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Comparison of Different Approaches To Quantify *Staphylococcus aureus* Cells by Real-Time Quantitative PCR and Application of This Technique for Examination of Cheese

INGEBORG HEIN,[1]* ANGELIKA LEHNER,[1] PETRA RIECK,[1] KURT KLEIN,[2] ERNST BRANDL,[1] AND MARTIN WAGNER[1]

Institute of Milk Hygiene, Milk Technology, and Food Science, University of Veterinary Medicine,[1] and Roche Diagnostics GmbH,[2] 1210 Vienna, Austria

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Two different real-time quantitative PCR (RTQ-PCR) approaches were applied for PCR-based quantification of *Staphylococcus aureus* cells by targeting the thermonuclease (*nuc*) gene. Purified DNA extracts from pure cultures of *S. aureus* were quantified in a LightCycler system using SYBR Green I. Quantification proved to be less sensitive (60 *nuc* gene copies/μl) than using a fluorigenic TaqMan probe (6 *nuc* gene copies/μl). Comparison of the LightCycler system and the well-established ABI Prism 7700 SDS with TaqMan probes revealed no statistically significant differences with respect to sensitivity and reproducibility. Application of the RTQ-PCR assay to quantify *S. aureus* cells in artificially contaminated cheeses of different types achieved sensitivities from 1.5×10^2 to 6.4×10^2 copies of the *nuc* gene/2 g, depending on the cheese matrix. The coefficients of correlation between log CFU and *nuc* gene copy numbers ranged from 0.979 to 0.998, thus enabling calculation of the number of CFU of *S. aureus* in cheese by performing RTQ-PCR.

Food poisoning caused by the enterotoxins produced by *Staphylococcus aureus* is of major concern in food hygiene. Many outbreaks involving contaminated milk and milk products have been reported (2, 8, 12, 13, 21). The natural habitat of *S. aureus* is warm-blooded animals, including humans. Ten to 40% of people are asymptomatic carriers of *S. aureus*, mostly at the mucosal membrane (20). Food handlers with infected skin lesions and asymptomatic carriers are often sources of food contamination, although with milk and milk products the raw milk itself can be contaminated with *S. aureus*.

Once introduced into food-processing plants, bacteria can persist for a long time. CFU of *S. aureus* numbering 10^6 to 10^8 per g or ml of food must be present in order to produce sufficient amounts of toxins for poisoning (13). The bacteria are readily destroyed by heat, but the toxins are thermostable. Austrian national regulations concerning the presence of *S. aureus* in milk and milk products apply three-class plans that define *m* values to separate acceptable from marginally acceptable products and *M* values to separate marginally acceptable from defective-quality products; when numbers of bacteria are higher than *M* values, the presence of enterotoxins has to be investigated. The International Standardization Organization and International Dairy Federation methods (3, 4, 5, 6) apply conventional microbiological quantification techniques such as the plate count method and the most-probable-number (MPN) method. Some of these techniques require up to 6 days for quantification and detection, thus being time-consuming. There are rapid alternative methods for quantification of *S. aureus* cells. These comprise the application of MPN-PCR (17), a rather laborious procedure, and the quantification of the enzymatic activity of the bacterial phosphatase (10), which is not specific and can therefore be applied only to pure cultures.

Another rapid alternative is real-time quantitative PCR (RTQ-PCR) (11), which quantifies DNA and thus has the potential for accurate enumeration of microorganisms. To find the most convenient technique, two different approaches to RTQ-PCR-based quantification of the *nuc* gene in *S. aureus* cells were performed with a LightCycler system. This system combines an air thermocycler and a fluorimeter, enabling rapid-cycle PCR. The first approach applies the double-stranded DNA (dsDNA)-binding fluorescent dye SYBR Green I, and the second approach applies a fluorescent probe called the TaqMan probe. In addition, the sensitivity and repeatability of the results of the RTQ-PCR assay employing the TaqMan probe in the LightCycler were compared to those of the well-established ABI PRISM 7700 sequence detection system (SDS), a heat block thermocycler with an integrated fluorimeter. The application of different *Taq* DNA polymerases was tested. The optimized RTQ-PCR assay was used for quantification of the *nuc* gene of *S. aureus* cells in artificially contaminated cheeses of different types.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* DSM 1104 was used for preparing the quantification standards for RTQ-PCR as well as for contaminating the cheese samples. This strain was cultured at the Institute for Milk Hygiene, Milk Technology and Food Science of the University for Veterinary Medicine, Vienna, Austria, in a brain heart infusion medium (Merck, Darmstadt, Germany) at 37°C for 15 h.

Preparation of the quantification standard. One milliliter of a pure culture of *S. aureus* DSM 1104 was subjected to DNA isolation using a Puregene DNA isolation kit (Gentra Systems Inc., Minneapolis, Minn.) according to the manufacturer's instructions. DNA concentration was measured fluorimetrically using a HoeferDyNA Quant200 apparatus (Pharmacia Biotech, San Francisco, Calif.). The copy number of the *nuc* gene was determined by assuming that, based on the molecular weight of the 2.7- to 2.8-mbp-sized genome of *S. aureus* (18), 1 ng of

* Corresponding author. Mailing address: Institute of Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria. Phone: 431250773511. Fax: 431250773590. E-mail: Ingeborg.Hein@vu-wien.ac.at.

TABLE 1. Times and temperatures applied for the RTQ-PCR configuration using SYBR Green I and the LightCycler

Parameter	Temp(s) (°C)	Time(s) (s)	Slope (°C/s)	Acquisition mode
Denaturation	95	120/600[a]	20	None
Amplification (45 cycles)	95	2	20	None
	60/62	5	20	None
	72	12	20	Single
Melting	95	0	20	None
	68	15	20	None
	95	0	0.2	Step
Cooling	40	5	20	None

[a] Hot-start PCR.

TABLE 2. Times and temperatures applied for the RTQ-PCR configuration using the TaqMan probe and the LightCycler

Parameter	Temp (°C)	Time(s) (s)	Slope (°C/s)	Acquisition mode
Denaturation	95	120/600[a]	20	None
Amplification (45 cycles)	95	2	20	None
	62	60	10	Single
Cooling	40	5	20	None

[a] Hot-start PCR.

DNA equals 6×10^5 times the entire genome and that the *nuc* gene is a single-copy gene.

Oligonucleotides for RTQ-PCR. The primers used for amplifying a 269-bp fragment of the *nuc* gene in *S. aureus* cells have been previously published (9) and were purchased from MWG Biotech (Ebersberg, Germany). In our work, the sequence of the internal TaqMan probe was complementary to the sequence of the published hybridization probe (9). At the 5′ end, the probe was labeled with 6-carboxy-fluorescein (FAM), and at the 3′ end, it was labeled with 6-carboxy-tetramethyl-rhodamine (TAMRA). It was synthesized by Metabion GmbH (Martinsried, Germany).

RTQ-PCR. To quantify DNA by RTQ-PCR, an increase in fluorescence emission during PCR proportional to the initial copy number of the target gene is generated and detected. Both fluorescent dsDNA-binding dyes and fluorigenic probes (either hybridization probes or a TaqMan probe) can be applied. Fluorescence emission is detected for each PCR cycle. The threshold cycle (C_T) values must be determined. The C_T value is defined as the actual PCR cycle when the fluorescence signal increases above the background threshold. It indicates the number of target gene copies. PCR and fluorescence detection can be performed in a combined thermocycler and fluorimeter.

When SYBR Green I is used, the increase in fluorescence emission is due to binding of the dye to dsDNA. The identification of the PCR product is performed by determining the melting temperature (T_m) of the amplicon after PCR. A TaqMan probe is labeled at the 5′ end with the reporter dye and at the 3′ end with the quencher dye. Cleavage of the probe during PCR via the 5′-3′ exonuclease activity of *Taq* DNA polymerase displaces both dyes, resulting in an increase in reporter-specific fluorescence emission.

(i) SYBR Green I with the LightCycler. RTQ-PCR with SYBR Green I was performed using either the LightCycler-DNA Master SYBR Green I Kit or, for hot-start PCR purposes, the LightCycler-FastStart DNA Master SYBR Green I Kit (Roche Diagnostics GmbH, Mannheim, Germany) in a LightCycler. The 20-μl reaction mix contained 1× LightCycler-DNA Master SYBR Green I or LightCycler-FastStart DNA Master SYBR Green I; 5 or 4 mM $MgCl_2$; a 900, 600, or 300 nM concentration of each primer; and 5 μl of the template. Times and temperatures applied are shown in Table 1. For acquisition of the fluorescent signal, channel F1 was used and the gain of F1 was set at 5. For normalization of fluorescent data, the F1/1 ratio was applied.

(ii) TaqMan probe with the LightCycler and the ABI PRISM 7700 SDS. RTQ-PCR using a TaqMan probe was performed in a LightCycler by applying either the LightCycler-DNA Master Hybridization Probe Kit or, for hot-start PCR purposes, the LightCycler-FastStart DNA Master Hybridization Probe Kit (Roche Diagnostics GmbH). The 20-μl reaction mix contained a 1× concentration of LightCycler-DNA Master Hybridization probes or LightCycler-FastStart DNA Master Hybridization probes, 4 mM $MgCl_2$, a 300 nM concentration of the primer, a 100 nM concentration of the probe, and 5 μl of the template. Times and temperatures are shown in Table 2. Channel F1 was used for data acquisition, the gain of F1 was set at 2, and the F1 (FAM)/1 ratio was applied for normalization of fluorescent data.

In addition to using commercial kits, we used an in-house reaction mix for RTQ-PCR using a TaqMan probe in a LightCycler as well as in an ABI PRISM 7700 SDS (Perkin-Elmer, Foster City, Calif.). The 20-μl reaction volume contained 20 mM Tris-HCl; 50 mM KCl; 4 mM $MgCl_2$; a 300 nM concentration of each primer; a 100 nM concentration of the probe; 200 μM (each) dATP, dTTP, dGTP, and dCTP; 1.5 U of Platinum *Taq* DNA polymerase (GIBCO BRL, Life Technologies, Vienna, Austria), 0.25 mg of bovine serum albumin (New England Biolabs Inc., Beverly, Mass.) per ml, and 5 μl of the template. Times and temperatures in the LightCycler were the same as those in Table 2, with the exceptions that denaturation took place at 94°C instead of 95°C and the duration of the initial denaturation was 2 min instead of 10 min. Amplification in the ABI PRISM 7700 SDS following an initial denaturation at 94°C for 2 min was performed in 45 cycles at 94°C for 15 s and 62°C for 1 min.

Statistical procedures. When comparing the levels of performance of different reaction mixtures and different RTQ-PCR equipment, the C_T values were first tested for outliers by the method of Nalimov as discussed by Kaiser and Gottschalk (14). The presence or absence of statistically significant differences between the approaches were investigated by F-test comparison of variances of the C_T values.

Artificial contamination of cheese. Tilsiter-type semisoft cheese made from pasteurized milk and French Camembert made from raw milk were purchased at a local supermarket. Fresh cheese made from raw goat's milk was obtained from a local farm. All cheese types tested negative by RTQ-PCR for the presence of *S. aureus* DNA.

A pure culture of *S. aureus* DSM 1104 in brain heart infusion medium was subjected to a 10-fold dilution series in buffered peptone water, and the number of CFU of each dilution step was obtained by the plate count method using Baird Parker agar plates (Merck). One milliliter of each dilution step was added to 2 g of cheese with 45 ml of digestion buffer (100 mM Tris-HCl, 100 mM EDTA, 0.5% sodium dodecyl sulfate [pH 8.0]) diluted 1:10.

DNA isolation from artificially contaminated cheese. DNA isolation from artificially contaminated cheese was performed as previously described (1), apart from the following modifications. Two grams of cheese was added to 45 ml of digestion buffer diluted 1:10 and containing 12 U of pronase (Fluka Chemie, Buchs, Switzerland) per ml, homogenized in a Laboratory Blender Stomacher 400 (Seward, London, United Kingdom) for 1 min, and subsequently incubated at 40°C for 3 h. After centrifugation at 5,700 × *g* for 15 min at 4°C, the fat layer and aqueous phase were discarded and the pellet was washed three times with TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]) and once with PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl [pH 8.4]). Finally, the pellet was resolved in 25 μl (Tilsiter, cheese from goat's milk) or 250 μl (Camembert) of PCR buffer and 143 U of lysostaphin (Sigma-Aldrich GmbH, Steinheim, Germany) per ml was added. This solution was incubated at room temperature for 15 min. After addition of 8 U of proteinase K (Roche Diagnostics GmbH) per ml, samples were held at 60°C for 1 h and afterwards incubated for 15 min at 95°C. Cell debris was removed by centrifugation at 13,000 × *g* for 5 min. Five microliters of the supernatant was subjected to RTQ-PCR.

Calculation of the *nuc* gene copy number per 2 g of cheese. Calculation of the *nuc* gene copy number per 2 g of cheese was performed by multiplying the number of *nuc* gene copies per PCR with the ratio of the final suspension volume of the DNA isolated from 2 g of cheese and 5, as 5 μl of the final DNA suspension was subjected to PCR.

RESULTS

Setup of RTQ-PCR. A 269-bp fragment of the *nuc* gene was chosen as a target for detection and quantification in *S. aureus* cells by RTQ-PCR. This gene codes for an extracellular thermonuclease, which is produced at a frequency similar to that of the coagulase. The primers and probe had been used previously for amplification and identification of the fragment by

TABLE 3. Mean C_T values and standard deviations of a dilution series of the *nuc* gene of *S. aureus* using the TaqMan probe with the LightCycler or the ABI PRISM 7700 SDS and different reaction mixes

System	Mean C_T value ± SD				
	3×10^0 copies/μl	3×10^1 copies/μl	3×10^2 copies/μl	3×10^3 copies/μl	3×10^4 copies/μl
LightCycler with a commercial kit	36.72 ± 0.128	33.02 ± 0.240	29.80 ± 0.105	26.46 ± 0.167	22.32 ± 0.147
LightCycler with Platinum *Taq* DNA polymerase	34.65 ± 0.214	31.11 ± 0.243	27.99 ± 0.064	24.76 ± 0.068	21.29 ± 0.052
ABI PRISM 7700 SDS with Platinum *Taq* DNA polymerase	35.07 ± 0.367	31.49 ± 0.069	28.56 ± 0.208	25.74 ± 0.121	NM[a]

[a] NM, not measured.

conventional PCR. The specificities of the oligonucleotides have been demonstrated elsewhere (9). Preliminary testing proved these oligonucleotides to be suited for RTQ-PCR (data not shown).

Comparison of two different approaches for quantification of *S. aureus* cells by RTQ-PCR using a LightCycler. (i) SYBR Green I with the LightCycler. A 10-fold dilution series of pure DNA from *S. aureus* DSM 1104 ranging from 6×10^5 to 6×10^0 copies of the *nuc* gene/μl was quantified by RTQ-PCR with SYBR Green I. Detection of the amplicon was performed by examination of the T_ms of the amplicons after PCR. The T_m of the specific 269-bp amplicon was 81°C. Nonspecific side products and primer dimers showed lower T_ms. The detection limit was improved from 6,000 copies of the *nuc* gene/μl of the template to 60 copies by decreasing stepwise the concentrations of the primers from 900 to 300 nM and of $MgCl_2$ from 5 to 4 mM and increasing the annealing temperature from 60 to 62°C. For samples containing less than 60 copies/μl of the template, no specific amplicon was generated. Application by hot-start PCR using a modified form of the thermostable recombinant *Taq* DNA polymerase, inactive at room temperature, improved the detection limit of the RTQ-PCR assay to 6 *nuc* gene copies/μl of the template. However, for samples containing less than 60 *nuc* gene copies/μl, more nonspecific side products than specific amplicons were generated. Binding of SYBR Green I to both the undesired PCR side products and the specific amplicon masked the signal of the latter, thus hindering accurate quantification of low copy numbers. Therefore, further experiments concentrated on the performance of a TaqMan probe for the quantification of *S. aureus*.

(ii) TaqMan probe with the LightCycler. The detection limit of the TaqMan probe was 60 copies of the *nuc* gene/μl. Once again, application of hot-start PCR improved the detection limit to 6 *nuc* gene copies/μl of the template DNA. Contrary to what occurred in SYBR Green I-based quantification, the signal generation through cleavage of the TaqMan probe was strictly specific. Therefore, an accurate quantification of low copy numbers was possible. All further experiments were then conducted by applying the TaqMan probe.

Quantification sensitivity and repeatability of RTQ-PCR results using either a commercial kit or an in-house reaction mix formula. To this point, all experiments had been performed using commercially available RTQ-PCR kits. The same quantification sensitivity as that obtained with the TaqMan probe and the kit for hot-start PCR was achieved when we used a PCR mix including Platinum *Taq* DNA polymerase (6 *nuc* gene copies/μl of the template DNA). This enzyme is inactive at room temperature as well. Further experiments compared the repeatability of the results obtained with the commercially available kit with those obtained with the PCR mix containing Platinum *Taq* DNA polymerase.

For each step of a 10-fold dilution series ranging from 6×10^4 to 6×10^0 copies/μl, the *nuc* gene of *S. aureus* was quantified six times after both polymerases were applied. Mean C_T values and standard deviations are shown in Table 3. No statistically significant differences between the methods were observed. However, mean C_T values were 1.03 to 2.07 lower when we used Platinum *Taq* DNA polymerase, indicating higher RTQ-PCR efficiency. Although both PCR mixtures worked satisfactorily, the in-house procedure was used for all further experiments due to budget limitations.

TaqMan probe with the LightCycler versus TaqMan probe with the ABI PRISM 7700 SDS. As the LightCycler system is rather new equipment, its performance was compared to that of the well-established ABI PRISM 7700 SDS. Experiments to measure the quantification sensitivity and repeatability of results were conducted on both systems. Mean C_T values and standard deviations for the measurements (repeated six times) of a 10-fold dilution series of the *nuc* gene of *S. aureus* ranging from 6×10^3 to 6×10^0 copies/μl are shown in Table 3. Regarding repeatability, no significant distinction was found between the two machines. The sensitivity of the RTQ-PCR assay in the ABI PRISM 7700 SDS was 6 copies of the *nuc* gene. The mean C_T values were 0.38 to 0.98 higher than in the LightCycler, suggesting a slightly lower efficiency for the RTQ-PCR.

In summary, the TaqMan probe-LightCycler configuration was found to be the most appropriate for quantification of the *nuc* gene of *S. aureus*. Therefore, it was used for applicability testing in a cheese model.

Quantification of *S. aureus* in artificially contaminated cheese with the TaqMan probe-LightCycler configuration. Cheeses of three different types (Tilsiter-style cheese, French Camembert made from raw milk, and fresh cheese made from raw goat's milk) were artificially contaminated with different concentrations of a pure culture of *S. aureus* DSM 1104. The samples were then subjected to DNA isolation in triplicate, and *nuc* gene copy numbers were measured twice by RTQ-PCR (Table 4). Mean *nuc* gene copy numbers were compared to the numbers of CFU determined by the plate count method. Quantification of *S. aureus* cells in Tilsiter and goat's cheese consistently revealed *nuc* gene copy numbers comparable to

TABLE 4. Quantification of the number of copies of the *nuc* gene of *S. aureus* in artificially contaminated cheeses of three different types by RTQ-PCR

Sample	No. of *nuc* gene copies or CFU/2 g of:					
	Tilsiter		Camembert		Goat's cheese	
	Copies	CFU	Copies	CFU	Copies	CFU
1	2.6×10^8	3.4×10^7	2.6×10^8	2.1×10^7	8.4×10^7	2.5×10^7
2	7.4×10^6	4.3×10^6	2.2×10^7	2.0×10^6	8.6×10^6	3.4×10^6
3	4.6×10^5	2.2×10^5	3.6×10^6	2.0×10^5	5.2×10^5	3.8×10^5
4	4.0×10^4	3.0×10^4	3.0×10^5	1.6×10^4	2.2×10^4	2.9×10^4
5	9.2×10^3	2.0×10^3	1.5×10^4	2.1×10^3	2.2×10^3	3.8×10^3
6	3.8×10^2	3.5×10^2	2.4×10^3	1.5×10^2	1.5×10^2	2.9×10^2
7	—[a]	2.0×10^1	6.4×10^2	2.0×10^1	—	3.6×10^1

[a] —, inconsistent quantification data.

numbers of CFU. Using Camembert, copy numbers were 1 log unit higher than numbers of CFU. The calculated coefficients of correlation (r^2) between log copy numbers of the *nuc* gene and log CFU were 0.979 for Tilsiter, 0.996 for Camembert, and 0.998 for goat's cheese. The limit of quantification was 3.8×10^2 *nuc* gene copies/2 g of Tilsiter, 6.4×10^2 copies/2 g of Camembert, and 1.5×10^2 copies/2 g of goat's cheese. Samples containing lower copy numbers did not reveal consistent quantification results.

DISCUSSION

SYBR Green I with the LightCycler versus the TaqMan probe with the LightCycler. The dsDNA-binding dye SYBR Green I and a TaqMan probe were tested in different RTQ-PCR systems (LightCyler and ABI PRISM 7700 SDS) to determine the number of copies of the *S. aureus nuc* gene.

Accurate quantification of low copy numbers of the *nuc* gene of *S. aureus* using SYBR Green I was hindered due to the accumulation of primer dimers and nonspecific side products which were bound by the SYBR Green I dye. Measurement of the ratio of specific to nonspecific PCR products by determining the melting peak integration enabled quantification of low copy numbers (22). However, this procedure requires further post-PCR analysis of the data. In another publication, fluorescence data collection at a temperature above the T_ms of nonspecific PCR products minimized biased quantification of low copy numbers of the gene (19). In our study, the T_ms of nonspecific side products exceeded 72°C. Thus, the procedure would have required another PCR step subsequent to primer extension. To improve the sensitivity, an addition of tRNA to the PCR mix (23) may further reduce nonspecific side products.

To overcome the shortcomings of SYBR Green I for the quantification of the *nuc* gene of *S. aureus*, a TaqMan probe was applied.

Optimization included testing two different *Taq* DNA polymerases with respect to the 5′-3′ exonuclease activities of the enzymes for cleavage of the probe. The FastStart *Taq* DNA polymerase, included in a commercial kit designed to be used in conjunction with hybridization probes (15) (which represents a slightly different approach to RTQ-PCR using fluorogenic probes), proved to be sufficient for sensitive quantification of the *nuc* gene using the TaqMan probe. Application of an in-house PCR mix containing Platinum *Taq* DNA polymerase also enabled sensitive quantification, with which, according to the product information, no 5′-3′ exonuclease activity should be present. Moreover, C_T values were 1.03 to 2.07 lower when this enzyme was applied, suggesting a higher efficiency of cleavage of the TaqMan probe. A recently published study used the same DNA polymerase for quantification of *bcr* and *abl* fusion transcripts by a TaqMan probe (15). When our assay configuration was applied to the LightCycler, repeatability was in accordance with recently published data (7, 16).

TaqMan probe with the LightCycler versus TaqMan probe with the ABI PRISM 7700 SDS. The performance of the air-heated LightCycler was compared to that of the well-established ABI PRISM 7700 SDS 96-well block cycler. The algorithms for calculation of the initial target copy number and acquisition of fluorescence emission data differ substantially between both systems. In the ABI PRISM 7700 SDS, the increase in fluorescence emission of the reporter dye, generated by cleavage of the probe, is normalized either to the fluorescence emission of an additional fluorescent dye or to that of the quencher dye. Normalization to the emission of a separately added fluorescent dye is not feasible in the LightCycler. Excitation and data acquisition in the ABI PRISM 7700 SDS are performed continuously in parallel for all samples via fiber optic cables. In the LightCycler a high-precision stepper motor positions each sample above the fluorimetric optics, thus enabling data acquisition from each sample by the same optical device. However, both approaches led to comparable results in the present study. For high-throughput applications, the 96-well machine may be advantageous. For establishing new RTQ-PCR assays or examining smaller numbers of samples, the 32-capillary LightCycler may be preferable as it enables performance of PCR in less than 1 h and is much less expensive.

Quantification of *S. aureus* cells in cheese by RTQ-PCR. When we compared *nuc* gene copy numbers in *S. aureus* cells and numbers of CFU, matrix-dependent effects were observed. In the case of Tilsiter and fresh goat's cheese, CFU and *nuc* gene copy numbers were nearly identical. In the Camembert, about 1 log unit more *nuc* gene copies than CFU was detected. This result suggests different extraction efficiencies. Application of MPN-PCR for quantification of the enterotoxin *c*1 gene in fresh cheese revealed a copy number 2 log units higher than the number of CFU (17). Mäntynen et al. explained the bias by the detection of dead and stressed unculturable bacterial cells

through PCR. The difference of 1 log unit in the Camembert suggests that only 10% of bacterial cells were culturable and therefore detectable by the plate count method. As fresh overnight cultures were applied for contamination, this should not have been the case in our experiments. Another explanation may be that *S. aureus* formed clusters during multiplication. They may not have been sufficiently broken up before the samples were plated onto agar plates. In this case 1 CFU would originate from more than one bacterial cell. However, the coefficients of correlation between copy numbers and numbers of CFU ranged from 0.979 to 0.998 for all cheese matrices tested. Therefore, the calculation of the number of CFU of *S. aureus* via determination of *nuc* gene copy numbers is possible.

The quantification limits of 20 and 290 CFU/2 g in raw milk Camembert and fresh goat's cheese, respectively, are sufficient for examination according to the national regulations (m = 1,000/g and M = 10,000/g). On the other hand, as Tilsiter is made from pasteurized milk, an m of 100/g and an M of 1,000/g apply. Therefore, the sensitivity of 350 CFU/2 g is sufficient only for detecting a transgression of the M value. When applying PCR-based quantification methods to heat-processed products, it must be considered that the DNAs from bacteria are detected whether they are alive or dead. In foodstuffs *S. aureus* is reliably killed when it is subjected to appropriate pasteurization conditions. However, the enterotoxins are still harmful due to their heat stability. Therefore PCR-based quantification methods which display the total contamination level of a toxin-producing pathogen present a distinct advantage over conventional microbiological methods.

In conclusion, the suitability of RTQ-PCR for fast quantification of *S. aureus* cells in cheese using this particular technique was demonstrated. With regard to the different RTQ-PCR configurations, it was shown (i) that TaqMan probes are superior to SYBR Green I with respect to quantification of low copy numbers of the target gene by RTQ-PCR and (ii) that both types of RTQ-PCR equipment, the LightCycler and the ABI PRISM 7700 SDS, lead to comparable results despite differences in hardware and software.

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