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Research Note

Diversity and Enterotoxigenicity of *Staphylococcus* spp. Associated with Domiati Cheese

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ABSTRACT

A total of 87 samples of fresh and stored Domiati cheese (an Egyptian soft cheese) were examined for the presence of *Staphylococcus* spp. Fifteen *Staphylococcus* isolates identified as *S. aureus* (2 isolates), *S. xylosus* (4), *S. caprae* (4), and *S. chromogenes* (5) were recovered from 15 cheese samples. The *S. aureus* isolates were resistant to penicillin G and ampicillin, and one isolate was also resistant to tetracycline. *S. aureus* isolates harbored classical staphylococcal enterotoxin (SE) genes (*sea* and *seb*) and recently characterized SE-like genes (*selg, seli, selm,* and *selo*). One *S. aureus* isolate contained a single SE gene (*sea*), whereas another isolate contained five SE genes (*seb, selg, seli, selm,* and *selo*). These results suggest that Domiati cheese is a source for various *Staphylococcus* species, including *S. aureus* strains that could be enterotoxigenic.

Staphylococcal food poisoning (SFP) is one of the major causes of gastroenteritis worldwide *(14, 19, 33).* Various species of *Staphylococcus* can produce staphylococcal enterotoxins (SEs), many of which are considered superantigens, which can cause immunological modulation leading to shock, immunosuppression, and other systematic disorders of toxic shock syndrome *(3, 7, 13, 14, 29).* Although SEs are part of the family of pyrogenic toxins, which also includes toxins produced by streptococci, SEs are unique in their ability to induce an emetic response upon oral ingestion and thus are solely responsible for SFP *(14).* Human intoxication is caused by ingestion of enterotoxins produced in food by strains of *Staphylococcus aureus*. Several foodstuffs have been implicated in SFP, including meat and meat products, poultry and egg products, salads, bakery products such as cream-filled pastries, cream pies, and chocolate eclairs, sandwich fillings, and milk and dairy products *(19, 29, 31).* Preparation of these foods usually requires considerable handling. The final product may be kept at slightly elevated temperatures during or after processing, which encourages the growth of staphylococci and production of toxins *(16).*

Domiati cheese is a traditional Egyptian soft cheese that is highly salted and enzyme coagulated and is widely consumed in the Middle East; approximately 75% of cheese produced and consumed in Egypt is Domiati cheese *(34).* The application of advanced technologies such as ultrafiltration and hazard analysis critical control point systems to the preparation of this cheese in large processing plants has eliminated several risks associated with its production. However, Domiati cheese is still prepared in small and medium-size processing facilities, where milk is not pasteurized but is only warmed for dissolving the salt and for coagulation. Human handling occurs often during cheese processing and packaging in these facilities. These practices can result in contamination of the cheese with diverse foodborne bacteria, as revealed in a recent study in which high microbial diversity was found in Domiati cheese prepared in small processing facilities *(12).* In previous studies, similar white soft cheeses produced in different countries were a source of enterotoxigenic *Staphylococcus* spp. *(1, 2, 32).* Staphylococci that naturally exist on the skin and within the nose, throat, or perineum of even healthy food handlers *(16, 17)* may gain access to Domiati cheese. El-Baradei et al. *(12)* reported the presence of staphylococci in Domiati cheese but did not characterize the detected isolates in terms of their antibiotic resistance and enterotoxigenicity.

The present study was designed to characterize staphylococci associated with Domiati cheese, considering their diversity, antibiotic resistance, and enterotoxigenicity. The multiplex PCR assays designed by Omoe et al. *(25, 26)* for the detection of SE genes were used to further characterize the strains of *S. aureus* recovered from Domiati cheese samples. These improved PCR assays allowed the detection of almost all known SE genes, which is not currently possible with commercial *Staphylococcus* enterotoxin reversed passive latex agglutination (SET-RPLA) kits.

MATERIALS AND METHODS

Detection and identification of *Staphylococcus* spp. A total of 87 Domiati cheese samples, including 53 samples of stored

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Domiati cheese (salted at 10 to 14% milk weight) and 34 samples of fresh Domiati cheese (salted at <10% milk weight), were randomly collected from the local markets in Mansoura and in villages in the vicinity within the Nile Delta region of Egypt. Each sample was aseptically transferred to a sterile plastic bag, placed in an ice box, and transported to the laboratory within 2 h. Cheese samples were stored at 4°C and analyzed within 24 h of collection.

Tenfold serial dilutions of the cheese samples (25 g) were prepared in maximum recovery diluent (Oxoid, Basingstoke, UK), plated on Baird-Parker agar (Oxoid), and incubated at 35°C for 24 h. Black suspect colonies surrounded by white clear zones were picked and subjected to Gram staining and the catalase test. Additional discriminatory tests included coagulase production using BBL coagulase plasma (Becton Dickinson, Sparks, MD), yellow pigmentation on brain heart infusion (BHI) agar (Oxoid), anaerobic fermentation of mannitol in mannitol salt agar (Oxoid), and hemolysin production on blood agar (Oxoid). Isolates were also tested using the API Staph miniaturized kits (bioMérieux, Marcy l'Etoile, France) and Dry Spot Staphytect Plus latex kits (Oxoid).

Antibiotic susceptibility assay. *Staphylococcus* isolates were examined for their susceptibility to tetracycline (30 μg), penicillin G (100 units), ampicillin (10 μg), methicillin (5 μg), oxacillin (1 μg), co-trimoxazole (25 μg), cefotaxime (30 μg), nalidixic acid (30 μg), amoxicillin plus clavulanic acid (30 μg), streptomycin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), trimethoprim (5 μg), ciprofloxacin (5 μg), and kanamycin (30 μg) on Mueller-Hinton agar (MHA; Oxoid) using the Kirby-Bauer disc diffusion method *(4, 10).* An overnight culture of *Staphylococcus* spp. was spread plated on MHA, and then antibiotic-impregnated discs (Oxoid) were placed on the agar surface. The plates were incubated at 37°C for 24 h for all antibiotics except methicillin and oxacillin, for which the plates were incubated at 30°C *(18).* Inhibition zones were measured with calipers after 18 and 24 h, and strains were categorized as sensitive, intermediate, or resistant, depending on the diameter of the inhibition zone. Resistance to methicillin and oxacillin was also examined on MHA containing 2% NaCl. A multiplex PCR assay able to identify five of the main known staphylococcal cassette chromosomes (SCCmec) carrying the *mecA* gene responsible for methicillin resistance in *S. aureus* (MRSA) strains was also performed *(9, 30).*

Multiplex PCR and identification of genes encoding SEs. Single colonies of each isolated strain were inoculated with a sterile loop into 5 ml of BHI broth at 37°C. DNA was extracted from the cultures using an Ultraclean Microbial DNA Isolation kit (MoBio, Solana Beach, CA) according to the manufacturer's instructions and quantified spectrophotometrically (GENEQUANT *pro*, GE Healthcare, Milan, Italy). Multiplex PCR assays as described by Omoe et al. *(25, 26)* were performed in 50-μl reaction mixtures that contained 0.3 μM concentrations of each primer and 30 ng of DNA in 2× QIAGEN Multiplex PCR Master Mix (QIAGEN Spa, Milan, Italy). For PCR sets 1 and 4, the PCR mix also contained 5 μl of 5× Q Solution (QIAGEN) *(29).* Thermal cycling conditions were 95°C for 15 min and then 35 cycles of 95°C for 30 s, 57°C (for sets 1 and 2) or 58°C (for sets 3 and 4) for 90 s, and 72°C for 90 s. Primers specific to *S. aureus* for amplifying *femA* and *femB* genes were used as an internal positive control *(22, 27).* The PCR was stopped at 72°C for 10 min and then cooled to 4°C. PCR fragments were resolved by gel electrophoresis by applying 10 μl of the sample to 2.0% agarose gels. Gels were run for about 40 min at 90 V in 1× TAE buffer (0.04 M Tris-acetate and 1 mM EDTA). A 200-bp ladder (Promega, Madison, WI) was used as a standard marker. After electrophoresis, the gel was stained with 1 μg ml^{-1} ethidium bromide and washed for 10 min, and the gel image was acquired by a Versa Doc 4000 (Bio-Rad Laboratories, Hercules, CA). All experiments were run in duplicate.

TABLE 1. Staphylococcus *spp. associated with stored and fresh Domiati cheese*

Sample type	No. of samples	No. (%) of positive samples	*Staphylococcus* species (no. of isolates)
Stored cheese	53	9 (16)	*S. aureus* (2), *S. xylosus* (3), *S. caprae* (2), *S. chromogenes* (3)
Fresh cheese	34	5 (14)	*S. xylosus* (1), *S. caprae* (2), *S. chromogenes* (2)
Total	87	15 (17)	

Immunological assay. Both *S. aureus* isolates that were positive for classical SE genes *sea* through *sed* were incubated in tryptone soy broth (Oxoid) for 24 h at 37°C and tested for SEs A through D with the SET-RPLA kit (Oxoid) in accordance with the manufacturer's instructions. The standard enterotoxins A, B, C, and D were used as positive controls. For a negative control, control latex rather than latex sensitized with antienterotoxins was used.

RESULTS

Fifteen *Staphylococcus* isolates were recovered from 15 samples of stored and fresh Domiati cheese (Table 1). These *Staphylococcus* isolates were identified as *S. aureus* (2 isolates), *S. xylosus* (4), *S. caprae* (4), and *S. chromogenes* (5). The biochemical characteristics of these isolates are reported in Table 2. All isolates were catalase positive, and all except isolates FSMP261 and FSMP262 were coagulase negative. These two strains were isolated from two different stored Domiati cheese samples collected from two different markets.

All isolates were further tested for resistance to 15 antibiotics. Only *S. aureus* strains FSMP261 and FSMP262 were resistant to penicillin G and ampicillin; strain FSMP261 was also resistant to tetracycline (Table 3). All isolates were susceptible to the remaining antibiotics. No isolates were resistant to methicillin or oxacillin, as indicated by the disc diffusion method with cultures on both 2% salt-supplemented MHA and nonsupplemented MHA (data not shown). These results were also confirmed by PCR amplification of methicillin resistance genes (data not shown).

All 15 *Staphylococcus* isolates recovered from the Domiati cheese samples were assessed for the presence of 18 SE genes using a recently developed multiplex PCR assay *(26).* Among the isolates examined, only *S. aureus* FSMP261 and FSMP262 contained SE genes. Isolate FSMP262 contained only one SE gene, *sea*, whereas isolate FSMP261 contained five SE genes: *seb, selg, seli, selm,* and *selo* (Fig. 1). PCR fragments were obtained from isolate FSMP262 with PCR primer set 1 (*sea*) and from isolate FSMP261 with PCR primer sets 1 (*seb*), 2 (*seli* and *selg*), and 3 (*selm* and *selo*). No amplification fragments were detected in isolate FSMP262 when PCR primer sets 2 and

TABLE 2. *Biochemical characteristics of* Staphylococcus *isolates recovered from Domiati cheese*

Isolate	Coagulase	Dry Spot Staphytect Plus	Anaerobic fermentation of mannitol	Yellow pigmentation	Catalase	Hemolysin
S. aureus FSMP261	+	+	+	+	+	+
S. aureus FSMP262	+	+	+	+	+	+
S. xylosus FSMP1	−	−	+	−	+	+
S. xylosus FSMP6	−	−	+	−	+	+
S. xylosus FSMP11	−	−	+	−	+	+
S. xylosus FSMP13	−	−	+	−	+	+
S. caprae FSMP7	−	−	−	−	+	+
S. caprae FSMP14	−	−	−	−	+	+
S. caprae FSMP280	−	−	−	−	+	+
S. caprae FSMP289	−	−	−	−	+	+
S. chromogenes FSMP4	−	−	+	−	+	−
S. chromogenes FSMP5	−	−	+	−	+	−
S. chromogenes FSMP8	−	−	+	−	+	−
S. chromogenes FSMP9	−	−	+	−	+	−
S. chromogenes FSMP12	−	−	+	−	+	−

3 were used. Primer set 4 produced no PCR fragments with either FSMP261 or FSMP262. However, FSMP261 and FSMP262 yielded *femB* and *femA* PCR fragments, thus confirming these two isolates as *S. aureus*. The SET-RPLA assay also confirmed the presence of SEA and SEB in isolates FSMP262 and FSMP261, respectively (Table 3).

DISCUSSION

The aim of this work was to evaluate the occurrence of *Staphylococcus* spp. and potentially enterotoxigenic staphylococci in Domiati cheese, a traditional Egyptian cheese widely consumed in the Middle East. The overall prevalence of staphylococci in stored and fresh Domiati cheese samples was low and relatively similar (Table 1). The majority of these *Staphylococcus* isolates were coagulase negative, and the species were identified as *S. xylosus, S. caprae,* and *S. chromogenes*. These species have been associated with foodstuffs *(6, 7),* and *S. chromogenes* is a common cause of subclinical mastitis *(15).* However, two coagulase-positive *S. aureus* isolates were detected in two stored Domiati cheese samples. The resistance of both isolates to at least two antibiotics (penicillin G and ampicillin) and the resistance of one of these isolates also to tetracycline suggest that antibiotic resistance in *S. aureus* isolates associated with Domiati cheese may be common. Resistance to penicillin G and ampicillin, which are beta-lactam antibiotics, could be attributed to the reported ability of *S. aureus* to produce beta-lactamases *(15).* However, none of the isolates were resistant to methicillin or oxacillin or contained methicillin resistance genes. MRSA and oxacillin-resistant *S. aureus* (ORSA) strains have emerged since the 1960s and have caused numerous deadly SFP cases worldwide *(21).* It is reassuring that neither MRSA nor ORSA isolates were detected in Domiati cheese samples examined in this study.

PCR amplification of SE genes has been a useful method for genotyping isolates for epidemiological studies and for providing information used in risk assessments involving coagulase-negative *Staphylococcus* spp. *(6, 7).* Based on the multiplex PCR analysis, only the *S. aureus* isolates from stored Domiati cheese were potentially able to produce enterotoxins (Fig. 1). These isolates harbored classical SE genes (*sea* and *seb*) but also contained SE-like genes (*selg, seli, selm,* and *selo*) that had not been detected frequently in foodborne staphylococci. Genome sequence analyses have led to a rapid expansion of the *se* alphabet and description of several SE-like genes *(5, 8, 9, 11, 19, 23, 24).* Some of the new SEs reportedly lack emetic activity, a defining property of SEs *(26, 28),* whereas others have yet to be tested for emetic activity *(6, 20).* However, information regarding the SE-like genes is mainly associated with *S. aureus* isolates from clinical samples. Researchers have suggested that the SE-like genes and relative genetic variants are also widespread in foodborne *Staphy-*

TABLE 3. *Antibiotic resistance and SEs of* Staphylococcus aureus *strains FSMP261 and FSMP262*

S. aureus strain	Antibiotics[a]: AMP	SXT	CXT	NAL	AUG	STR	CHL	TET	GEN	OX	METH	PG	TMP	CIP	KAN	SE
FSMP261	R	S	S	S	S	S	S	R	S	S	S	R	S	S	S	SEB
FSMP262	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S	SEA

[a] AMP, ampicillin; SXT, co-trimoxazole; CXT, cefotaxime; NAL, nalidixic acid; AUG, amoxicillin plus clavulanic acid; STR, streptomycin; CHL, chloramphenicol; TET, tetracycline; GEN, gentamicin; OX, oxacillin; METH, methicillin; PG, penicillin G; TMP, trimethoprim; CIP, ciprofloxacin; KAN, kanamycin. R, resistant; S, sensitive.

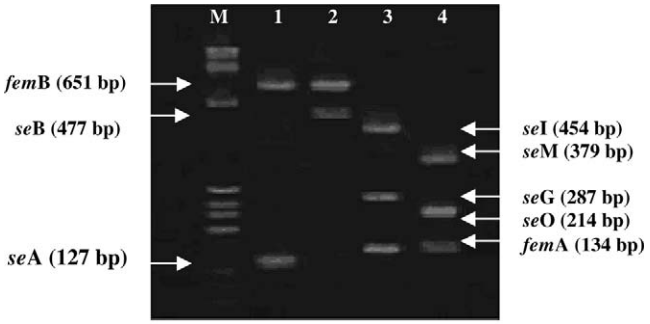

FIGURE 1. *Examples of multiplex PCRs performed with DNA extracted from* Staphylococcus aureus *isolates FSMP261 and FSMP262. Total DNA from* S. aureus *FSMP261 and FSMP262 was amplified with four sets of multiplex PCR primers. Lanes 1 and 2, amplification fragments from isolates FSMP262 and FSMP261, respectively, obtained with PCR primer set 1. Lanes 3 and 4, amplification fragments from isolate FSMP261 obtained with PCR primer sets 2 and 3 (no fragments were detected for isolate FSMP262 with these primer sets). Primer set 4 produced no fragments for either isolate. M, Bench Top ϕX174* Hin*dIII.*

lococcus strains and that the potential role of these genes in food poisoning cases has been underestimated *(8).* Consistent with these observations, we found SE-like genes in *S. aureus* isolates recovered from Domiati cheese, but the role of these SE genes in SFP has still to be clarified.

The present study revealed that Domiati cheese prepared in small and medium-size processing facilities can be a source of diverse *Staphylococcus* species and enterotoxigenic *S. aureus.* Because *S. aureus* is not known for its resistance to food preservation regimes, these findings suggest that poor hygienic practices are used in the manufacture of cheese in these facilities. SE-like genes were also detected in the foodborne *S. aureus* isolates. However, further work is needed to assess the risk associated with the presence of SE-like genes.

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REFERENCES

1. Adesiyum, A. A., L. A. Webb, and H. T. Romain. 1998. Prevalence and characteristics of *Staphylococcus aureus* strains isolated from bulk and composite milk and cattle handlers. *J. Food Prot*. 61:629–632.
2. Arau'jo, V. S., V. A. Pagliares, M. L. P. Queiroz, and A. C. Freitas-Almeida. 2002. Occurrence of *Staphylococcus* and enteropathogens in soft cheese commercialized in the city of Rio de Janeiro, Brazil. *J. Appl. Microbiol.* 92:1172–1177.
3. Ash, M. 1997. *Staphylococcus aureus* and staphylococcal enterotoxins, p. 313–332. *In* A. D. Hocking, G. Arnold, I. Jenson, K. Newton, and P. Sutherland (ed.), Foodborne microorganisms of public health significance. Australian Institute of Food Science and Technology Inc., North Sydney.
4. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45:493–496.
5. Blaiotta, G., A. Casaburi, and F. Villani. 2005. Identification and differentiation of *Staphylococcus carnosus* and *Staphylococcus simulans* by species-specific PCR assays of sodA genes. *Syst. Appl. Microbiol*. 286:519–526.
6. Blaiotta, G., D. Ercolini, C. Pennacchia, V. Fusco, A. Casaburi, O. Pepe, and F. Villani. 2004. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of *seg* and *sei* in *S. aureus* AB-8802, *J. Appl. Microbiol.* 97:719–730.
7. Blaiotta, G., V. Fusco, C. von Eiff, F. Villani, and K. Becker. 2006. Biotyping of enterotoxigenic *Staphylococcus aureus* by enterotoxin gene cluster (*egc*) polymorphism and spa typing analyses. *Appl. Environ. Microbiol.* 72:6117–6123.
8. Boerema, J. A., R. Clemens, and G. Brightwell. 2006. Evaluation of molecular methods to determine enterotoxigenic status and molecular genotype of bovine, ovine, human and food isolates of *Staphylococcus aureus*. *Int. J. Food Microbiol*. 107:192–201.
9. Boye, K., M. D. Bartels, I. S. Andersen, J. A. Møller, and H. Westh. 2007. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I–V. *Clin. Microbiol. Infect.* 13:725–727.
10. British Society for Antimicrobial Chemotherapy. 2008. Standardized disc testing method. Available at: http://www.bsac.org.uk/. Accessed 10 June 2008.
11. Dinges, M. M., P. M. Orwin, and P. M. Schlievert. 2000. Enterotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev*. 13:16–34.
12. El-Baradei, G., A. Delacroix-Buchet, and J.-C. Ogier. 2007. Biodiversity of bacterial ecosystems in traditional Egyptian Domiati cheese. *Appl. Environ. Microbiol.* 73:1248–1255.
13. Fitzgerald, J. R., S. R. Monday, T. J. Foster, G. A. Bohach, P. J. Hartigan, W. J. Meaney, and C. J. Smyth. 2001. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. *J. Bacteriol.* 183:63–70.
14. Jablonski, L. M., and G. A. Bohach. 2001. *Staphylococcus aureus,* p. 353–375. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
15. Jarp, J. 1991. Classification of coagulase-negative staphylococci isolated from bovine clinical and subclinical mastitis. *Vet. Microbiol.* 27:151–158.
16. Jay, M. J., M. J. Lossner, and D. A. Golden. 2005. Modern food microbiology. Springer, NY.
17. Kloos, W. E. 1980. Natural populations of the genus *Staphylococcus*. *Annu. Rev. Microbiol.* 34:559–592.
18. Kluytmans, J., W. van Leeuwen, W. Goessens, R. Hollis, S. Messer, L. Herwaldt, H. Bruining, M. Heck, J. Rost, and N. van Leeuwen. 1995. Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping. *J. Clin. Microbiol.* 33: 1121–1128.
19. Leloir, Y. L., F. Baron, and M. Gautier. 2003. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* 2:63–76.
20. Letertre, C., S. Perelle, F. Dilasser, and P. Fach. 2003. Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. *J. Appl. Microbiol*. 95:38–43.
21. Lina, G., G. A. Bohach, S. P. Nair, K. Hiramatsu, E. Jouvin-Marche, and R. Mariuzza. 2004. Standard nomenclature for the superantigens expressed by *Staphylococcus*. *J. Infect. Dis.* 189:2334–2336.
22. Matagene, A., J. Lamotte-Brasseur, and J.-M. Frere. 1998. Catalytic properties of class A β-lactamases: efficiency and diversity. *Biochem. J.* 330:581–598.
23. Mehrotra, M., G. Wang, and W. M. Johnson. 2000. Multiplex PCR for detection for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J. Clin. Microbiol.* 38:1032–1035.
24. Nassib, T. A., M. Z. El-Din, and W. M. El-Sharoud. 2003. Assessment of the presence of *Salmonella* spp. in Egyptian dairy products using various detection media. *Lett. Appl. Microbiol*. 37:405–409.
25. Omoe, K., D.-L. Hu, H. Takahashi-Omoe, A. Nakane, and K. Shinagawa. 2003. Identification and characterization of a new staphylococcal enterotoxin–related putative toxin encoded by two kinds of plasmids. *Infect. Immun.* 71:6088–6094.

26. Omoe, K., D.-L. Hu, H. Takahashi-Omoe, A. Nakane, and K. Shinagawa. 2005. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. *FEMS Microbiol. Lett.* 246:191–198.
27. Orwin, P. M., J. R. Fitzgerald, D. Y. M. Leung, J. A. Gutierrez, G. A. Bohach, and P. M. Schlievert. 2003. Characterization of *Staphylococcus aureus* enterotoxin L. *Infect. Immun.* 71:2916–2919.
28. Orwin, P. M., D. Y. M. Leung, T. J. Tripp, G. A. Bohach, C. A. Earhart, D. H. Ohlendorf, and P. M. Schlievert. 2002. Characterisation of a novel staphylococcal enterotoxin-like superantigen, a member of the group V subfamily of pyrogenic toxins, *Biochemistry* 41: 14033–14040.
29. Peles, F., M. Wagner, L. Varga, I. Hein, P. Rieck, K. Gutser, P. Keresztúri, G. Kardos, I. Turcsányi, B. Béri, and A. Szabó. 2007. Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. *Int. J. Food Microbiol.* 118:186–193.
30. Perez-Roth, E., F. Claverie-Martın, J. Villar, and S. Mendez-Alvarez. 2001. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. *J. Clin. Microbiol.* 39:4037–4041.
31. Poli, A., E. Guglielmini, S. Sembeni, M. Spiazzi, F. Dellaglio, F. Rossi, and S. Torriani. 2007. Detection of *Staphylococcus aureus* and enterotoxin genotype diversity in Monte Veronese, a protected designation of origin Italian cheese. *Lett. Appl. Microbiol*. 45:529–534.
32. Temelli, S., S. Anar, C. Sen, and P. Akyuva. 2006. Determination of microbiological contamination sources during Turkish white cheese production. *Food Control* 17:856–861.
33. Thomas, D., S. Chou, O. Dauwalder, and G. Lina. 2007. Diversity in *Staphylococcus aureus* enterotoxins. *Chem. Immunol*. *Allergy* 93: 24–41.
34. Zhang, X., R. L. Kilmer, and A. Muhammad**.** 2003. A descriptive analysis of Egypt and Saudi Arabia who import United States dairy products. Monograph MGTC 03-8. International Agricultural Trade and Policy Center, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.