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# Fate of enterotoxigenic *Staphylococcus aureus* and staphylococcal enterotoxins in Feta and Galotyri cheeses

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In this study the fate of enterotoxigenic *Staphylococcus aureus* and staphylococcal enterotoxins in Feta and Galotyri cheeses were studied. Initially, the enterotoxigenic abilities of four *Staph. aureus* LHA, LHB, LHC and LHD strains isolated from raw ovine milk were examined in both BHI broth and ovine milk. In BHI broth, the *Staph. aureus* LHA, LHB, LHC and LHD strains were found toxigenic at 37 °C producing the staphylococcal enterotoxins (SEs) serotypes SEA, SEB, SEC and SED, respectively, whereas in ovine milk at 37 °C, *Staph. aureus* LHD was found to produce only SED, while no SE production was observed for the other examined strains. Thus, the fate of only *Staph. aureus* LHD and SED were examined in Feta and Galotyri cheeses. The cheeses were made from raw ovine toxic milk with preformed SED or raw ovine milk contaminated with high (ca 6 log cfu/ml) and low inocula (ca 3 log cfu/ml) of *Staph. aureus* LHD. Results showed that the pathogen was eliminated at slower rate in Galotyri cheese than in Feta cheese, for the high (5 d vs. 16 d) or the low (1 d vs. 12 d) inoculum trials. In both cheeses produced from the toxic milk, SED was detected during manufacturing and storage. SED was also detected in the curd (2 h), when *Staph. aureus* LHD populations had reached ca 7 log cfu/g, and up to the end of storage for the high inoculum trials of both cheeses. No SED was observed for the low inoculum trials of either cheese.

**Keywords:** Cheeses, Feta, Galotyri, staphylococcal enterotoxins, *Staphylococcus aureus*.

## Introduction

*Staphylococcus aureus* is an opportunistic Gram positive pathogen and the causative agent of many diseases ranging from skin lesions to septicaemia or meningitis. Certain strains of *Staph. aureus* can produce staphylococcal enterotoxins (SEs) in foods and cause staphylococcal food poisonings (SFP). SEs are formed in foods during growth of *Staph. aureus*. The symptoms of SFP, abdominal cramps, nausea, vomiting and diarrhoea, develop 2–4 h after food intake and their seriousness depends on individual health status. To date, 21 antigenic serotypes of SEs have been described. Among SEs, the classical serotypes SEA, SEB, SEC and SED are most often isolated in SFP outbreaks (Balaban & Rasooly, 2000). The occurrence of *Staph. aureus* strains producing SEs in raw milk or cheeses has been reported in many countries (Hein et al. 2005; Cremonesi et al. 2007; El-Sharoud & Spano, 2008). SFP outbreaks due to consumption of cheese products have also been reported worldwide (Balaban & Rasooly, 2000), and in Greece as well (Jelastopulu et al. 2006).

Feta and Galotyri are two traditional Greek cheeses. Feta cheese is made from ovine milk or mixtures of ovine and caprine milk, with the latter not exceeding 30%. Galotyri cheese is also made from ovine or caprine milk or mixtures of both. According to European Union regulation 1107/96, Feta and Galotyri cheeses are designated as cheeses of Protected Denomination of Origin (PDO).

The fate of *Staph. aureus* and the production of SEs have been studied in various cheese types during manufacturing, ripening and storage (Meyrand et al. 1998; Vernozy-Rozand et al. 1998; Hamama et al. 2002; Rilla et al. 2004, Delbes et al. 2006; El-Sharoud & Spano, 2008). The results obtained have varied due to various factors such as cheese characteristics, starters or *Staph. aureus* strains. For this

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reason, no generalisations should be made about the fate of *Staph. aureus* in different cheese products (Vernozy-Rozand et al. 1998). Besides, the toxigenic ability of *Staph. aureus* strains, isolated from raw ovine milk, have not been yet investigated in cheeses produced from ovine milk.

Feta and Galotyri are two different cheese types with different starter cultures, pH, and moisture and may offer the opportunity to investigate the effects of different competitions in the cheese ecology for the growth of food-borne pathogens like *Staph. aureus*. In addition, Feta cheese requires a maturation step of at least 2 months, while Galotyri cheese may be consumed fresh. Therefore, these cheeses are good models for studying *Staph. aureus* survival through cheese making process and subsequent storage. The aim of present work was to investigate the fate of enterotoxigenic *Staph. aureus* strains isolated from raw ovine milk, as well as the fate of produced SEs in Feta or Galotyri cheeses during manufacturing, ripening and storage.

## Materials and Methods

### *Bacterial strains*

*Staph. aureus* LHA, LHB, LHC and LHD strains from our Laboratory stock were used. All *Staph. aureus* strains were isolated from raw ovine milk produced in local farms. Each of the four *Staph. aureus* strains was toxigenic by producing a different type of SE. The *Staph. aureus* LHA, LHB, LHC and LHD strains were chosen, since they were toxigenic by producing the most common classical SEs (Balaban & Rasooly, 2000) of SEA, SEB, SEC and SED, respectively. The toxigenic ability of *Staph. aureus* strains was examined, as described below.

Each strain was grown separately in 50 ml Brain Heart Infusion broth (BHI) (Oxoid, Basingstoke, UK) for 24 h at 37 °C, with two consecutive transfers. The bacterial cells were pelleted by centrifugation at 5000 ***g*** for 15 min at 5 °C and washed twice with 10 ml 0·1 M phosphate buffer saline (PBS) (Oxoid), pH 7·0, and diluted to $1{\cdot}0 \times 10^8$ cfu/ml in PBS. Cell counts were determined by serial dilution and subsequent enumeration on Baird-Parker agar (Merck, Darmstadt, Germany) supplemented with egg yolk tellurite emulsion (BPA + EYT), (Merck). The final suspension was serially diluted in PBS for the preparation of two levels of inocula. A low (ca. $10^3$ cfu/ml) or a high (ca. $10^6$ cfu/ml) level inoculum of each strain was used for the low or high contamination trials of the milk or cheese, respectively.

A mesophilic starter culture (lyophilized type R 704, Chr. Hansen A/S, Horlom, Denmark) of a mixture of lactic acid strains (*Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris,* 1:1) was used for the manufacture of Feta cheese. A thermophilic starter culture (lyophilized type CH-1, Chr. Hansen) of a mixture of lactic acid strains (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus,* 1:1) was used for the manufacture of Galotyri cheese. The starter cultures were prepared according to manufacturer's instructions.

### *Ovine milk*

Raw ovine milk with total viable counts (TVC) of ca 5·1 log cfu/ml from a selected local farm was used for the initial screening tests of SEs production of our *Staph. aureus* strains, as well as the manufacture of Feta and Galotyri cheeses. The absence of *Staph. aureus* in the raw ovine milk was verified by microbiological analysis, as described below.

### *Detection of toxigenic ability of* Staph. aureus *strains in* in-vitro *tests and ovine milk*

*Staph. aureus* LHA, LHB, LHC and LHD strains were examined for toxigenic ability during their growth in BHI broth and in raw or pasteurized (65 °C for 30 min) ovine milk, for the *in-vitro* and ovine milk tests, respectively. Each *Staph. aureus* strain was inoculated (ca 3 log cfu/ml) in BHI and raw or pasteurized ovine milk and grown at 37 °C, under static conditions. Analysis for the presence of SEs was made, and verified by PCR, as described below. The toxigenic *Staph. aureus* strains, which proved to produce SE in ovine milk, were used for the contamination trials in Feta or Galotyri cheeses.

### *Detection of* Staph. aureus *virulence genes by PCR*

The *Staph. aureus* LHA, LHB, LHC and LHD strains were screened by PCR for the presence of *sea, seb, sec* and *sed* genes, encoding the production of SEA, SEB, SEC and SED, respectively. Analysis for presence of the SEs genes was carried out by using previously published primer sequences for *sea* (Tsen & Chen, 1992) and *seb–sed* genes (Johnson et al. 1991). DNA extraction from the *Staph. aureus* strains was conducted using the QIAamp DNA minikit (Qiagen, Hilden, GmbH) according to the manufacturer instructions. DNA amplification was performed in a Perkin–Elmer GeneAmp 2400 thermo cycler (Applied Biosystems, Warrington, UK). The amplification conditions and reagents for the PCR assays were those described by Akineden et al. (2008). PCR products were analysed by agarose gel electrophoresis and separated DNA bands were visualised using ethidium bromide staining under UV illumination.

### *Manufacture of cheeses and contaminations trials*

Raw ovine milk obtained the same day was split into six portions for the manufacturing of three batches for each type of cheese. The three batches of Feta or Galotyri cheeses were made from pasteurized toxic ovine milk, pasteurized ovine milk with a low inoculum of *Staph. aureus*, pasteurized ovine milk with a high inoculum of *Staph. aureus*.

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Toxic milk with SE was obtained by contaminating the raw ovine milk with high inoculum of *Staph. aureus* (ca 6 log cfu/ml) and incubation for 2 h at 37 °C. Both cheeses were manufactured in the pilot plant of a local cheese factory (Tyras SA, Trikala, Greece). Established methods were used for the manufacture of Feta (Govaris et al. 2002) and Galotyri cheeses (Kondyli et al. 2008). Both cheeses were prepared in stainless double-jacketed cheese vats with a milk capacity of 150 litres. The milk (raw or toxic) was pasteurized at 65 °C for 30 min. After pasteurization, the milk was cooled at 35 °C and the mesophilic and thermophilic starter cultures were added for the manufacturing of Feta and Galotyri cheeses respectively. At the same time, the low or high inocula of toxigenic *Staph. aureus* strains were also added to the milk, for the batches of low or high contamination trials, respectively. The toxigenic strains were selected among our *Staph.aureus* strains, as described above.

After 30 min of incubation at 35 °C, the milk of both cheese types was curdled using a rennet (Hala, Chr. Hansen) at a dose of 2·5 g/100 l milk. After the completion of curdling, drainage of the curd was carried out with different procedures for each type of cheese. The curd of Feta cheese was cut after 30 min and drained in polypropylene hoops until next morning (24 h) at 20 °C. The Feta cheese blocks (21 × 11 × 8 cm) of ca 2 kg were placed in a tin pack (24 × 24 × 32 cm) in four layers, filled with 3 l 5·6% salt brine and placed at 16 °C. Ripening at 16 °C continued until the pH of the cheese blocks decreased to 4·60 (16 d), and then the cheese packs were stored at 4 °C. The curd of Galotyri cheese was cut, after 6 h. Then the curd was placed in a cloth bag and drained at 20 °C for ca 6 h. After the whey drainage, the curd of Galotyri cheese was kept at 20 °C until its pH reached 4·30 (ca 24 h). Then, the Galotyri cheese (2 kg) was salted (1·7%w/w), packed in plastic containers and stored at 4 °C.

### *Analysis of SEs*

In *in-vitro* and ovine milk tests, samples of BHI broth and raw or pasteurized ovine milk were obtained for SEs analysis at 0 h and at 2 h intervals up to the 12 h incubation. In Feta or Galotyri cheeses tests, samples of cheese milk, curd and cheese were taken for SEs analysis at the same sampling times as those described in microbiological analysis of Feta or Galotyri cheeses.

Samples of BHI broth (25 ml), milk (25 ml), curd (25 g) or cheese (25 g) were examined for the presence of SEs by using an ELISA test kit for combined detection of *Staph. aureus* enterotoxins SEA, SEB, SEC, SED and SEE (RIDASCREEN® SET Total, R4105, R-Biopharm AG, Darmstadt, Germany), according to manufacture's recommendations. Samples found positive for the presence of SEs were further examined for the determination of the toxin type (A-E), by using an ELISA test kit (RIDASCREEN® SET A,B,C,D,E, R4101, R-Biopharm AG), according to manufacture's recommendations. The detection limit of both test kits was labelled as 0·25 ng/g.

### *Microbiological analysis*

*Staph. aureus*, and TVC populations were estimated at 2 h intervals up to 12 h incubation, in BHI broth and raw or pasteurized ovine milk.

In Feta cheese tests, *Staph. aureus* and LAB populations were estimated at: milk (0 h), curd before cutting (2 h), curd in hoops after 2 h of cutting (4 h), curd after salting and drainage (10 h), cheese after 24 h, and then 4 d intervals up to the end of ripening (16 d), and on 30 and 60 d of the refrigerated storage. In Galotyri cheese tests, *Staph. aureus* and LAB populations were estimated at: milk (0 h), curd (2 h), curd before cutting (6 h), curd after six hours of whey drainage (12 h), cheese after 24 h, cheese on 5 d and then 5 d intervals up to the end of the refrigerated storage (30 d).

At each sampling time, samples of BHI broth (25 ml), milk (25 ml), curd or cheese (25 g) were placed in the stomacher bags and aseptically filled with 225 ml peptone water 0·1%. The content was macerated in the stomacher for 2 min at room temperature. Resulting slurries were serially diluted (1:10) in 0·1% sterile peptone water and plated on appropriate growth media. *Staph. aureus* populations were counted on Baird-Parker agar (Merck, Darmstadt, Germany) supplemented with egg yolk tellurite emulsion (Merck) (BPA + EYT) according to FIL-IDF standard n. 45 (International Dairy Federation, 1990). When *Staph. aureus* populations were below the detection limit ($10^2$ cfu/g), the presence or absence of the pathogen was verified by enrichment of the cheese sample (25 g) in (225 ml) Baird Parker broth (Merck) at 37 °C for 48 h, and subsequent plating (0·1 ml) on (BPA + EYT) agar. LAB were estimated by plating appropriate dilutions (0·1 ml) on deMan Rogosa Sharpe agar (Oxoid), as described by Govaris et al. (2002), at 40 or 28 °C for thermophilic and mesophilic LAB, respectively. Selected colonies from plates with the higher dilution were confirmed with API 50 CH test strips (Biomerieux, Marcy l' Etoile, France). TVC were enumerated on Plate Count Agar (Oxoid), after incubation at 30 °C for 48 h.

### *Physicochemical analysis*

Standard methods were used for the determination of fat, moisture, and sodium chloride in Feta or Galotyri cheese samples (APHA, 2004). Samples for the chemical analysis were obtained on 16 d (after ripening), 30 and 60 d for the Feta cheese, and 24 h, 10 and 30 d for the Galotyri cheese. At each sampling time, pH values of cheese samples were also determined with a pH meter (WTW, type 525, Wissennchaftlich – Technische Werkstatten, GmbH, D 82 362 Weilheim, Germany).

### *Statistical analysis*

Data were subjected to analysis of variance in the general linear model using the SPSS 10·05 statistical package (SPSS Ltd., Woking, UK). A probability level of $P < 0{\cdot}05$ was used in testing the statistical significance of all experimental data.

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**Table 1.** Composition of Feta and Galotyri cheeses

| Cheese | Moisture%† | Fat%† | NaCl%† |
|---|---|---|---|
| Feta | 53·4 ± 0·3 | 26·1 ± 0·3 | 2·20 ± 0·1 |
| Galotyri | 74·2 ± 0·4 | 10·8 ± 0·2 | 1·71 ± 0·1 |

† Mean of six separate analyses of each cheese ± SD

## Results

### *Physicochemical analysis*

The chemical composition of Feta and Galotyri cheeses (Table 1) was typical for these cheese types (Greek Codex Alimentarius, 2003). The moisture, fat, and NaCl contents estimated on 16 d and 24 h did not change significantly ($P > 0·05$) up to the end of storage, for the Feta and Galotyri cheeses, respectively. The pH values were decreased to 4·6 on 16 d or 4·3 at 12 h with no significant changes ($P < 0·05$) up to the end of storage for the Feta and Galotyri cheeses, respectively. It is important to note that pH at each sampling time did not show significant differences ($P < 0·05$) between low and high inoculum trials in Feta and Galotyri cheeses, during manufacturing and up to the end of storage.

### *Detection of toxigenic ability of* Staph. aureus *strains in* in-vitro *tests and by PCR*

During growth in BHI, *Staph. aureus* LHA, LHB, LHC and LHD strains were found toxigenic by producing SEA, SEB, SEC and SED, respectively. All *Staph. aureus* strains showed production of SEs, when populations exceeded 7 log cfu/g (data not shown).

PCR tests confirmed the presence of the toxigenic genes *sea, seb, sec* and *sed* encoding SEA, SEB, SEC and SED in *Staph. aureus* LHA, LHB, LHC and LHD strains, respectively. PCR showed that *Staph. aureus* LHB strain also possessed the *sec* gene, which was not expressed in the BHI tests.

### *Examination of toxigenic ability of* Staph. aureus *strains in ovine milk*

The growth of *Staph. aureus* LHA, LHB, LHC and LHD strains in raw or pasteurized ovine milk at 37 °C is shown in Figs. 1 & 2, respectively. All *Staph. aureus* strains showed a similar growth pattern in raw and pasteurized ovine milk. The SED was detected as soon as populations of *Staph. aureus* LHD strain reached ca 7 log cfu/ml, after 6 h and 8 h for raw and pasteurized ovine milk, respectively. Subsequently, SED was also detected up to the end of the incubation time (12 h), with the populations of *Staph. aureus* LHD ranged between ca 7–9 log cfu/ml. In contrast, SEA, SEB and SEC were not detected throughout raw and pasteurized ovine milk tests, even when populations of *Staph. aureus* LHA, LHB and LHC strains reached the maximum growth level (ca 8–9 log cfu/ml). Growth of bacteria present initially in the raw milk (TVC ca 5·1 log cfu/ml; LAB ca 3·5 log cfu/ml) and pasteurized milk (TVC ca 3·8 log cfu/ml ; LAB ca 2·6 log cfu/ml) at 37 °C did not affect growth of *Staph. aureus* strains.

Therefore, only *Staph. aureus* LHD strain was used for the cheese contamination trials, since this strain was the only one found to produce enterotoxin (SED) in ovine milk.

### *Fate of* Staph. aureus *LHD and SED in Feta and Galotyri cheeses*

The changes in *Staph. aureus* LHD populations in Feta and Galotyri cheeses, produced from toxic milk, during the manufacture and up to the end of storage are shown in Figs.3 & 4, respectively. Populations of the pathogen, survived after the pasteurization of the milk, and decreased to undetectable levels after 24 h or 12 d for the Galotyri and Feta cheese, respectively. SED was detected in Feta and Galotyri cheeses, produced from toxic milk, during manufacture and up to end of storage of both cheeses.

The changes in *Staph. aureus* LHD populations during manufacture, ripening and storage of Feta cheese for the low or high inoculum trials are shown in Fig. 5. *Staph. aureus* LHD populations increased in the curd (4 h) and decreased to undetectable levels on 12 and 16 d of storage for the low and high inoculum trials, respectively. For the low inoculum trials, SED was not detected in the curd or the Feta cheese during manufacture, ripening and storage. For the high inoculum trials, SED was detected in the curd (2 h), when *Staph. aureus* LHD populations reached ca 7 log cfu/g, and in Feta cheese up to the end of storage.

The changes in the *Staph. aureus* LHD populations during manufacture and storage of Galotyri cheese for the low or high inoculum trials are shown in Fig. 6. During the initial steps of the Galotyri manufacture, the populations of *Staph. aureus* LHD increased in the curd (6 h) to 3·8 and 7·3 log cfu/g for the low and high inoculum trials, respectively. Subsequently, the pathogen decreased to undetectable levels by 24 h and 5 d for the low and high inoculum trials, respectively. For the low inoculum trials, no SED was detected in the curd or the Galotyri cheese during manufacture and storage. For the high inoculum trials, SED was initially detected in the curd (2 h) with toxigenic levels of *Staph. aureus* LHD populations estimated at ca 7 log cfu/g, as in Feta cheese. SED was also detected during subsequent sampling times and up to the end of storage.

The LAB populations (ca 5·2 log cfu/ml) in both cheese milks for the low or high inoculum trials, increased to (ca 8·6 log cfu/g) or (ca 8·8 log cfu/g) at 4 d and 12 h and kept almost stable up to the end of storage for the Feta and Galotyri cheeses, respectively. It is important to note that LAB populations at each sampling time did not show significant differences ($P < 0·05$) between low and high inoculum trials in Feta and Galotyri tests. A similar growth pattern of LAB was also observed in Feta or Galotyri cheeses produced from toxic milk.

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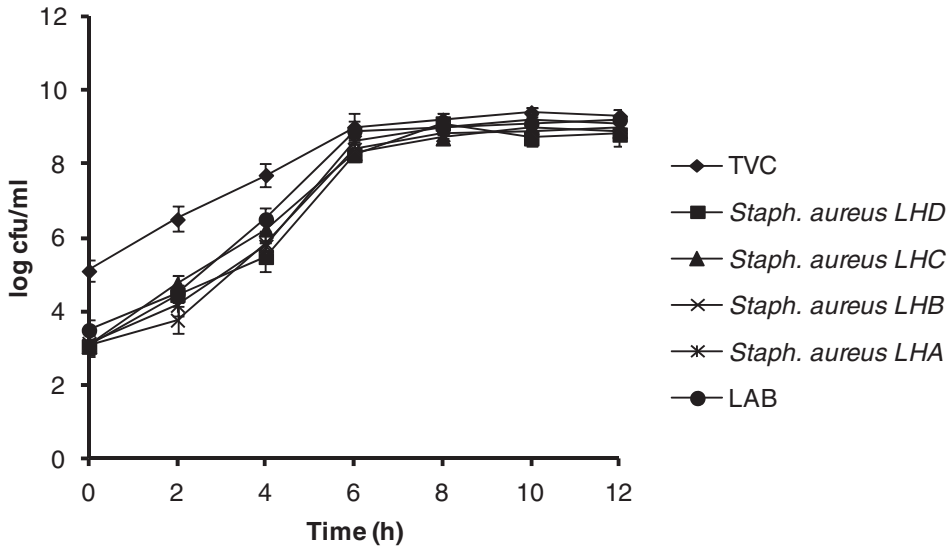


**Fig. 1.** Growth of *Staph. aureus* LHA, LHB, LHC and LHD strains TVC and LAB in raw ovine milk at 37 °C. Each data point represent the mean of six separate analyses of each cheese ± SD

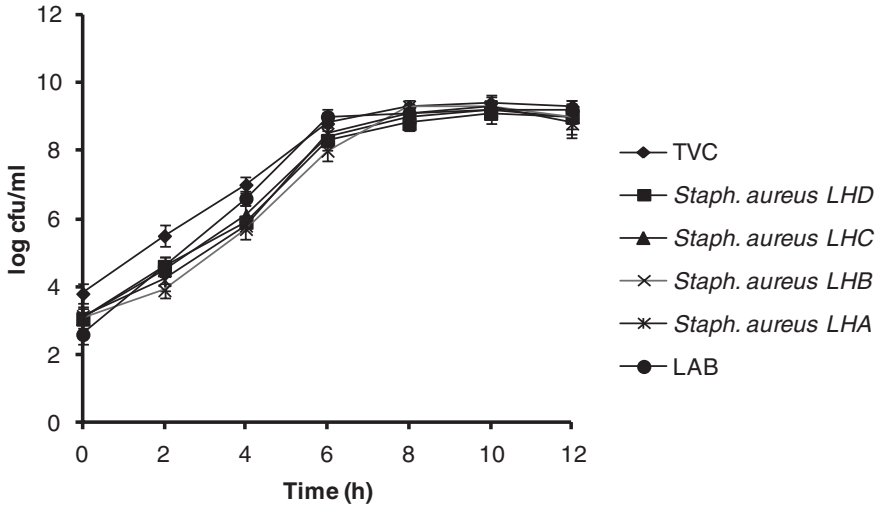


**Fig. 2.** Growth of *Staph. aureus* LHA, LHB, LHC and LHD strains, TVC and LAB in pasteurized ovine milk at 37 °C. Each data point represent the mean of six separate analyses of each cheese ± SD

## Discussion

According to PCR tests, all our *Staph. aureus* strains possessed the relative toxigenic genes of SEs. The non-phenotypic expression of *sec* gene of *Staph. aureus* LHB strain is in accordance with observations in previous studies (Cremonesi et al. 2007; Poli et al. 2007). This finding may be due to lower sensitivity of immunoassay methods (Morandi et al. 2007), or to the fact that *se* gene detection in *Staph. aureus* strains does not necessarily indicate enterotoxin production (Loncarevic et al. 2005).

*Staph. aureus* strains showed a different toxigenic ability in ovine milk than in BHI. Among examined toxigenic *Staph. aureus* strains, only *Staph. aureus* LHD was able to produce detectable amounts of SED in ovine milk. In our views, such a toxigenic behaviour of *Staph. aureus* strains of raw ovine milk origin, during their growth in BHI and ovine milk, has not been yet observed in previous studies. The inability of our toxigenic *Staph. aureus* LHA, LHB and LHC strains to produce SEs in ovine milk could be due to factors like antagonistic activity of milk microflora, competition for nutrients or production of hydrogen peroxide by the milk lactoperoxidase system (Vernozy-Rozand et al. 1998).

The toxigenic ability of *Staph. aureus* isolates from raw bovine milk, during their growth in laboratory media as well

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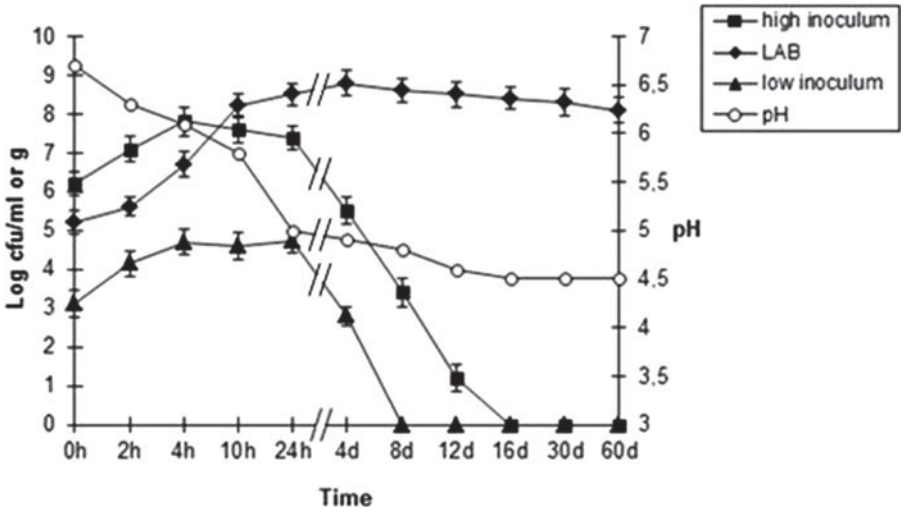


**Fig. 3.** Changes in *Staph. aureus* LHD and LAB populations and pH in Feta cheese, produced from toxic milk, during the manufacture, ripening and storage. Each data point represent the mean of six separate analyses of each cheese ± SD

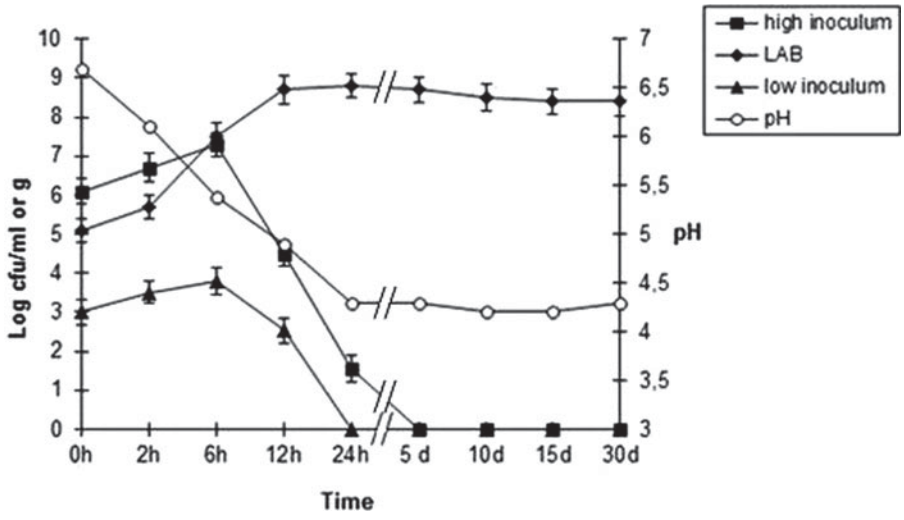


**Fig. 4.** Changes in *Staph. aureus* LHD and LAB populations and pH in Galotyri cheese, produced from toxic milk, during the manufacture and storage. Each data point represent the mean of six separate analyses of each cheese ± SD

as in raw or pasteurized milk, has not been adequately studied (Loncarevic et al. 2005). Donnelly et al. (1968) reported the production of SEA in raw or pasteurized milk, after inoculation with a *Staph. aureus* strain of cheese origin ($10^4$–$10^6$ cfu/ml) and incubation at 20, 25, 30 and 35 °C. Medvedova et al. (2009a) examined the toxigenic activity of three *Staph. aureus* strains during their growth in ultra-pasteurized milk at 12, 15, 18 and 21 °C and observed that one strain of human origin was only able to produce SED. It is also important to note that SED and SEA are most frequently isolated from cheeses implicated in cases of food poisoning (Balaban & Rasooly, 2000; Normanno et al. 2007). Poli et al. (2007) found that the gene encoding SED was among the predominant SEs genes in the *Staph. aureus* isolates from dairy products in Italy.

Present findings for the toxigenic ability *Staph. aureus* LHD at a population level of ca 7 log cfu/g are in agreement with previous observations for SE production in growth media (Pereira et al. 1991; Le Loir et al. 2003) or cheese products (Gomez-Lucia et al. 1992; Meyrand et al. 1998; Vernozy-Rozand et al. 1998; Hamama et al. 2002). The addition to cheesemilk of the mesophilic starter culture of (*Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris,* 1:1) for the Feta cheese and thermophilic starter culture (*Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus,* 1:1) for Galotyri cheese proved sufficient to keep population of *Staph. aureus* LHD below the toxigenic level (7 log cfu/g) only for the low inoculum trials. The initial ratio of population levels of *Staph. aureus* LHD to starter cultures in the milk could affect the growth of the pathogen

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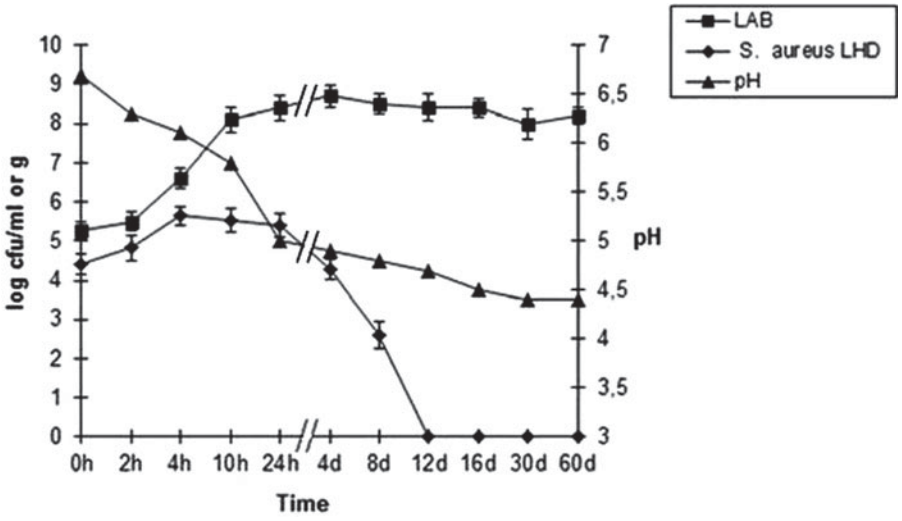


**Fig. 5.** Changes in *Staph. aureus* LHD populations during manufacture, ripening and storage of Feta cheese for the low or high inoculum trials. Each data point represent the mean of six separate analyses of each cheese ± SD

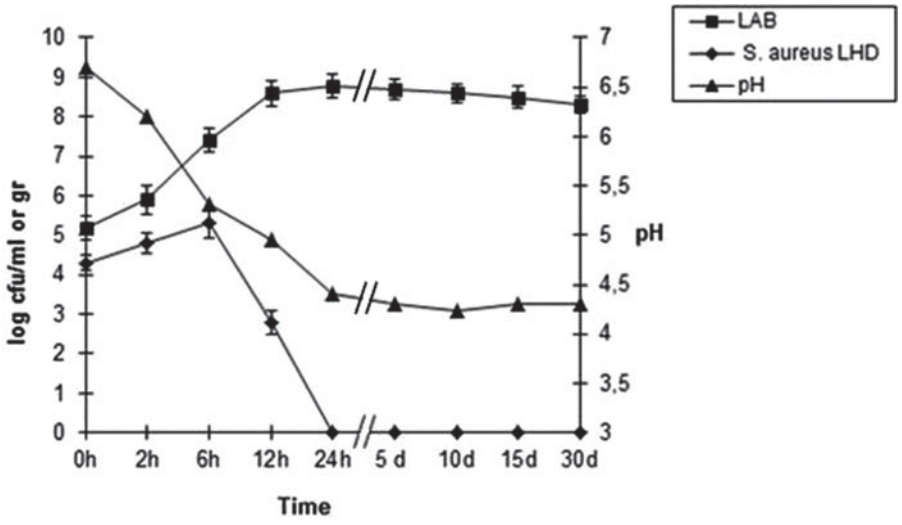


**Fig. 6.** Changes in the *Staph. aureus* LHD populations during manufacture and storage of Galotyri cheese for the low or high inoculum trials. Each data point represent the mean of six separate analyses of each cheese ± SD

to toxigenic levels for both examined cheeses and is in accordance with previous observations in other cheese products (Meyrand et al. 1998; Hamama et al. 2002; Lindqvist et al. 2002; Charlier et al. 2009). According to our results, the initial 6 h of cheesemaking for both cheeses products were crucial for the production of SED. Other workers also observed that SEs were usually produced during the initial 6 h of cheesemaking of cheese types like French cheeses Saint Nectaire and Saler (Delbes et al. 2006), camembert type cheeses (Meyrand et al. 1998), or Turkish Herby cheese from pasteurized milk (Akkaya & Sancak, 2007).

In Feta cheese, populations of *Staph. aureus* LHD were rather stable between 4–24 h of manufacturing. This fact may be due to a rather slow decrease of *Staph. aureus* LHD and the entrapment of the pathogen in the curd, after the whey drainage at this time. Other workers also observed that *Staph. aureus* was more heavily concentrated in the curd than the whey of goat's milk lactic cheeses (Vernozy-Rozand et al. 1998) or camembert type cheeses (Meyrand et al. 1998).

The decrease in *Staph. aureus* LHD populations during the ripening time of Feta cheese and the end of manufacturing of Galotyri cheeses may be due to combined effect of different microbial hurdle factors like the inhibitory effect of LAB, low pH, NaCl content or low water activity ($a_w$). The inhibitory effect of LAB on *Staph. aureus* has also been attributed to metabolic end products such as organic acids, diacetyl,

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hydrogen peroxide, and bacteriocins as well as the decrease in pH and competition for nutrients (Charlier et al. 2009). Various LAB (Millette et al. 2007; Alomar et al. 2008; Le Marc et al. 2009) *Lc. lactis* (Alomar et al. 2008; Charlier et al. 2008) or *Lc. lactis* subsp. *cremoris* (Nicolaou et al. 2011) showed an inhibitory activity against *Staph. aureus* in milk. *Lc. lactis* proved to be an efficient inhibitor of *Staph. aureus* in Cameros cheese (Olarte et al. 2000), Portuguese-style traditional ewe's cheeses (Pereira et al. 2009), or Moroccan Jben cheese (Hamama et al. 2002) during ripening.

The pathogen was also eliminated in a shorter time in Galotyri cheese than Feta cheese, for the high (5 d vs. 16 d) or the low (1 d vs. 12 d) inoculum trials. This difference in the decrease rate of *Staph. aureus* LHD, may be due to the lower pH values observed in Galotyri cheese than Feta cheese, although other factors like the antagonistic activity of LAB may also be involved. Previous workers reported that growth of *Staph. aureus* was completely inhibited at a pH of 4·4–4·5. Charlier et al. 2008, 2009). In agreement with present work, populations of *Staph. aureus* were rapidly decreased in pH lower than 4·5 in other cheese products like lactic goat cheese (Meyrand et al. 1998), Moroccan Jben cheese (Hamama et al. 2002) or Afuega'l pitu cheese (Rilla et al. 2004). Although *Staph. aureus* is salt tolerant, NaCl can act antagonistically against the pathogen in the low $a_w$ and low pH of the cheese products (Charlier et al. 2009; Medvedova et al. 2009b).

SED was not detected in Feta and Galotyri cheeses when the milk had been contaminated with *Staph. aureus* LHD at $10^3$ cfu/ml, but it was detected when the milk had been contaminated with the pathogen at $10^6$ cfu/ml. These results lead support to the European Union standards (EC regulations no. 2073/2005, 1441/2007) concerning the presence of SEs in cheeses from milk contaminated with *Staph. aureus*.

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