

Detection of the Genes for *Staphylococcus aureus* Enterotoxin By PCR

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Abstract

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The aim of this study was to determine the presence of genes for staphylococcal enterotoxins in group of 32 isolates of *Staphylococcus aureus* from 75 samples (sheep, cow and goat raw milk) collected from sub-clinically and clinically sick sheep, goats and cows raised in different agricultural farms in the Eastern region of Slovakia and also from 12 samples of sheep cheeses. Fifteen out of 32 isolates of *S. aureus* carried gene for enterotoxin C (SEC): 12 from sheep raw milk and one from each of goat and cow raw milk and sheep cheese, too. Fifteen out of 32 isolates carried gene for enterotoxin D (SED): 12 from sheep raw milk, two from cow raw milk and one from sheep cheese. Two out of 32 isolates carried genes for enterotoxin A (SEA) and B (SEB): one positive isolate for SEA from sheep raw milk and the other SEB from sheep cheese.

Cheese, cow, goat, raw milk, sheep

Staphylococcus aureus produces different extra-cellular protein toxins and virulence factors, which stipulate its pathogenity due to their enterotoxins. The enterotoxins pass from the digestive tract to the blood circulation and consecutively activate the nerve centre of the emetic reflex, causing nausea, vomiting, abdominal cramps and diarrhea, which can lead to dehydration and the collapse of the organism (Gouloumes et al. 1996).

There are several types of staphylococcal enterotoxins: SEA, SEB, SEC₁, SEC₂, SEC₃, SED and SEE. It was found that some strains of *Staphylococcus aureus* produce more than one type of enterotoxins (Halpin-Dohnálek and Marth 1989). Therefore the presence of *Staphylococcus aureus* in the food can be potential risk for health, particularly, if the food is not stored at the prescribed temperature.

There are several procedures to detect staphylococcal enterotoxins. The production of enterotoxin needs long incubation time (20 h). Some factors, which affect the incubation period, are the pH, the water activity and the used substrates (Sharma et al. 2000). Numerous methods are based on the evidence of the enterotoxins directly in the food (ELISA, reversal passive latex agglutination and others), with a possibility to detect nanogram amounts of enterotoxins in one gram or in one mili-liter of food (Burdová et al. 1994; Gouloumes et al. 1996; Strachan et al. 1997). The advantage of these methods is that enterotoxins are detected even if the producer *Staphylococcus aureus* should not be identified by the classical bacteriological procedure, because it is usually devitalized by temperature.

DNA amplification methods (polymerase chain reaction, PCR) can show the presence of enterotoxigenic strains of *Staphylococcus aureus* before the expression of enterotoxins on the base of specific gene sequences and in this way detect the potential source of contamination. The advantage of the PCR methods is that it is able to detect genes which code the production of staphylococcal enterotoxins also from heat treatment of food, because the DNA remains unchanged (Tsen and Chen 1992; Holečková et al. 2000).

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Sharma et al. (2000) published multiplex PCR for the detection of all staphylococcus enterotoxins. They used one universal and five specific primers in one reaction. The result showed that this type of one-step PCR is very useful for detection of different staphylococcal enterotoxin genes. The aim of this work was to detect enterotoxins in sets of *Staphylococcus aureus*, isolated from the cow, sheep and goat raw milk and also sheep cheese.

Materials and Methods

Bacterial strains

Samples of *Staphylococcus aureus* were isolated by standard bacteriological guideline (EN ISO 6888) from the cow, sheep and goat raw milk and sheep cheese and stored at -20 °C in 50% (vol/vol) glycerol, 50% Brain heart infusion (BHI; Oxoid Ltd., England). Bacterial isolates were grown at 37 °C in BHI broth before the extraction of the DNA.

DNA isolation

Sediment obtained after centrifugation (3 min, 3500 x g) of 1.5 ml of broth culture (24 h, 37 °C) was washed with 100 l 0.5% Triton X-100 (Koch-Light Lab. Ltd., England). Sediment was resuspended in 100 l 0.5% Triton X-100. The isolates were incubated at 95 °C for 10 min and centrifuged at 3500 x g for 5 min. The supernatant was used as a source of DNA.

Oligonucleotide primers

Oligonucleotide primers were used for PCR reaction according to Sharma et al. (2000).

DNA amplification

PCR reaction was done according to Sharma et al. (2000).

Detection of amplified DNA

For detection, 10 l of PCR reaction mixture was electrophoresed on a 2% w/v agarose gel (Gibco BRL, USA), diluted in 1xTAE buffer (Sambrook et al. 1989), stained with ethidium bromide (Amresco, USA) in concentration 0.1 µg/ml and viewed under ultraviolet light. The 100 bp DNA Ladder (New England BioLabs, England) was used as a standard of molecular weight.

Results

We have examined 87 isolates of *S. aureus* collected from food samples: 53 from sheep raw milk, 21 from cow raw milk, one from goat raw milk and 12 from sheep cheese in which we detected each of the toxin gene product by multiplex PCR (Plate XV, Fig. 1).

The maximum number of staphylococcal isolates (75 isolates) was collected from two farms: No. 2 (54 isolates) and No. 3 (21 isolates). We detected a high number of enterotoxin-positive isolates from farm No. 2, where 24 (47%) isolates of *S. aureus* from sheep raw milk and one (33.3%) from sheep cheese were positive for SED, SEC and SEA enterotoxin genes (Table 1). On the other hand, three (14.3%) isolates of *S. aureus* from cow raw milk samples at farm No. 3 were positive for SED and SEC enterotoxin genes only.

The maximum numbers of staphylococcal isolates were positive for SEC and SED genes. We found that 15 isolates of *S. aureus* (17.2%) possess gene for enterotoxin SEC, 12 from sheep raw milk and one from each of the goat and cow raw milk and sheep cheese, respectively. The gene for enterotoxin D (SED) possesses the same amount of isolates *S. aureus* (17.2%), 12 from sheep raw milk, 2 from cow raw milk and one from sheep cheese. The genes for SEC and SED were detected mainly in staphylococci isolated from the sheep milk samples.

The gene for enterotoxin B (SEB) was detected only in one isolate (1.1%) of *S. aureus* from sheep cheese and the gene for enterotoxin A (SEA) was detected in one isolate (1.1%) from sheep raw milk, too (Table 1 and 2).

Table 1
Detection of staphylococcal enterotoxin genes in *S. aureus* isolates from food samples collected from different farms in the Eastern region of Slovakia

Type of food sample	Farm No.	No. of isolates (n)	Enterotoxin gene		Type
			No. of positive		
			(n)	%	
Sheep raw milk	1	2	1	50	SEC
	2	51	1	2	SEA
			11	21.6	SEC
			12	23.5	SED
Cow raw milk	3	21	1	4.8	SEC
			2	9.5	SED
Goat raw milk	4	1	1	100	SEC
Sheep cheese	2	3	1	33.3	SEC
	5	2	-	-	
	6	1	-	-	
	7	2	1	50	SEB
	8	1	-	-	
	9	1	-	-	
	10	1	-	-	
	11	1	1	100	SEC, SED
Total	11	87	32	36.8	

Table 2
Prevalence of staphylococcal enterotoxin genes in *S. aureus* isolates from food samples

Origin of <i>S. aureus</i>	No. of tested (n)	No. of enterotoxin positive samples					
		SEA	SEB	SEC	SED	SEE	%
Sheep raw milk	53	1	0	12	12	0	47.2
Goat raw milk	1	0	0	1	0	0	100
Cow raw milk	21	0	0	1	2	0	14.3
Sheep cheese	12	0	1	1	1	0	25
Total (n)	87	1	1	15	15	0	36.8
%		1.1 %	1.1 %	17.2 %	17.2 %	0	

Discussion

Many authors use PCR for detecting staphylococcal enterotoxin genes (Tornadijo et al. 1996; Holečková et al. 2000). All of them found a high variability (75-80%) in the presence of enterotoxin genes. Our experiments confirmed this fact as well (Table 1 and 2).

We detected the genes for four types of enterotoxin in our set of staphylococci. Three types of them (SEA, SEC and SED) were detected in bacteria isolated from samples of sheep raw milk; three types of enterotoxin genes (SEB, SEC and SED) were detected in bacteria isolated from samples of sheep cheese; one type of enterotoxin gene (SEC) was detected in bacteria isolated from samples of goat raw milk and two genes (SEC and SED) were detected in bacteria isolated from samples of cow raw milk. Majority of enterotoxin positive staphylococci (93.8 %) carry genes for enterotoxin SEC and SED. These isolates of *S. aureus* were collected from sheep raw milk (Table 2).

Some data published by other authors show that staphylococcal enterotoxin type C is the most frequent type in the cow and sheep milk (Tornadijo et al. 1996; Stephan et al. 1999).

In conclusion, we detected the presence of enterotoxin genes in *S. aureus* isolates using PCR. They were collected from the samples of cow, sheep and goat raw milk from subclinically and clinically sick animals from different agricultural farms in the Eastern region of Slovakia. Relatively high prevalence of enterotoxin positive strains, 29 out of 75 isolates (38.6 %), was probably in direct correlation to the health status of the mammary gland of these animals. Relatively high prevalence of enterotoxigenic isolates from the sheep raw milk in farm No. 2, 24 out of 51 isolates (47%), may probably influence the prevalence of enterotoxigenic *S. aureus* in other types of sheep food products, e.g. sheep cheese.

PCR is a rapid and extremely sensitive procedure, which is a very good tool for the detection of enterotoxin genes in clinical isolates of *S. aureus*. It can be used for specifying the staphylococcal infection of the mammary gland and to speed up the diagnosis of the hazardous staphylococcal strains.

Detekcia enterotoxínových génov *Staphylococcus aureus* pomocou PCR

Cieľom predloženej práce bolo stanoviť prítomnosť génov pre stafylokokové enterotoxíny v skupine 32 izolátov *Staphylococcus aureus* izolovaných zo 72 vzoriek (surové mlieko oviec, kráv a kôz) odobratých zo subklinických a klinický chorých oviec, kráv a kôz pochádzajúcich z rôznych poľnohospodárskych fariem východného Slovenska a tiež z 12 vzoriek ovčieho syra. 15 z 32 izolátov *S. aureus* malo gény pre enterotoxín C (SEC): 12 zo surového mlieka oviec, po jednom zo surového mlieka kôz, kravy a z ovčieho syra. 15 z 32 izolátov malo gény pre enterotoxín D (SED): 12 zo surového mlieka oviec a 2 zo surového kravského mlieka a 1 z ovčieho syra. 2 z 32 izolátov mali gény pre enterotoxin A (SEA) a B (SEB): jeden izolát zo surového ovčieho mlieka pozitívny pre SEA a druhý izolát z ovčieho syra pozitívny pre SEB.

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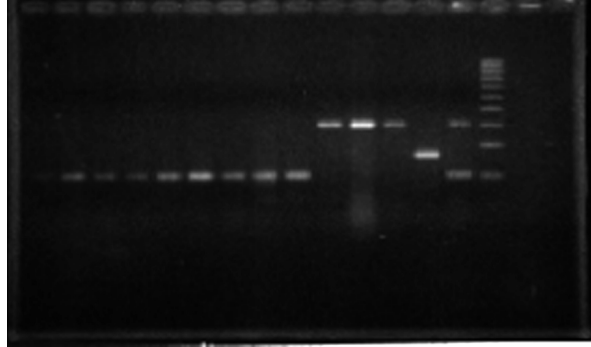


Fig. 1. Gel analysis of PCR-amplified toxin gene sequences. The individual toxin gene products were characterized by comparing with standard molecular size marker. Lane 1-9, SEC (102 bp); lane 10-12, SED (306 bp); lane 13, SEB (165 bp); lane 14, SEC and SED (102 bp and 306 bp). Lane 15, 100 bp DNA ladder.