gram positive and gram negative bacteria, yeasts, moulds and protozoa, probably by inhibition of ribonucleotidreductase [1,2]. Lactic acid bacteria produce a variety of metabolic products that are capable of interfering with the growth of other microbes. These bacterial end products have been applied to food systems to prevent the growth of certain undesirable bacteria. The ability of lactic acid bacteria to produce antibacterial substances, which are active against certain pathogenic and spoilage organisms [3,4]. The antagonistic effects have been attributed to both the production of primary metabolites, such as lactic acid and hydrogen peroxide and the secretion of specific bacteriocins their activities are directed towards a wide range of organisms including those associated with food poisoning such as *Listeria*, *Clostridium* and *Bacillus species* [5]. There are different mechanisms of action for bacteriocins: alteration of enzymatic activity, inhibition of spore germination and inactivation of anionic carriers through the formation of selective and non-selective pores. Holzapfel et al. [6] stated that the metabolic products of lactic acid bacteria with antimicrobial properties are: organic acids (lactic acid and acetic acid), metabolites of oxygen ($H_2O_2$ and free radicals), enzymes (lacto peroxidase system with $H_2O_2$ and lysozyme), low molecular weight metabolites (CO2, reutrin, diacetyl, acetaldehyade) and bactrocines (nisin and others).

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and/or their antibacterial products [7]. Lactic acid bacteria have a major potential for use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods. The cell-free filtrate from *Lc. lactis subsp. lactis* AI 62 was contained approximately 350 ppm $H_2O_2$ for antimicrobial activity against *Enterococcus faecalis* and *S. aureus*, after 1 h of incubation at 30°C in the cell-free filtrate, the initial viable cell counts of the target bacteria (5.53-6.00 log cfu/mL) were reduced by 0.12-5.00 log units, except in the case of enterococci. The sensitivity varied with the bacterial species and pH. They concluded that $H_2O_2$ accumulated by lactic acid bacteria in a cell suspension is very effective in reducing the viable cell count of *S. aureus* [8].

The preservative ability of LAB in foods is attributed to the production of anti-microbial metabolites including organic acids and bacteriocins. Bacteriocins generally exert their anti-microbial action by interfering with the cell wall or the membrane of target organisms, either by inhibiting cell wall biosynthesis or causing pore formation, subsequently resulting in death. The incorporation of bacteriocins as a biopreservative has been shown to be effective in the control of pathogenic and spoilage microorganisms. However, a more practical and economic option of incorporating bacteriocins into foods can be the direct addition of bacteriocin-producing cultures into food. Eduardo et al. [9] pointed that probiotics were bactericidal for *S. aureus* and *P. aeruginosa*, but were inhibitory for *S. typhi*. They also inhibited the growth of *C. albicans*.

Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains of microorganism and are produced by both Gram-positive and Gram-negative bacteria. The bacteriocins from the Generally Recognized as Safe (GRAS) lactic acid bacteria have a great deal of attention to control pathogens in foods. Lactic acid bacteria are capable of producing substances, known as bacteriocin-like substances (BLS) [10]. An example of this class of molecule is reuterin, produced by some strains of Lactobacillus reuteri during anaerobic fermentation of glycerol. It is water-soluble, active over a wide range of pH values and resistant to proteolytic and lipolytic enzymes, so its being a suitable compound for food biopreservation. Kabak and Var [11] pointed that the lactic acid bacteria are of special interest as preservation organisms, since they have a long history of use in food and are generally regarded as safe organisms to reduce aflatoxin contamination in various food materials. Probiotic bacteria produce lactic and acetic acids as a

metabolic by-product which plays a complementary role in inhibiting pathogenic and spoilage bacteria [12].

This work aimed to control the growth of *Pseudomonas fluorescence* by using *B. longum* In Vitro and in laboratory prepared Domiati cheese.

## Materials and Methods

### Starter culture

Probiotics bacteria *Lactobacillus acidophilus* P109, *Lactobacillus plantarum* P164 and *E. durans* P174 were identify by Mahrous [13], was used in this study. The strains were isolated from, breast-feeding infant (15 days old) and selected as probiotic in previous studies. *B. longum* CHRS also used in this study. The strains were maintained on MRS-agar (E. Merck, Darmstadt, Germany) at 4-6°C. *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus fecalis* isolated from Domiati cheese. The strains were maintained on medium (nutrient broth, Oxoid).

### Antibacterial activity of used probiotics

Antibacterial activity was determined in agar well-diffusion assay against target organism as described in the previous work of Mahesh and Satish [14]. Plates were prepared by adding 2 ml(~105 CFU/ml) from an overnight culture of *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus fecalis* obtained during the study to 200 ml of plate count agar medium (PCA, Oxoid) held at 45°C. The agar was then immediately dispensed into round sterile 8.5 cm diameter Petri dishes and after solidification; wells (3 mm diameter) were made by removing the agar by a sterile metal borer. Subsequently, 30 µL of neutralized and filter-sterilized supernatants of culture obtained from overnight cultures of the tested strains include strains (*Lb. acidophilus* P106, *Lactobacillus plantarum* P164, *E. durans* P174 and *Bifidobacterium longum* CHRS, grown in MRS broth at 37°C, were dispensed in individual wells. The plates were incubated for 2 h at 4°C and subsequently overnight at 37°C after which the diameter of the inhibition zones was measured.

### Cheese manufacture

**Source and maintenance of culture:** *Pseudomonas fluorescens* was obtained during this study. It was maintained by subcultring on nutrient broth for 18 h at 30°C. The culture of *Bifidobacterium longum* CHRS was cultured twice on de Man, Rogosa and Sharpe (MRS) broth with 0.05% cysteine. Incubated for 18 h at 37°C under anaerobic condition. Both cultures were subcultured 3 times immediately before used in experiments.

**Preparation of culture:** The strains were statically grown for 18-20 h without agitation at 37°C to reach the early stationary phase. Bacteria were harvested by centrifugation (15,000 x g, 10 min), and washed twice with phosphate buffer saline (PBS; pH 7.2). The optical density of the bacterial suspensions at 600 nm was adjusted with PBS to 0.5 ± 0.02, giving approximately ($10^6$-$10^8$) CFU/ml.

**Inoculation of culture and preparation of white soft cheese:** Four batches of mixed raw cow's and buffalo's milk (ten liter each) were used in this study. All batches were pasteurized at 75°C for 15 second, warmed to 40°C and then calcium chloride (0.03% w/w) & rennet (liquid calf rennet, strength 1:6000) were added. Chesse was manufactured with some modification according to Abou-Donia [15]. 1st batch is control, 2nd batch added 2% of the culture of *Bifidobacterium longum* BL-CHRS ($10^6$-$10^8$), 3rd batch added 2% of both culture of *Pseudomonas fluorescens* and *Bifidobacterium longum* CHRS(1:1) and 4th added 2% culture of *Pseudomonas fluorescens*. All the batches were left to coagulate in 2-3 hr at 40°C. The curd was scooped and whey into molds, lined with coarse cloth (netting), to drain. The manufactured cheese was stored at 10°C in soldered tins, filled with boiled salted whey (10%) and analyzed when fresh and after 7,14, 21, 28, 35, 42 days of storage for chemical and microbiological examination.

### Chemical analysis

All samples were chemically examined for pH, total nitrogen (TN), soluble nitrogen (SN) and total volatile fatty acid (T.V.F.F.A). All analysis of cheese samples were performed in triplicate.

**pH:** The values for pH were determined by the potentiometric method [16].

**Total nitrogen (TN):** Was determined by Kjeldahl method AOAC [16]. Weigh accurately about 5 gm of sample and transfer to the Kjeldahl flask. Digest with sulphuric acid by using Copper sulphate as catalyst and potassium sulphate as boiling point elevator to release nitrogen from protein and retain nitrogen as ammonium salt. Concentrated NaOH is added to release ammonia which is absorbed in HCl and back titrated.

**Soluble nitrogen (SN):** Was determined according to AOAC [16]. Ten gram of cheese mixed with deionized water and homogenized by using stomacher (50 mints. At 40°C), after 1 mint the suspension was again homogenized for 1 mint. The homogenate was then held for 1 hr at 40°C. The samples were centrifuged at 3000 rpm for 30 mints at 4°C or centrifuged for 30 mints at 20°C and then cooled to 4°C. The suspension finally filtrated through glass wall. 12% trichloroacetic acid soluble nitrogen: 25 ml of WSN extract was added to 25 ml of 240 gm/kg trichloroacetic acid solution. The suspension was held at room temperature for 2 hr and then filtrated through whatman No. 40 filter paper. The nitrogen content was then determined using the Kjeldahl method.

**Total volatile fatty acid (T.V.F.F.A):** Total volatile fatty acid in cheese is estimated by direct distillation method, as described by Kosikowski [17], ten grams of cheese were placed in a mortar with 10% sulphuric acid until the cheese become a complete emulsion, which quanitiavely to 750 ml. Kjeldahl flask with 25 ml of 10% sulphoric acid. About 35 gm of magnesium sulphate were added to the flask contents, followed by few glass beads and 250 ml of distilled water exactly. The flask was fitted to Kjeldhahl distillation apparatus and then distilled. Distillation was terminated when 280 ml. of distillate were collected, and then titrated with 0.1 N sodium hydroxide. The inside tube of the condenser was washed with small quantity of neutral alcohol and then titrated by the same alkaline. The sum of the two titrations equals the total volatile acidity of the cheese.

### Microbiological examination

**Preparation of serial dilution [18]:** 11 g. of cheese sample was added to 99 ml of 2% Sod. Citrate solution in sterile bottles and thoroughly homogenized to prepare a dilution of 1/10 from which decimal dilutions were prepared using buffering peptone water.

**Total colony count [19]:** One ml from each dilution was transferred into duplicate sterile Petri dishes and mixed with about 15 ml of sterile

melted and cooled to 45°C Standard Plate Count Agar medium. After solidification, cultured plates as well as control one were incubated at 37 o C for 48 hrs in an inverted position. Plates with a range of 30 to 300 colonies were counted. Results were calculated and recorded as a total aerobic plate count/ml.

**Enumeration of *Pseudomonas fluorescens* [20]:** 0.1 ml from each previously prepared dilution of samples under investigation was transferred and evenly distributed over a dry surface of Asparagine agar medium by a bent glass rod. Inoculated plates were incubated in an inverted position at 30°C for 48 hours. The colonies which showed fluorescence were enumerated as Pseudomonas fluorescens. The number per gram was calculated and recorded.

**Enumeration of *Bifidobacterium longum* [21]:** One ml. quantities from each of previously prepared dilutions were plated in duplicates Petri-dishes and thoroughly mixed with about 15 ml of melted and cooled to 45 o C of de Man, Rogosa and Sharpe (MRS) agar (Biokar, Diagnostics, France) containing 0.25% L-cysteine and incubated at 37°C for 48 h in an anaerobic chamber (MAC500; Down Whitley Scientific, West Yorkshire, UK) containing an atmosphere of 85% N2 , 10% H2 and 5% CO2.

### Statistical analysis

Results from the facial hedonic scale record sheets were collated and input into SPSS version 15 database, mean, standard deviations and p-values were calculated for each sample. P-values less than 0.05 were considered statistically significant.

## Results and Discussion

### Antimicrobial activities of some probiotics bacteria on some isolated strains

Table 1 showed that *Lactobacillus plantarum* P164 and *B. longum* CHRS were effective to inhibit the growth of *Pseudomonas fluorescens*. *Staphylococcus aureus* was inhibited by *Lactobacillus acidophilus* P109, *E. durans* P174 and *B. longum* CHRS. *Bacillus cereus* was inhibited by *Lactobacillus plantarum* P164, *E. durans* P174 and *B. longum* CHRS. While *Enterococcus fecalis* was inhibited by *Lactobacillus acidophilus* P109 and *B. longum* CHRS.

| Probiotics bacteria Isolated strains | *Lactobacillus acidophilus* P109 | *Lactobacillus plantarum* P164 | *E. durans* P174 | *B. longum* CHRS |
|---|---|---|---|---|
| *Ps. fluorescens* | 0 | 5 ± 0.15 | 0 | 10 ± 0.1 |
| *S. aureus* | 2 ± 0.5 | 0 | 3 ± 0.2 | 5 ± 0.01 |
| *B. cereus* | 0 | 2 ± 0.3 | 2 ± 0.1 | 4 ± 0.4 |
| *E. fecalis* | 5 ± 0.1 | 0 | 0 | 3 ± 0.3 |

**Table 1**: Antibacterial activities of some probiotics bacteria on some isolated strains (Diameter of the inhibition zone (mm)).

Osman and shatta [22] examined 62 *Lactobacillus* species isolated from milk products and 15 other *Lactobacillus* species for their antimicrobial activity against 30 target bacteria (18 isolates of *S. aureus*, 5 of *E. coli*, 1 each of *Ent. Aerogenes*, *Micrococcus variance*, *B. subtilis* and *B. mycoides* and 3 *B. cereus*). They found that no one of *Lactobacillus* species completely inhibited growth of target bacteria and they reported that the acid accumulation by Lactobacillus species played an important role in inhibition of target bacteria. Yang et al. [23] reported that thirteen *Lactobacillus* species were shown to produce an antimicrobial agent, 2-pyrolidone-5 carboxilic acids (PCA). It inhibited many spoilage bacteria particularly *E. cloacae* 1575, *Ps. fluorescence* KJLG and *Ps. putida* 1560-2. The antimicrobial of PCA did not change at high temperature. However, the activity was destroyed rapidly by neutralization with Ammonium hydroxide. PCA showed slightly lower antimicrobial activity than lactic acid bacteria. O'Riordan and Fitzgerald [24] examined twelve strains of Bifidobacteria which exhibited a broad spectrum of antagonistic activity against both Gram-positive and Gram-negative indicators, especially Pseudomonas species, using deferred antagonism spot plate assays. Inhibitory action was shown to be unrelated to hydrogen peroxide production and not solely dependent on acidity. However, attempts to detect inhibitory activity in cell-free supernatant fluids from these strains were unsuccessful.

Nearly similar results obtained by Eduardo et al., [9], Røssland et al., [25], Zinedine and Faid [21], Lengkey and Adriani [26] and Kives et al. [27]. The degree of inhibition of psychrotrophs depends on amount of bacteriocin produced by the lactic acid bacteria [28].

### Effect of *B. longum* CHRS on the growth of *Ps. fluorescens* in excrementally manufactured Domiati chesse

For studying the impact of *B. longum* CHRS on the growth of *Ps. fluroscence* in the Domiati cheese, added *B. longum* CHRS and *Ps. fluroscence* both individually to follow up their ability to grow in Domiati cheese and added the two strain together to determine the antibacterial effect of *B. longum* CHRS on *Ps. fluroscence* and the control Domiati cheese without any bacteria.

### Microbiological effect

Counts of *Ps. fluroscence* and *B. longum* in Domiati cheese during storage period were illustrated in Table 2. It was noted that the presence of *Ps. fluroscence* alone happened increase in growth clearly it reached the maximum during the period 28-35 days and then began to decrease at the end of storage period. Our study in the case of addition *B. longum* CHRS observed that the growth of *Ps. fluroscence* has been controlled and happened a slight increase in growth during the early stages of manufacture, then there almost constant in their growth during the period 21-28 days and then growth began to decrease, indicating that the addition of the *B. longum* CHRS affect the growth of *Ps. fluroscence* if contamination has occurred to the milk or through the manufacturing. It is generally believed that antagonism by bifidobacteria results primarily from the acetic and lactic acids produced from the metabolism of glucose. However, several reports have demonstrated that specific antimicrobial compounds are elaborated by members of this genus, including 'bifidin' and 'bifilong' produced by Bifidobacterum and *Bif. longum* strains, respectively [29,30]. In addition, Gibson and Wang [4] provided additional evidence that acidity may not be the sole mechanism of inhibition. Concerning the control cheese which not contain *Ps. fluroscence* and *B. longum* CHRS, there is increasing in total colony count (TCC) throughout the storage period, this increasing in TCC can be explained by the sufficient change in the environmental condition which happen during cheese storage and allow the growth and multiplication of microorganisms [31] Results obtained showed the

potential advantages of using the above probiotic strain to produce safe and healthy cheese. Kives et al. [26] stated that *Lactococcus lactis ssp*. cremoris was effective for reducing growth of *Ps. fluorescens*. Chapman et al. [32] mentioned that commercial interest in functional food containing probiotic strains has consistently increased due to the awareness of the benefits for gut health, disease prevention and therapy. However, this explains the reason for a rising interest in probiotic health based products. Effat et al. [33] demonstrate that combination among dextrin or litesse as prebiotics with *Lactobacillus* strains (*Lb. hilgardii* NRRL B-1843, *Lb. johnsonii* NRRL B-2178 and *Lb. curvatus* NBIMCC-3452) as probiotics can be used for manufacturing functional white soft cheeses with high quality and with potential health benefits. Zinedine and Faid [21] pointed that *B. longum* were able to inhibit the growth of pathogenic bacteria and the antibacterial compounds produced could be identified as bacteriocins. Mélika et al. [28] stated that *L. lactis* is a bacteriocin producer, specifically of Nisin that is used as a natural preservative in some foods. Cagrili [34] mentioned that lactobacilli produce substances that inhibit the growth of pathogens in vitro and in vivo. Lactobacillus dietary supplement alleviation intestinal infection in the Gl tract in both humans and animal among the various by-products formed during lactobacilli growth, are certain substances, such as hydrogen peroxide, Lactolin, Lactocidin and Acidolin. Lengkey and Adriani [26] stated that *Bifidobacterium spp*. and *Lactobacillus acidophilus*, showed sensitivity reaction on *Ps. aeruginosa* and *S. aureus*, but *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed sensitivity reaction only to *Staphylococcus aureus*, but no sensitivity to *Ps. aeruginosa*; because *Bifidobacterium spp*. and *Lactobacillus acidophilus* has the ability as bacteriocin. Tharmaraj and Shah [35] Stated that the inhibitory affect of all probiotic bacteria was weakest against *E. coli* and strongest against *B. cereus*. *S. aureus* was inhibited to a greater extend by *B. animalis* and by *L. rhamnosus*. They found varying quantities of organic acids (acetic, lactic, formic, propionic, butyric, benzoic and phenyllactic) which were responsible for the inhibition. Deeb, Azza, and Ahmed [36] recorded that ten batches of white soft cheese were prepared from cow's milk containing 5% Sodium chloride and inoculated with *Ps. fluorescence*, the first batch was a control (containing no probiotics bacteria or potassium sorbate), the following four batches (2-5) containing different concentration of potassium sorbate (0.02, 0.05, 0.1 and 0.2%). The sixth batch contains *Bif. Longum*, the rest four batches (7-10) containing both potassium sorbate at different concentration 0.02, 0.05, 0.1 and 0.2% and *Bif. longum*. The cheese batches were examined physically and bacteriologically at zero time, after 3, 9, 12, 18, 21, 27 and 30 days. The reduction percent of Ps. fluorescence at the end of storage period (30 days) were 93.6, 94.6, 97.78 and 99.5 for cheese containing potassium sorbate at different concentration.02, 0.05, 0.1 and 0.2%, respectively. While the reduction percent in case of cheese containing *Bifidobacterium longum* only was 96.25%. But in case of combined addition potassium sorbate and *Bif. longum* the reduction percent were 96.39, 97.6, 99.4 and 99.4% for 0.02, 0.05, 0.1 and 0.2% added potassium sorbate and *Bifidobacterium longum*, respectively. In the same time, in control batch, the reduction percent of pseudomonas count at the end of storage period (30 days) was 87.50%. Sorbate above 0.1% although highly effective , cause unobjectionable sweet flavour, a condition that acts is an effective check against excessive use of the preservative. In conclusion the combined addition of potassium sorbate at concentration of 0.1% and Bif. longum had great inhibitory effect upon existing micro organisms and also improved organoleptic quality of cheese. Juffs and Babel [37] mentioned that certain commercial multi-strain cultures (lactic acid-producing *streptococci* plus *Leuconostoc cremoris*) were the most effective in restricting psychrotrophic growth. The degree of inhibition varied with the lactic culture, the initial population of psychrotrophs, the psychrotroph culture, storage temperature and time. Inhibition due to lactic culture was decreased by addition of catalase, suggesting that hydrogen peroxide was the inhibitor.

| Control | *Ps. fluroscence & B. longum* | | *B. longum* | *Ps. fluroscence* | Time |
|---|---|---|---|---|---|
| | *B. longum* | *Ps. fluroscence* | | | |
| $1.46 \times 10^4 \pm 0.12 \times 10^{4bc}$ | $3 \times 10^3 \pm 0.58 \times 10^{3c}$ | $1.1 \times 10^4 \pm 0.06 \times 10^{4b}$ | $5.3 \times 10^3 \pm 0.88 \times 10^{3bc}$ | $2.2 \times 10^4 \pm 0.05 \times 10^{4c}$ | Fresh |
| $3.97 \times 10^5 \pm 0.01 \times 10^{5c}$ | $4.7 \times 10^3 \pm 0.67 \times 10^{3c}$ | $2.3 \times 10^4 \pm 0.88 \times 10^{4b}$ | $8.0 \times 10^3 \pm 0.58 \times 10^{3b}$ | $8.0 \times 10^4 \pm 0.58 \times 10^{4bc}$ | 7 days |
| $1.16 \times 10^7 \pm 0.09 \times 10^{7b}$ | $1.5 \times 10^4 \pm 0.09 \times 10^{4b}$ | $2.4 \times 10^3 \pm 0.09 \times 10^{4b}$ | $1.1 \times 10^3 \pm 0.09 \times 10^{4a}$ | $5.3 \times 10^5 \pm 0.88 \times 10^{5b}$ | 14 days |
| $5.33 \times 10^7 \pm 1.7 \times 10^{7ab}$ | $4.0 \times 10^4 \pm 0.58 \times 10^{4ab}$ | $3.5 \times 10^4 \pm 0.09 \times 10^{4a}$ | $1.5 \times 106 \pm 0.09 \times 10^{4a}$ | $1.2 \times 106 \pm 0.09 \times 106^{b}$ | 21 days |
| $2.00 \times 108 \pm 0.57 \times 108^{a}$ | $6.7 \times 10^4 \pm 0.33 \times 10^{4a}$ | $3.0 \times 10^4 \pm 0.58 \times 10^{4a}$ | $2.2 \times 106 \pm 0.09 \times 10^{4b}$ | $2.0 \times 10^5 \pm 0.58 \times 10^{7a}$ | 27 days |
| $2.45 \times 108 \pm 0.05 \times 108^{a}$ | $9.4 \times 10^4 \pm 0.033 \times 10^{4a}$ | $4.5 \times 10^3 \pm 0.03 \times 10^{3c}$ | $1.5 \times 10^4 \pm 0.09 \times 10^{4a}$ | $4.0 \times 10^7 \pm 0.58 \times 10^{7a}$ | 34 days |
| $1.3 \times 109 \pm 0.12 \times 109^{b}$ | $2.3 \times 10^4 \pm 0.33 \times 10^{4b}$ | $2.3 \times 10^3 \pm 0.33 \times 10^{3c}$ | $2.3 \times 102 \pm 0.33 \times 10^{3c}$ | $1.4 \times 10^7 \pm 0.06 \times 10^{7b}$ | 41 days |
| a.b. means in the same column that bearing different superscripts are significantly at (P<0.05). | | | | | |

**Table 2**: Effect of *B. longum* CHRS on the growth of *Ps. fluorescence* in excrementally manufactured soft cheese.

## Chemical effect

Domiati cheese samples were analyzed for pH, total volatile fatty acids and soluble nitrogen. Results in Table 3 indicated that the pH of the cheese show a slightly decreasing trend till the end of the storage period in all samples especially in the case of addition *Ps. fluroscence* only while in the case of addition *Ps. fluroscence*+*B. longum* the decrease in pH was less. The highest value was obtained at the beginning of the storage period, while the lowest at the end of the storage period. Wahba and El-Abbassy [38] reported that, the pH values progressively decreased during storage with a pronounced drop in the first month. The obtained results are in agree with those obtained by Magdoub et al. [39] they reported that the decrease in pH values may be due to the convert of residual lactose in cheese to lactic

acid and free fatty acid which had developed in the cheese at the end of storage period. Besides, Fooks et al. (1999) reported that the decrease in pH values may be due to short chain fatty acids which produced in varying quantities as metabolic end product of the probiotic bacteria. This might be attributed to the fact that storage tends to increase lactose fermentation which lead to a decrease in pH value.

| Days of storage | *Ps fluroscence* | | | | *B. longum* | | | | *Ps. fluroscence+B. longum* | | | | **Control** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | TVFA | SN (%) | DPI | pH | TVFA | SN (%) | DPI | pH | TVFA | SN (%) | DPI | pH | TVFA | SN (%) | DPI |
| Fresh | 6.85 ± 0.1 | 2.5 ± 0.1 | 0.070 ± 0.1 | 2.59 ± 0.1 | 6.85 ± 0.1 | 2.5 ± 0.1 | 0.070 ± 0.1 | 2.59 ± 0.1 | 6.85 ± 0.1 | 2.5 ± 0.1 | 0.070 ± 0.1 | 2.59 ± 0.1 | 6.85 ± 0.1 | 2.5 ± 0.1 | 0.070 ± 0.1 | 2.59 ± 0.1 |
| 14 days | 7.20 ± 0.1 | 3.1 ± 0.1 | 0.0995 ± 0.1 | 3.68 ± 0.1 | 6.81 ± 0.1 | 4.1 ± 0.1 | 0.1305 ± 0.1 | 4.83 ± 0.1 | 6.93 ± 0.1 | 2.8 ± 0.1 | 0.090 ± 0.1 | 3.33 ± 0.1 | 6.78 ± 0.1 | 2.7 ± 0.1 | 0.082 ± 0.1 | 3.03 ± 0.1 |
| 42 days | 7.01 ± 0.1 | 14.9 ± 0.1 | 0.207 ± 0.1 | 7.66 ± 0.1 | 6.97 ± 0.1 | 4.8 ± 0.1 | 0.1905 ± 0.1 | 7 ± 0.1 | 6.85 ± 0.1 | 5.6 ± 0.1 | 0.101 ± 0.1 | 3.74 ± 0.1 | 6.29 ± 0.1 | 3.0 ± 0.1 | 0.093 ± 0.1 | 3.44 ± 0.1 |

**Table 3:** Impact of *B. longum* on the chemical parameters of experimentally manufactured Domiati cheese.

Regarding to the effect of adding *B. longum* CHRS it was noticed that the total volatile fatty acids contents average ranged between 2.5 mg/100 gm in the fresh cheese to 14.9 mg/100 gm in cheese treatment with *Ps. fluroscence* only while, there were slightly increase in the TVFA in the treatment *Ps. fluroscence+B. longum* CHRS (5.6 mg/100 gm) at the end of storage period this may due to the antimicrobial effect of *B. longum* CHRS against *Ps. fluroscence*. As shown in Table 3, the soluble nitrogen (SN) of Domiati cheese slightly increased in the treatment with *Ps. fluroscence+B. longum* CHRS, while there was significant increase in the treatment with *Ps. fluroscence* only. These results coincide with those obtained by Elewa et al., [40] who reported that the SN contents of white soft cheeses made with probiotics show an increase at the end of storage period. Concerning depth proteolysis index (DPI) which reflect protein decomposition as percent to which the large peptides are degraded into smaller molecules are high in case of cheese with *Ps. fluroscence* and cheese with *B. longum* CHRS as a result to proteolytic activities of both organisms. While cheese that treated with *Ps. fluroscence* and *B. longum* CHRS were low as a result of antimicrobial activity of *B. longum* CHRS. Cornering the control cheese which not contain any *Ps. fluroscence* and *B. longum* CHRS show lowering in TVFA, SN and DPI this indicate that the control cheese dose not contaminate with any lipolytic or proteolytic organisms.

## Conclusion

Raw milk should be stored at 2°C before processing into cheese. Implementing good hygienic practice during milk production at farm level and during cheese manufacture is very important before implementing pasteurization in order to produce milk with quality suitable for pasteurizations. Hygienic quality of raw milk on arrival in the cheese factory. Implementation of HACCP system to ensure the hygienic quality of milk and cheese. The conditions of storage and transportation of the retail product should ensure that quality is maintained. In this respect refrigerated storage and handling at retail points is important to complete the value chain from farm to the table. Addition of Nisin is recommended at concentration of 150 IU/ ml to inactivate *S. aureus* and B. cereus in soft cheese. Addition of *B. longum* is recommended to inactivate *Ps. fluorescens* in soft cheese.

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