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ORIGINAL ARTICLE

Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese

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Abstract

Aim: To develop an easy, rapid and efficient DNA extraction procedure for *Staphylococcus aureus* detection with a low number of steps and removing completely the PCR inhibitors, applicable to raw milk cheese samples, and to compare phenotypical and genotypical method to detect *Staph. aureus* isolates and staphylococcal enterotoxins (SEs) production.

Methods and Results: A total of 33 bovine and caprine raw milk cheese samples were analysed by means of both classic microbiological and molecular techniques. All samples were positive for *Staph. aureus* contamination. The DNA extraction protocol optimized was found to achieve a detection limit of 100 CFU g^{-1} for *Staph. aureus*. None of the samples tested with immunological assays contained SEs but in 14 of 33 samples a mixture of *se* positive (*sea*, *sec*, *sed*, *seg*, *sel*, *sej*) isolates were identified.

Conclusions: *Staphylococcus aureus* is a food-borne pathogen mainly detected in finished dairy products. The rapid and efficient detection of *Staph. aureus* isolates from dairy products is essential for consumer safety. The direct detection of pathogens from food is possible with careful attention to sample preparation and nucleic acid amplification optimization.

Significance and Impact of the Study: This study shows that raw milk cheese samples can be tested for *Staph. aureus* contamination with a rapid, simple and reproducible procedure.

Introduction

Staphylococcus aureus is an ubiquitous Gram-positive micro-organism commonly isolated from raw milk of dairy cattle suffering from mastitis. So its presence in raw milk is a major concern for the safety and the quality of traditionally produced cheeses (Delbes *et al*. 2006). In fact some *Staph. aureus* strains may produce up to 20 serologically distinct staphylococcal enterotoxins (SEs), which could be responsible for food poisoning. Among them, 11 major antigenic types of SEs have been recognized (Tamarapu *et al*. 2001). More recently further SEs have been identified but their role in food poisoning is still unclear (Omoe *et al*. 2002; Letertre *et al*. 2003).

Symptoms, such as nausea, emesis, abdominal cramps and diarrhoea, develop 2–4 h after food intake and their seriousness depends on the amount of ingested toxin and on individual state of health. In order to cause a food-borne intoxication, Staphylococci must be able both to grow and produce enterotoxins. Sometimes the amount of produced SE may be insufficient for food intoxication. In addition, there are many factors affecting enterotoxin production in food such as NaCl concentration, temperature, pH, climatic conditions and the presence of a competitive flora (Brasca *et al*. 2005).

As previously described by Loncarevic *et al.* (2005), the detection of *Staph. aureus* and SEs in food is often difficult because food processing may kill the bacteria without destroying SEs, which are stable to high temperature and to inactivation by gastrointestinal proteases such as pepsin. The currently available methods for SE detection are based on microbiological culture of milk and milk products and they have been developed according to three immunological methods: enzyme-linked immunosorbent assay (ELISA), enzyme-linked fluorescent assay (ELFA) and reverse passive latex agglutination (RPLA).

However, there are several disadvantages associated with microbiological culture such as time consumption, cost and detection limits of SE higher than the level required for staphylococcal intoxication. Two or three days are required for growth, isolation and identification of pathogen rendering the methods impractical for the detection and identification of a large group of related toxins. In addition, new European standards, applicable as of 1 January 2006 (CEE Law no. 2073), lay down strict criteria for *Staph. aureus* in raw milk, cheeses, milk powder and frozen milk products.

Therefore, there is still a need for a rapid and specific method for simultaneous detection of *Staph. aureus* and SEs in bulk milk and raw milk products. Polymerase chain reaction (PCR)-based analytical methods for ascertaining the occurrence of pathogenic or toxigenic micro-organisms in food are widely recognized as capable of decreasing detection time and increasing the specificity and sensitivity (Ercolini *et al.* 2004). Unfortunately, large sample volumes (≥25 ml or g) compared with small amplification volumes (10–50 μl), residual food components that inhibit enzymatic reactions, such as calcium ions, plasmin and protein (Wilson 1997), low levels of contaminating pathogens, and the presence of competitive microflora which may interfere with amplification and detection reactions, have been consistent stumbling blocks to the widespread use of nucleic acid amplification for pathogen detection in food (Bej and Mahbubani 1994). PCR has been often experimented in milk and cheeses for direct detection of *Staph. aureus* (Kim *et al.* 2001; Tamarapu *et al.* 2001) but none of these approaches is ideal, and in many cases, a technique optimized for one food system or micro-organism is not readily adaptable to others (Baruzzi *et al.* 2005). The objectives of this study were (i) to develop an easy, rapid and efficient DNA extraction procedure for *Staph. aureus*, applicable to raw milk cheese samples, with a low number of steps and removing completely the PCR inhibitors, and (ii) to compare phenotypical and genotypical method to detect *Staph. aureus* isolates and SEs production.

Materials and methods

Cheese samples and bacteriological analyses

A total of 23 bovine and 10 caprine raw milk cheese samples provided by large and small scale Italian producers and including fresh, soft, semi-hard and hard cheese (Table 1) were analysed for the presence of *Staph. aureus*. To our knowledge, none of the samples had been involved in food poisoning. Ten grams of each milk product was added to 90 g of sterile tryptone phosphate water (Sharlau Microbiology, Barcelona, Spain) and stomached for 30–90 s. Then three 10-fold dilutions were made and 0·1 ml of each step was inoculated on Baird Parker RPF Agar (Biolife, Milan, Italy) to detect coagulase-positive *Staph. aureus*, as required by the rule ISO 6888. The plates were incubated for 48 h at 37°C. Typical colonies were counted after 24 and 48 h. In accordance with CEE law No. 2073 (in force since January 2006), cheese samples having more than 10^5 CFU of *Staph. aureus* per gram were then tested for their ability to produce enterotoxins. According to the manufacturer's instructions, the ELFA VIDAS SET Test (bioMèrieux, RCS Lyon, France) was used to detect enterotoxin production of SEA to SEE without distinguishing individual toxins while a reversed passive latex agglutination assay (SET RPLA; Oxoid, Milan, Italy) was utilized in order to distinguish SEA, SEB, SEC and SED enterotoxins.

DNA extraction

DNA was extracted directly from fresh, soft, semi-hard and hard cheeses following the procedure previously described for milk samples (Cremonesi *et al.* 2006) with minor modifications. Briefly, we have diluted 100 mg of cheese sample with 500 μl of lysis buffer (3 mol l^{-1} guanidine thiocyanate, 20 mmol l^{-1} EDTA, 10 mmol l^{-1} Tris–HCl, pH 6·8, 40 mg ml^{-1} Triton X-100, 10 mg ml^{-1} DTT) and vortexed for 30 s to obtain an emulsified solution. After addition of 500 μl of binding solution (40 mg ml^{-1} silica from Sigma Aldrich, Milan, Italy, directly suspended in the lysis buffer), the sample was incubated for 5 min at room temperature. After a centrifugation of 30 s at 500 ***g*** the supernatant was discarded. After this sample treatment, the procedure was the same described in Cremonesi *et al.* (2006) starting from step 3. Briefly, the silica-DNA pellet obtained was subsequently washed twice with 200 μl of lysis buffer, twice with 200 μl of washing solution (25% absolute ethanol, 25% isopropanol, 100 mmol l^{-1} NaCl, 10 mmol l^{-1} Tris–HCl, pH 8) and once with 200 μl of absolute ethanol. After every washing and vortexing, the silica-DNA pellet was centrifuged for 30 s at 500 ***g*** and the supernatant was

Table 1 Source, *Staphylococcus aureus* concentration and presence of staphylococcal enterotoxin genes (*se*) and proteins (SE) in bovine and caprine raw milk cheese samples detected by use of SET-RPLA and multiplex-PCR. Phenotype was determined only for samples having *Staph. aureus* concentration higher than 10^5 CFU g^{-1}

Sample	Source	*Staph. aureus* (CFU g^{-1})	Genotype (PCR)	Phenotype (SET-RPLA)
1	Bovine	1300	Non-enterotoxigenic	ND
2	Bovine	<100	*sea*	ND
3	Bovine	<100	Non-enterotoxigenic	ND
4	Bovine	600	*sed*	ND
5	Bovine	1800	Non-enterotoxigenic	ND
6	Bovine	<100	*sea, sed, sej*	ND
7	Bovine	2300	Non-enterotoxigenic	ND
8	Bovine	20 000	Non-enterotoxigenic	Negative
9	Bovine	100	Non-enterotoxigenic	ND
10	Bovine	1000	*sea, sed, sej*	ND
11	Bovine	>30 000	Non-enterotoxigenic	Negative
12	Bovine	<100	*sed*	ND
13	Bovine	<100	*sea*	ND
14	Bovine	100 000	Non-enterotoxigenic	Negative
15	Bovine	6200	*sea, sed, sej*	ND
16	Bovine	<100	*sea*	ND
17	Bovine	6300	*sea, sed*	ND
18	Bovine	<100	Non-enterotoxigenic	ND
19	Bovine	<100	*seg*	ND
20	Bovine	100	Non-enterotoxigenic	ND
21	Bovine	2400	*sea*	ND
22	Bovine	180 000	*sea, sed, sej*	Negative
23	Bovine	200	Non-enterotoxigenic	ND
24	Caprine	2600	Non-enterotoxigenic	ND
25	Caprine	1100	Non-enterotoxigenic	ND
26	Caprine	7000	Non-enterotoxigenic	ND
27	Caprine	1 100 000	*sec, sel*	Negative
28	Caprine	790 000	Non-enterotoxigenic	Negative
29	Caprine	1100	*sec, sel*	ND
30	Caprine	200	Non-enterotoxigenic	ND
31	Caprine	46 000	Non-enterotoxigenic	Negative
32	Caprine	790 000	Non-enterotoxigenic	Negative
33	Caprine	5400	Non-enterotoxigenic	ND

ND, not determined.

discarded. The pellet was vacuum-dried at 56°C in a heat block for 10 min. After addition of 100 μl of elution buffer (10 mmol l^{-1} Tris–HCl, pH 8·0, 1 mmol l^{-1} EDTA), the silica-DNA pellet was gently vortexed and incubated for 15 min at 65°C. After a 5 min centrifugation at 500 ***g***, the supernatant containing the DNA was stored at −20°C until use.

DNA extraction from cheese samples was carried out also following the protocol described by Baruzzi *et al.* (2005). Briefly, 1 ml of 1:10 cheese suspension in 2% sodium citrate solution was homogenized at 10 000 rpm for 2 min in a food processor and centrifuged at 14 000 ***g***. The pellet, dissolved in 500 μl of 1:39 Triton X-100 solution, was shaked and heated at 70°C for 10 min, centrifuged at 14 000 ***g*** for 3 min and rinsed twice in sterile distilled water. Then the Wizard Genomic DNA Purification Kit (Promega Italia, Milan, Italy) was utilized according to the manufacturer's instructions.

The quantity and quality of DNA samples were measured using a NanoDrop ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA).

PCR amplification

First, to test the efficiency of two extraction procedures for *Staph. aureus* detection, the DNA obtained from different caprine and bovine source was amplified by means of the PCR reaction described in Cremonesi *et al.* (2006).

Then, the 23S rRNA, *coa*, *nuc* genes with *sea*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej*, *sel* enterotoxin genes were amplified by a multiplex PCR; primers and reaction conditions have been described in Cremonesi *et al.* (2005). All the amplifications were visualized by standard gel electrophoresis in a 4% agarose gel (GellyPhor; Euroclone, Milan, Italy), stained with ethidium bromide (0·05 μg μl^{-1}; Sigma Aldrich, Milan, Italy). The gels were photographed under

ultraviolet light using the BioProfile system (Mitsubishi, Tokyo, Japan). Molecular size marker (100-bp ladder; Finnzymes, Espoo, Finland) was included in each agarose gel.

To evaluate the specificity of the DNA extraction protocol, the PCR products obtained from cheese samples were sequenced by CRIBI Services (CRIBI, Padova, Italy) on an ABI37 sequencer by using the ABI PRISM dye-terminator cycle sequencing ready reaction kit with Amplitaq DNA polymerase (Applied Biosystem, Foster City, CA).

Results

We analysed 33 fresh, soft, semi-hard and hard raw milk cheese samples by means of both classic microbiological and molecular techniques, to detect *Staph. aureus* and SEs. In agreement with microbiological tests, all samples gave positive results to 23S rRNA, *coa* and *nuc* genes, confirming the presence of *Staph. aureus*. The PCR products sequenced were aligned with those of known 23S rRNA variants using ClustalW, confirming the bacterial specificity.

In accordance with CEE law No. 2073, cheese samples having *Staph. aureus* concentration higher than 10^5 CFU g^{-1} were tested for enterotoxin production by reversed passive latex agglutination (SET-RPLA) assay (Oxoid) and also by ELFA VIDAS SET Test. No enterotoxins (SEs) were detected in the 33 samples tested (Table 1). However, isolates from 14 of the 33 (42%) samples harboured the *se* genes (Table 1). *Staph. aureus* strains possessing the *sea*, *sed* or combination of *sea*, *sed* and *sej* genes were detected in 10 bovine cheese samples, while a combination of *sec* and *sel* genes was detected in two caprine cheese samples. Only one *Staph. aureus* strain harboured the *seg* gene (Fig. 1).

The DNA extraction procedure described in this work was found to achieve a detection limit of 100 CFU g^{-1} for *Staph. aureus* better than 1000 CFU g^{-1} obtained on the same samples with the protocol described by Baruzzi *et al.* (2005) (Table 2).

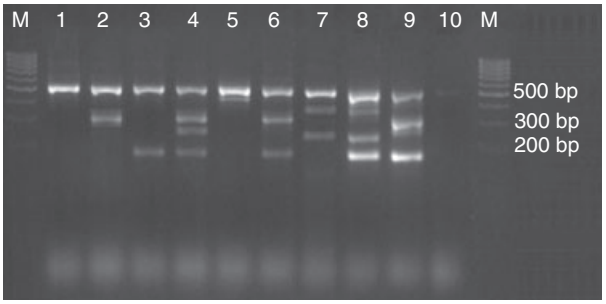

Figure 1 Examples of multiplex PCR results for *Staphylococcus aureus* strains isolated from raw milk cheese samples. Lanes 1–6, bovine samples; lane 7, caprine sample; lanes 8 and 9, positive controls; lane 10, negative control. All the samples were positive for 23S rRNA gene (499 bp). The sample in lane 1 (sample 5, semi-hard cheese, in Table 1) is non-enterotoxigenic while the other samples contain *se* genes: lane 2, sample 4 (soft cheese) *sed* gene (343 bp); lane 3, sample 2 (hard cheese) *sea* gene (180 bp); lane 4, sample 10 (semi-hard cheese) *sea* (180 bp), *sej* (306 bp) and *sed* (343 bp) genes; lane 5, sample 19 (hard cheese) *seg* (432 bp) gene; lane 6, sample 17 (soft cheese) *sea* (180 bp) and *sed* (343 bp) genes; lane 7, sample 27 (soft cheese) *sel* (240 bp) and *sec* (371 bp) genes; lanes 8 and 9 reference strain ATCC 700699D (genes *sea*, *sec*, *seg*, *sei*, *sel*) and ATCC 23235 (genes *sea*, *sed*, *seg*, *sei*, *sej*), respectively. M: 100 bp DNA ladder (Finnzymes).

Table 2 Efficiency comparison of DNA extraction methods tested on bovine and caprine cheese samples with different *Staphylococcus aureus* concentration (CFU g^{-1}). To detect the presence of *Staph. aureus* DNA, the 23S rRNA gene was PCR amplified

		PCR results	
Source	*Staph. aureus* (CFU g^{-1})	Baruzzi *et al.* (2005)	Our procedure
Caprine	1100 000	+	+
Bovine	100 000	+	+
Bovine	20 000	+	+
Bovine	6200	+	+
Caprine	5400	+	+
Bovine	1300	+	+
Caprine	1100	+	+
Bovine	1000	+	+
Bovine	600	−	+
Caprine	200	−	+
Bovine	100	−	+
Bovine	<100	−	+

Discussion

Dairy food products are compositionally complex and represent one of the more challenging matrices for which molecular-based pathogens detection strategies have to be developed. Numerous previous works (Rossen *et al.* 1992; Makino *et al.* 1995) underlined a decreased sensitivity of the PCR reaction because of an inefficient DNA extraction and/or high levels of background and competing microflora, both of which can be problematic for compositionally complex dairy products. In addition, more researches attempting direct detection (without cultural enrichment) of pathogens from food matrices report poorer detection limits in comparison with methods that are preceded by cultural enrichment. Furthermore, cheeses are often regarded as difficult matrices to be assayed by PCR because of the high fat content (Ercolini *et al.* 2004), which can affect the DNA extraction and PCR amplification. By the protocol used in this study, previously shown to be effective in extracting microbial

DNA from milk samples (Cremonesi *et al.* 2006), efficient lysis of cells and removal of inhibitors had been obtained combining the guanidine thiocyanate and lysis buffer solution, avoiding sample's enrichment. For the direct detection of pathogens in cheese samples, this rapid, simple and reproducible procedure for DNA preparation showed a detection limit well below the minimum level required by law, whereas other authors obtained similar or better results using higher amounts of samples (McKillip *et al.* 2000; Stevens and Jaykus 2004) or pre-enrichment procedures (Longhi *et al.* 2003). A possible limit of this procedure is that molecular methods are only able to demonstrate the existence of the genes encoding for SEs in the micro-organism and cannot prove that production of SE protein occurs (Rodriguez *et al.* 1996) during food processing and/or storage. To demonstrate the capability of the strain to produce an amount of SE protein that is sufficient to induce disease, bioassay or immunological methods for the detection of the SE protein must be used. Nowadays, the laboratory methods used to detect SEs from bacterial strains underestimate the potentially SE production of the isolates. Availability of DNA sequence information of all described *se* and development of PCR methods has, however, given the opportunity to overcome this problem.

As underlined by other authors (Ercolini *et al.* 2004), in case of food matrix, a PCR detection of toxin encoding genes coupled with the specific detection of the producing species, indicates the potential to find toxins in the food and, depending on the level of contamination, the possibility to have hazardous food products. Nevertheless, we stress that even if not expressed in food enterotoxins represent a potential risk for the consumer. The sensitivity achieved makes the protocol described in this work effective in revealing potentially hazardous *Staph. aureus* strains throughout the dairy products chain.

Multiplex PCR assays, allowing rapid and simultaneous detection of staphylococcal enterotoxigenic strains and toxin genes, gave good results even though DNA was extracted directly from a complex foodstuff such as cheese. In agreement with previous studies (Akineden *et al.* 2001; Scherrer *et al.* 2004) we found mainly *sea*, *sed* and *sej* genes in bovine samples, while in caprine samples we found *sec* and *sel* genes. Moreover, PCR allowed detection of *seg*, *sej* and *sel* genes not identifiable using ELISAs currently on the market. Previous work (McLauchin *et al.* 2000) reported significant occurrence of the SEs G, H and I and their encoding genes in strains of *Staph. aureus* involved in food poisoning outbreaks.

Our conclusions are that the rapid and efficient detection of *Staph. aureus* isolates from finished dairy products is essential for consumer safety. The direct detection of pathogens from food is possible with careful attention to sample preparation and nucleic acid amplification optimization.

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References

Akineden, Ö., Annemüller, C., Hassan, A.A., Lämmler, C., Wolter, W. and Zschöck, M. (2001) Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clin Diagn Lab Immunol* **8**, 959–964.

Baruzzi, F., Matarante, A., Caputo, L. and Morea, M. (2005) Development of a culture-independent polymerase chain reaction-based assay for the detection of Lactobacilli in stretched cheese. *J Rapid Meth Aut Mic* **13**, 177–192.

Bej, A.K. and Mahbubani, M.H. (1994) Detection of foodborne microbial pathogens. In: *PCR Technology: Current Innovations* ed. Griffin, H. and Griffin, A. pp. 341–365. Boca Raton, FL: CRC Press.

Brasca, M., Morandi, S., Vanoni, L., Colombo, L. and Lodi, R. (2005) The influence of different cultural conditions on the development and toxinogenesis of *Staphylococcus aureus*. *Scienza Tecnica Lattiero Casearia* **56**, 105–115.

Cremonesi, P., Luzzana, M., Brasca, M., Morandi, S., Lodi, R., Vimercati, C., Agnellini, D., Caramenti, G., *et al.* (2005) Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. *Mol Cell Probes* **19**, 299–305.

Cremonesi, P., Castiglioni, B., Malferrari, G., Biunno, I., Vimercati, C., Moroni, P., Morandi, S. and Luzzana, M. (2006) Improved method for rapid DNA extraction of mastitis pathogens directly from milk. *J Dairy Sci* **89**, 163–169.

Delbes, C., Alomar, J., Chougui, N., Martin, J.F. and Montel, M.C. (2006) *Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese from cows raw milk. *J Food Prot* **69**, 2161–2167.

Ercolini, D., Blaiotta, G., Fusco, V. and Coppola, S. (2004) PCR-based detection of enterotoxigenic *Staphylococcus aureus* in the early stages of raw milk cheese making. *J Appl Microbiol* **96**, 1090–1098.

Kim, C.-H., Khan, M., Morin, D.E., Hurley, W.L., Tripathy, D.N., Kehrli, M.J., Oluoch, O.A. and Kakoma, I. (2001) Optimisation of the PC for detection of *Staphylococcus aureus nuc* gene in bovine milk. *J Dairy Sci* **84**, 74–83.

Letertre, C., Perelle, S., Dilasser, F. and Fach, P. (2003) Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. *J Appl Microbiol* **95**, 38–43.

Loncarevic, S., Jørgensen, H.J., Løvseth, A., Mathisen, T. and Rørvik, L.M. (2005) Diversity of *Staphylococcus aureus* enterotoxin types within single samples of raw milk and raw milk products. *J Appl Microbiol* **98**, 344–350.

Longhi, C., Maffeo, A., Penta, M., Petrone, G., Seganti, L. and Conte, M.P. (2003) Detection of *Listeria monocitogenes*: Italian-style soft cheeses. *J Appl Microbiol* **94**, 879–885.

Makino, S.I., Okado, Y. and Maruyama, T. (1995) A new method for direct detection of *Listeria monocytogenes* from foods by PCR. *Appl Environ Microbiol* **61**, 3745–3747.

McKillip, J.L., Jaykus, L.A. and Drake, M.A. (2000) A comparison of methods for the detection of *Escherichia coli* O157:H7 from artificially-contaminated dairy products using PCR. *J Appl Microbiol* **89**, 49–55.

McLauchin, J., Narayanan, G.L., Mithani, V. and O'Neill, G. (2000) The detection of enterotoxins and toxic shock syndrome toxin genes in *Staphylococcus aureus* by polymerase chain reaction. *J Food Prot* **63**, 479–488.

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, DL., Ueda, S. and Shinagawa, K. (2002) Detection of *seg*, *seh* and *sei* genes in *Staphylococcus aureus* isolates and determination of enterotoxin productivities of *S. aureus* isolates harbouring *seg*, *seh* or *sei* genes. *J Clin Microbiol* **40**, 857–862.

Rodriguez, M., Nunez, F., Cordoba, J.J., Bermudez, E. and Asensio, M.A. (1996) Gram-positive, catalase-positive cocci from dry cured Iberian ham and their enterotoxigenic potential. *Appl Environ Microbiol* **62**, 1897–1902.

Rossen, L., Norskov, P., Holmstrom, K. and Rasmussen, O.F. (1992) Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *Int J Food Microbiol* **17**, 37–45.

Scherrer, D., Corti, S., Muehlherr, J.E., Zweifel, C. and Stephan, R. (2004) Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. *Vet Microbiol* **101**, 101–107.

Stevens, K.A. and Jaykus, L.A. (2004) Direct detection of bacterial pathogens in representative dairy products using combined bacterial concentration-PCR approach. *J Appl Microbiol* **97**, 1115–1122.

Tamarapu, S., McKillip, J.L. and Drake, M. (2001) Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. *J Food Prot* **64**, 664–668.

Wilson, I.G. (1997) Inhibition and facilitation of nucleic acid amplification. *Appl Environ Microbiol* **63**, 3741–3751.

