FEMS Microbiology Letters 252 (2005) 267–272

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk

Hannah J. Jørgensen [a], Tone Mathisen [a], Astrid Løvseth [a], Katsuhiko Omoe [b], Kristina S. Qvale [c], Semir Loncarevic [a,*]

[a] *Department for Feed and Food Hygiene, National Veterinary Institute, PB 8156 dep, 0033 Oslo, Norway*
[b] *Department of Veterinary Microbiology, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan*
[c] *Labpartner IKS, Elverum, Norway*

Received 8 August 2005; received in revised form 2 September 2005; accepted 2 September 2005

First published online 26 September 2005

Edited by J-I. Flock

Abstract

Mashed potato made with raw bovine milk was suspected to have been the source of a food poisoning outbreak. Almost 8×10^8 *Staphylococcus aureus* CFU g^{-1} were detected in the mashed potato. *S. aureus* was also found in bulk milk from the farm that had supplied milk for the mashed potato. Isolates from mashed potato and bulk milk were positive for the gene encoding staphylococcal enterotoxin H (*seh*), and the corresponding protein toxin, SEH, was detected by ELISA in the mashed potato. Production of SEH by *S. aureus* isolates from mashed potato ($n = 4$) and bulk milk ($n = 4$) was also demonstrated by ELISA. Sequencing of *seh* from one mashed potato isolate and one bulk milk isolate confirmed that the gene was a variant *seh*, and that the genes in both isolates were identical. Macrorestriction of chromosomal DNA with *Sma*1 followed by pulsed-field gel electrophoresis of *seh*-positive *S. aureus* from mashed potato and bulk milk revealed indistinguishable banding patterns between isolates from both sources. It seems likely that raw bovine milk used in the preparation of mashed potato contained *S. aureus* that subsequently produced sufficient SEH in the mashed potato to cause food poisoning.

Keywords: Staphylococcal food poisoning; Staphylococcal enterotoxin; SEH; Raw milk

1. Introduction

Staphylococcal food poisoning (SFP) is caused by ingestion of food containing preformed staphylococcal enterotoxin (SE), produced by some strains of *Staphylococcus aureus* and occasionally by other staphylococci [1,2]. Symptoms have a rapid onset (1–6 h), and typically include vomiting, diarrhoea and stomach cramps [1]. An outbreak of SFP is usually suspected if more than one person is affected with these symptoms shortly after eating. However, food poisoning caused by the emetic toxin of *Bacillus cereus* is an important differential to SFP because it causes similar symptoms also with a rapid onset [3].

To date, 18 SEs have been described and designated SEA–SEE, SEG–SER and SEU [4–11]. Some of the SEs lack the ability to cause emesis (SEL, SEQ) [11,12], and others have yet to be tested for emetic potential (SEJ, SEK, SEM–SEP, SER and SEU). These SEs are more appropriately referred to as SE-like proteins (SEl) [13], and the role of the SEls in SFP is unclear. Remaining SEs have been implicated in outbreaks of SFP in various reports [14–19].

* Corresponding author. Tel.: +47 23216248; fax: + 47 23216202.
E-mail address: semir.loncarevic@vetinst.no (S. Loncarevic).

0378-1097/$22.00
doi:10.1016/j.femsle.2005.09.005

Because SEs are stable with respect to heat and storage, they may be present in foods where viable *S. aureus* are absent [1]. Moreover, not all strains of *S. aureus* produce SE. Therefore, a conclusive diagnosis of SFP should be based on the demonstration of SE in the food. To date, commercial kits for identification of SEs are only available for SEA–SEE, and if none of these are present in suspected foods the identification of SE-genes in available *S. aureus* isolates by PCR may be useful.

In December 2003, five children aged 2–6 years, and three adults became sick after eating lunch together in a kindergarten. The symptoms occurred approximately 1 h after eating and included vomiting, diarrhoea and abdominal cramping. All eight persons were fully recovered 24 h after onset of disease. For the lunch, sausage and mashed potato, leftovers from a Christmas party held the night before, had been re-heated. The mashed potato had been prepared with raw milk. Not all the affected persons had eaten sausage, but all had eaten mashed potato. Based on the history, SFP from the mashed potato was suspected. The aim of the present study was to identify the cause of the food poisoning.

2. Materials and methods

Preliminary bacteriological analyses of mashed potato and sausage were performed at Labpartner, Elverum, Norway. Bacteriological analysis of bulk milk, detection of SEA–SEE and SE-genes, genotyping, and sequencing were performed at the National Veterinary Institute in Oslo (NVI). Detection of SEH was performed at the Department of Veterinary Microbiology, Faculty of Agriculture, Iwate University in Japan.

2.1. Samples

Samples of sausage and mashed potato (scraps from the lunch) were collected, chilled and submitted to Labpartner IKS for bacteriological analyses. A sample of bulk milk from the dairy farm that had supplied raw bovine milk for the mashed potato was collected in a sterile plastic vial, and sent chilled overnight to NVI.

2.2. Bacteriological analyses

Mashed potato was streaked on glass slides, air-dried and analysed by direct microscopy using Gram staining (Difco, Sparks, MD, USA). Serial 10-fold dilutions of mashed potato and sausage were prepared in peptone water and analysed by standard methods for *S. aureus* [20], using sheep blood agar (BA) (Oxoid, Basingstoke, UK) and Baird Parker agar with a rabbit plasma fibrinogen supplement (BP + RPF) (bioMérieux, Marcy-l'Etoile, France), and for *B. cereus* [21], using *B. cereus* agar (Oxoid) and BA. Bulk milk was analysed for *S. aureus* as described above, but using BA with bovine blood. Fourteen and nine *S. aureus* isolates from mashed potato and raw milk, respectively, and one isolate of *B. cereus* from mashed potato, were stored in Heart Infusion Broth (Difco) with 15% glycerine at −80 °C until further analyses.

2.3. Analyses for SEA–SEE in food samples

Mashed potato, sausage and bulk milk were tested for SEA–SEE by the Transia plate-staphylococcal enterotoxin kit (Diffchamb, Västra Frölunda, Sweden) according to the manufacturer's recommendations. The method is based on an ELISA performed on the resulting supernatant after homogenisation of 25 g of a food sample with distilled water, adjustment of pH depending on sample-type, and centrifugation. Supernatants from samples of milk and milk products are, according to the protocol, additionally subjected to dialysis against polyethylene glycol (PEG) before the ELISA. The mashed potato sample was subjected to this dialysis step because it was prepared with milk.

2.4. Analysis of toxin production by bacterial isolates

S. aureus isolates from mashed potato ($n = 14$) and from bulk milk ($n = 9$) were tested for production of SEA–SED by SET-RPLA (Oxoid). Before testing, isolates were plated on BA and incubated aerobically at 37 °C for 24 h. One colony was picked from the BA plate and inoculated into Tryptic Soy Broth (Difco) and incubated aerobically at 37 °C for 24 h. The SET-RPLA kit was then used according to the manufacturer's instructions.

Detection of *B. cereus* toxins was performed at the reference laboratory for Gram-positive spore-forming food pathogens at the Norwegian School of Veterinary Science in Oslo. One *B. cereus* isolate from mashed potato was tested by a Vero cell cytotoxicity assay [22] and a sperm motility test [23] for *B. cereus* enterotoxin and emetic toxin, respectively.

2.5. Detection of SE-genes in S. aureus isolates

S. aureus from mashed potato ($n = 14$) and from bulk milk ($n = 9$) were tested for the presence of SE-genes (*sea–see*, *seg–sej*) and the toxic shock syndrome toxin (TSST-1) gene (*tst*). DNA extraction using CTAB, and multiplex PCR (m-PCR) was performed as described previously [24]. The method includes primers for 16S rRNA for control of DNA isolation, and four positive control strains (FRI 913, 3169, R5460, R5010) that together include all the genes detected by the m-PCR. MilliQ water was used as negative control.

2.6. ELISA for detection of SEH

A sandwich ELISA was performed to determine the concentration of SEH in mashed potato as described previously [25], with slight modifications. The extract obtained from 25 g mashed potato for the Transia method, as described above, was subjected to this assay. Sandwich ELISA was performed using microtiter plates coated with rabbit anti-SEH immunoglobulin G (IgG) and a horseradish peroxidase-labelled anti-SEH monospecific antibody previously obtained from rabbit immunisation [25]. The concentration of SEH in the mashed potato extract was determined by converting the absorbance value of the corresponding concentration by use of a standard curve. In order to estimate an interference of protein A, another ELISA was performed using IgG from non-immunised rabbits as coating antibody.

In order to test the ability of *seh*-positive *S. aureus* isolates to produce SEH, the same ELISA procedure was used to detect SEH in culture supernatants from *seh*-positive isolates from mashed potato ($n = 4$) and bulk milk ($n = 4$). The isolates were grown in double concentration heart infusion broth (Difco) for 24 h. Culture supernatants were pre-incubated in normal rabbit serum at 4 °C overnight and then diluted in phosphate buffered saline–Tween 20 to avoid non-specific reaction caused by protein A.

2.7. Sequencing

DNA from two of the *seh*-positive *S. aureus* strains from mashed potato (VI 50671) and bulk milk (VI 50695), respectively, were used for sequencing *seh*. Primers are numbered and presented in Table 1, some of these were described previously while others were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Primers 1 and 2 were used for the initial PCR. Each reaction mixture contained 3 units of AmpliTaq Gold® DNA polymerase and 1× AmpliTaq Gold® buffer, 4 mM $MgCl_2$, 400 μM dNTP-mix (all from Applied Biosystems, Foster City, CA, USA), 300 nM of each primer, milliQ water to a total volume of 45 μl, and 5 μl of DNA (ca. 10 ng μl^{-1}). The amplification was performed in an MJ Research thermocycler PTC 225 (GMI, Ramsey, MN, USA), with initial denaturation at 95 °C for 10 min, followed by 31 cycles of 95 °C for 1 min, 54 °C for 45 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min.

PCR products were purified using ExoSAP-IT (Amersham Biosciences, Freiburg, Germany). Sequencing was performed both in the forward and in the reverse direction using the primers 1–5 and BigDye terminator reaction mix v 3.1 (Applied Biosystems) with PCR conditions as recommended by the manufacturer. The sequence reactions were precipitated using the ethanol/EDTA protocol recommended by Applied Biosystems. Purified sequence reactions were loaded on the ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems), and the results were analysed using DNA Sequencing Analysis Software v 3.7 (Applied Biosystems). Sequences were aligned in BioEdit Version 7.0.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) (April 2005) and submitted to EMBL (http://www.ebi.ac.uk/embl/Submission/webin.html).

Obtained sequences were translated into amino acid sequences using ExPASy (http://au.expasy.org), and aligned in BioEdit with respect to nucleic acid and amino acid sequences with two *seh* variants from *S. aureus* strains FRI 137 and D4508 (GenBank Accession Numbers AY345144 and U11702, respectively).

2.8. Pulsed-field gel electrophoresis of S. aureus

The *seh*-positive *S. aureus* isolates from mashed potato ($n = 14$) and from bulk milk ($n = 5$) were genotyped by pulsed-field gel electrophoresis (PFGE). Preparation of chromosomal DNA, enzymatic digestion with *Sma*1 and electrophoresis were performed as described by McDougal et al. [26], but with an 18 h electrophoresis time. Identification of PFGE banding patterns was performed by a combination of visual inspection and computer analysis (BioNumerics v 4.00, Applied Maths, Kortrijk, Belgium). Isolates with indistinguishable banding patterns were assigned to the same pulsotype (PT) and designated Arabic numbers.

3. Results

Direct microscopy of mashed potato revealed Gram-positive bacilli and cocci. *S. aureus* was found at levels

Table 1
DNA sequences of the primers used for sequencing *seh*

No.	Primer name	Primer sequence (5′–3′)	Reference	Genbank Accession No.
1	*seh*-1, forward	TCCATTCTAACTACTATAGCAACTGAT	Present study	U11702
2	*seh*-1, reverse	GAAATTTTCATTGATTATACTTTTT	Present study	U11702
3	*Bam*HI-2, forward	ATATGGATCCATGGAAGATTTACACGATAAAAGT	[36]	AY345144
4	*seh*-2, forward	GGAAAGGGTGATTGGTGCTA	Present study	U11702
5	*seh*-3, reverse	TCATTGCCACTATCACCTTGA	Present study	U11702

of 7.8×10^8 and 2×10^2 CFU g^{-1} in mashed potato and in sausage, respectively. *B. cereus* was found at levels of 1.8×10^6 and 2.5×10^3 CFU g^{-1} in mashed potato and sausage, respectively. A concentration of 9×10^2 CFU $ml^{-1}$of *S. aureus* was identified in bulk milk.

Enterotoxins SEA–SEE were not detected in samples of mashed potato, sausage or bulk milk, and none of the 19 *S. aureus* isolates tested by RPLA produced SEA through SED. The *B. cereus* isolate was negative for emetic-like toxin and enterotoxin. All 14 *S. aureus* isolates from mashed potato and five of the nine isolates from bulk milk were positive for fragments of *seh* by m-PCR.

By sandwich ELISA, SEH was detected at a level of 277.8 ng ml^{-1} in the mashed potato dialysate. Since 5 ml of dialysate was retrieved from a 25 g sample of mashed potato, it was estimated that the mashed potato contained approximately 55.5 ng g^{-1} of SEH. In addition, no significant signal was observed in the ELISA assay with non-immunised rabbit IgG coated microtiter plate, suggesting that the interference of protein A was negligible. All eight *S. aureus* isolates tested produced significant amounts of SEH. The four isolates from mashed potato produced between 98 and 108 ng ml^{-1}, and the four isolates from bulk milk produced between 96 and 99 ng ml^{-1}.

The obtained full-length sequences for VI 50671 and VI 50695 were identical and were assigned GenBank accession numbers AJ937548 and AJ937549, respectively. Alignment of the present sequence with the *seh* of FRI 137 and D4508 revealed three and one base pair differences, respectively. The predicted amino acid sequence of the present gene was identical to that of D4508, but differed by one amino acid from the SEH of FRI 137.

The 19 *seh*-positive *S. aureus* isolates were typed by PFGE. All 14 isolates from mashed potato and four of the bulk milk isolates belonged to the same pulsotype (PT A). One bulk milk isolate belonged to a different pulsotype (PT B) (Fig. 1).

4. Discussion

An outbreak of SFP was suspected after eight persons became ill with vomiting, stomach cramps and diarrhoea shortly after eating lunch together. Almost 8×10^8 CFU *S. aureus* and 2×10^6 CFU *B. cereus* were enumerated per gram of mashed potato. The sausage was most likely contaminated by the mashed potato since they were stored together. Because foods with levels of SE-producing *S. aureus* greater than 10^5 CFU g^{-1} are considered a risk with respect to SFP [1], *S. aureus* was believed to be the cause of the outbreak. The detection of *seh* in *S. aureus* isolates prompted further investigations that ultimately revealed SEH in the mashed potato.

The concomitant finding of *B. cereus* in the food samples initially complicated diagnosis, but since the tested isolate produced neither emetic toxin nor enterotoxin *B. cereus* is unlikely to have caused the outbreak. Compared to SFP, food poisoning caused by *B. cereus* emetic toxin is rare in Norway. While the Norwegian reference laboratory for *S. aureus* food poisoning detected SE in 24 food samples suspected of causing SFP between 2000 and 2003 [27], the reference laboratory for

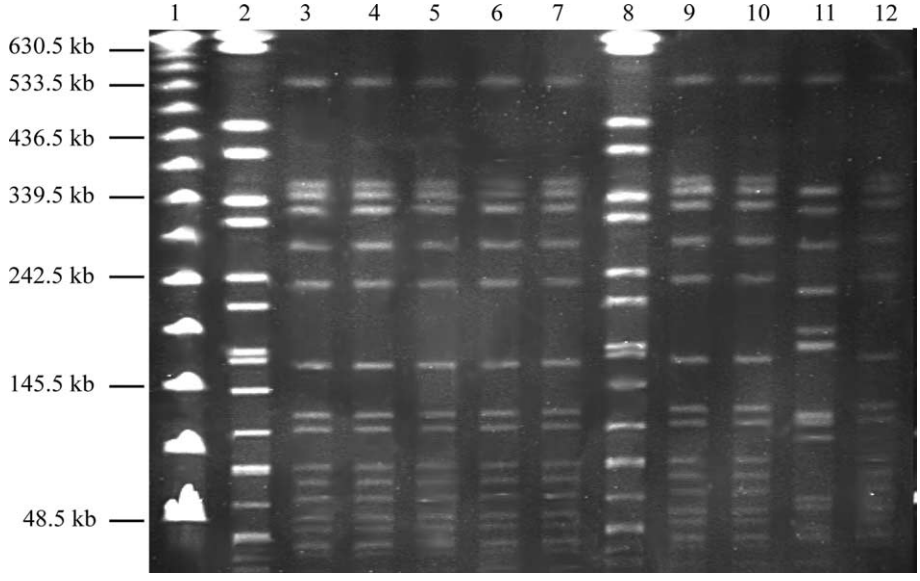

Fig. 1. *Sma*1 restriction profiles as shown by PFGE of 9 *seh*-positive *S. aureus* isolates. Lane 1: lambda ladder; Lanes 2 and 8: internal control (*Salmonella* braenderup); Lanes 3–7: *S. aureus* isolates from mashed potato; Lanes 9–12: *S. aureus* isolates from bovine bulk milk. Isolates in lanes 3–7 and 9, 10 and 12 have the same banding pattern (pulsotype A), while the isolate in lane 11 is different (pulsotype B).

Gram-positive spore-forming food pathogens has reported four emetic toxin-positive *B. cereus* strains from food poisoning outbreaks since 2002 (P.E. Granum, pers. commun.).

The amount of SE necessary to cause symptoms of SFP depends on individual susceptibility and SE type. The emetic dosages (ED_{50}) of SEA–SEE in monkeys vary between 5 and 20 μg per animal [28], but humans may be more sensitive. A total dose of 100–200 ng (0.1–0.2 μg) SEA per person was estimated to have caused symptoms in an outbreak of SFP [16]. In one study, 30 μg of SEH elicited emesis when administered orally to a monkey [29], in the present outbreak, ingestion of a small portion (50 g) of mashed potato would have provided a dose of ca. 2.8 μg of SEH. It was confirmed that *seh*-positive *S. aureus* were able to produce significant quantities of SEH in broth, which is in agreement with previous observations [25].

SEH was first described by Ren et al. [30], but the toxin was not tested for its ability to cause emesis in primates. A variant SEH was later shown to be emetic to monkeys [29], and *seh* has since been detected in *S. aureus* isolates from food poisonings [25,31]. One report from 1996 implicated SEH in an outbreak of SFP from cheese on the basis that *S. aureus* strains isolates from the cheese produced SEH [18]. In a recent study, SEA and SEH were detected together in reconstituted milk involved in an outbreak of SFP in 2000 [32].

Sequencing confirmed that the two *S. aureus* isolates from mashed potato and from bulk milk contained an identical SE-gene. The gene was confirmed to be a variant of *seh* because its sequence differed less than 10% from two previously reported *seh* variants (Accession Nos.: AY345144 and U11702) [13].

Isolates of *S. aureus* with identical PFGE restriction profiles were found in mashed potato and in bulk milk from the farm that had supplied milk for the mashed potato. This finding strongly suggests that bulk milk was the source of *S. aureus* in the mashed potato. A food poisoning outbreak very similar to the present outbreak was described in Norway in 1951 [33]. Twelve persons who had eaten sausages, and mashed potato made with raw milk became ill with typical symptoms of SFP. Coagulase-positive staphylococci were found in the mashed potato, and in milk from a mastitic mammary quarter of a cow that had supplied milk used for preparing the mashed potato. Bacterial isolates from mashed potato and mastitic milk were identical phage types.

S. aureus is a common cause of bovine mastitis. The bacteria may be shed in the milk of symptomatic and asymptomatic carrier animals and thus contaminate bulk milk [34]. In a recent Norwegian study, *S. aureus* was identified in 75% of bovine bulk milk samples, and approximately 55% of the tested isolates contained SE-genes. Out of these, 11% contained *seh* [35]. The PFGE profiles of isolates in the present study were compared with the PFGE profiles of 11 *seh*-positive *S. aureus* isolates collected from bovine bulk milk and raw milk products in Norway (unpublished data). Among these, three isolates had PFGE banding patterns indistinguishable from the banding patterns of isolates observed in the present study. Most commonly, food handlers are the source of *S. aureus* contamination in outbreaks of SFP [1], but in outbreaks involving raw milk and raw milk products dairy animals are possibly equally likely to be the source of the outbreak strain.

Based on high levels of *S. aureus* and the detection of SEH in the mashed potato, the food poisoning outbreak is considered to have been an incident of SFP. However, because nine SE-genes were not tested for, the possible presence of other SEs cannot be completely excluded. *S. aureus* in the raw milk used for preparing mashed potato appears to have caused the outbreak, and shows that *S. aureus* in bulk milk may pose a risk of SFP from foods prepared with raw milk. The present study also provides further evidence that *seh*-positive *S. aureus* may produce sufficient SEH to cause intoxication.

Acknowledgements

The authors are most grateful to Liv Marit Rørvik for reading the manuscript and Øyvin Østensvik for kindly testing *B. cereus* for toxins.

References

[1] Jablonski, L.M. and Bohach, G.A. (1997) *Staphylococcus aureus* In: Food Microbiology. Fundamentals and Frontiers (Doyle, L.R., Beuchat, L.R. and Montville, T.J., Eds.), pp. 353–375. American Society for Microbiology Press, Washington.

[2] Genigeorgis, C.A. (1989) Present state of knowledge on staphylococcal intoxication. Int. J. Food Microbiol. 9, 327–360.

[3] Granum, P.E. (1997) *Bacillus cereus* In: Food Microbiology Fundamentals and Frontiers (Doyle, M.P., Beuchat, L.R. and Montville, T.J., Eds.), pp. 327–336. American Society for Microbiology Press, Washington.

[4] Dinges, M.M., Orwin, P.M. and Schlievert, P.M. (2000) Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev. 13, 16–34.

[5] Fitzgerald, J.R., Monday, S.R., Foster, T.J., Bohach, G.A., Hartigan, P.J., Meaney, W.J. and Smyth, C.J. (2001) Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. J. Bacteriol. 183, 63–70.

[6] Jarraud, S., Peyrat, M.A., Lim, A., Tristan, A., Bes, M., Mougel, C., Etienne, J., Vandenesch, F., Bonneville, M. and Lina, G. (2001) *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J. Immunol. 166, 669–677.

[7] Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., Cui, L., Oguchi, A., Aoki, K., Nagai, Y., Lian, J., Ito, T., Kanamori, M., Matsumaru, H., Maruyama, A., Murakami, H., Hosoyama, A., Mizutani-Ui, Y., Takahashi, N.K., Sawano, T., Inoue, R., Kaito, C., Sekimizu, K., Hirakawa, H.,

Kuhara, V.G., Goto, S., Yabuzaki, J., Kanehisa, M., Yamashita, A., Oshima, K., Furuya, K., Yoshino, C., Shiba, T., Hattori, M., Ogasawara, N., Hayashi, H. and Hiramatsu, K. (2003) Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet 357, 1225–1240.

[8] 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.

[9] Omoe, K., Hu, D.L., Takahashi-Omoe, H., Nakane, A. and Shinagawa, K. (2003) Identification and characterization of a new staphylococcal enterotoxin-related putative toxin encoded by two kinds of plasmids. Infect. Immun. 71, 6088–6094.

[10] Orwin, P.M., Leung, D.Y., Donahue, H.L., Novick, R.P. and Schlievert, P.M. (2001) Biochemical and biological properties of staphylococcal enterotoxin K. Infect. Immun. 69, 360–366.

[11] Orwin, P.M., Leung, D.Y., Tripp, T.J., Bohach, G.A., Earhart, C.A., Ohlendorf, D.H. and Schlievert, P.M. (2002) Characterization of a novel staphylococcal enterotoxin-like superantigen, a member of the group V subfamily of pyrogenic toxins. Biochemistry 41, 14033–14040.

[12] Orwin, P.M., Fitzgerald, J.R., Leung, D.Y., Gutierrez, J.A., Bohach, G.A. and Schlievert, P.M. (2003) Characterization of *Staphylococcus aureus* enterotoxin L. Infect. Immun. 71, 2916–2919.

[13] Lina, G., Bohach, G.A., Nair, S.P., Hiramatsu, K., Jouvin-Marche, E. and Mariuzza, R. (2004) Standard nomenclature for the superantigens expressed by *Staphylococcus*. J. Infect. Dis. 189, 2334–2336.

[14] Bone, F.J., Bogie, D. and Morgan-Jones, S.C. (1989) Staphylococcal food poisoning from sheep milk cheese. Epidemiol. Infect. 103, 449–458.

[15] Cho, N., Shutou, A., Niibori, S., Takeda, Y., Ogawa, H., Hayashi, S., Funatogawa, K. and Hirai, Y. (2002) Food poisoning due to staphylococcal enterotoxins G and I. In: Conference Proceeding. 10th International Symposium on Staphylococci and Staphylococcal Infections, p. 80, Tsukuba, Japan.

[16] Evenson, M.L., Hinds, M.W., Bernstein, R.S. and Bergdoll, M.S. (1988) Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. Int. J. Food Micribiol. 7, 311–316.

[17] Schønberg, K.C. and Wåltorp, M. (2001) Staphylococcal food poisoning after consumption of goat cheese made from raw milk (in Norwegian). MSIS-rapport. 47, Norwegian Institute of Public Health, Oslo, Norway.

[18] Pereira, M.L., DoCarmo, L.S., dosSantos, E.J., Pereira, J.L. and Bergdoll, M.S. (1996) Enterotoxin H in staphylococcal food poisoning. J. Food Prot. 59, 559–561.

[19] Ewald, S. (1992) Staphylococcal food poisoning and detection of staphylococcal enterotoxins in food (In Norwegian). Nor. Vet. J. 104, 5–24.

[20] NMKL. (2003) Method No. 66, 4th Edn. *Staphylococcus aureus*, Enumeration in foods, Nordic Committee on Food Analysis.

[21] NMKL. (2003) Method No. 67, 5th Edn. *Bacillus cereus*, Determination in foods, Nordic Committee on Food Analysis.

[22] Sandvig, K. and Olsnes, S. (1982) Entry of the toxic proteins abrin, modeccin, ricin, and diphtheria toxin into cells. II. Effect of pH, metabolic inhibitors, and ionophores and evidence for toxin penetration from endocytotic vesicles. J. Biol. Chem. 257, 7504–7513.

[23] Andersson, M.A., Mikkola, R., Helin, J., Andersson, M.C. and Salkinoja-Salonen, M. (1998) A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores. Appl. Environ. Microbiol. 64, 1338–1343.

[24] Løvseth, A., Loncarevic, S. and Berdal, K. (2004) Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. J. Clin. Microbiol. 42, 3869–3872.

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

[26] McDougal, L.K., Steward, C.D., Killgore, G.E., Chaitram, J.M., McAllister, S.K. and Tenover, F.C. (2003) Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. 41, 5113–5120.

[27] Loncarevic, S., Mathisen, T. and Økland, M. (2004) Occurrence of *Staphylococcus aureus* enterotoxins in foods in Norway, In: Conference Proceeding, 5th World Congress on Foodborne Infections and Intoxications, p. 101, Berlin.

[28] Bergdoll, M.S. (1979) Staphylococcal intoxications In: Food-Borne Infections and Intoxications (Riemann, H. and Bryan, F.L., Eds.), 2nd Edn, pp. 443–494. Academic Press, Inc., New York.

[29] Su, Y.C. and Wong, A.C. (1995) Identification and purification of a new staphylococcal enterotoxin H. Appl. Environ. Microbiol. 61, 1438–1443.

[30] Ren, K., Bannan, J.D., Pancholi, V., Cheung, A.L., Robbins, J.C., Fischetti, V.A. and Zabriskie, J.B. (1994) Characterization and biological properties of a new staphylococcal exotoxin. J. Exp. Med. 180, 1675–1683.

[31] McLauchlin, 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.

[32] Ikeda, T., Tamate, N., Yamaguchi, K. and Makino, S. (2005) Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins a and h. Appl. Environ. Microbiol. 71, 2793–2795.

[33] Hauge, S. (1952) An outbreak of staphylococcus poisoning caused by staphylococci from infected cow (in Norwegian). Nord. Hyg. J. 3–4, 113–121.

[34] Hahn, G. (1996) Pathogenic bacteria in raw milk – situation and significanceBacteriological Quality of Raw Milk, pp. 67–83. International Dairy Federation, Brussels.

[35] Jørgensen, H.J., Mørk, T., Høgåsen, H.R. and Rørvik, L.M. (2005) Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. J. Appl. Microbiol. 99, 158–166.

[36] Gul'ko, L.B., Voyushin, K.E., Fluer, F.S., Okorokova, N.A., Krivenko, M.S., Veiko, V.P. and Debabov, V.G. (2003) The obtaining of the tumor-addressed genetically engineered drug for cancer immunotherapy. II. Cloning a gene of the pro-enteroxin H (*seh*) from *Staphylococcus aureus*, its expression in *Escherichia coli*. Investigation of the enterotoxin H secretion by *E. coli* cells (in Russian). Biotekhnologiia 6, 72–78.