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Detection of Enterotoxigenic Staphylococcus aureus in Schizothorax zarudnyi Using PCR Method

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Abstract

***Background*:** Enterotoxins of *Staphylococcus aureus* are the main factors which cause food poisoning.
***Materials and Methods*:** Thirty five samples of *Schizothorax zarudnyi* were cultured by bacteriological methods and *S. aureuses* were identified. The isolated bacteria were evaluated by PCR for diagnosis of the gene encoding of SEA and SEB. Data were analyzed using Stata software.
***Results*:** PCR is a rapid, sensitive, specific and inexpensive method for detecting Staphylococcal enterotoxins in food.
***Conclusion*:** 37.2% of fishes were contaminated by *S. aureus*. The PCR results showed that 14.3% of *S. aureus* isolates possessed the SEA gene, 8.5% had the SEB gene and 5.7% possessed both genes.

Introduction

Staphylococcus aureus is one of the common agents of food poisoning [1]. *S. aureus* produce a number of protein toxins and extracellular virulence factors that one of the most important of them is enterotoxin that cause food poisoning by this species of bacteria. *S. aureus* enterotoxin has been classified into 18 serotypes A to U based on biological and serological properties [2, 3].

The toxins enter from the alimentary tract into the blood circulation, stimulates the vomiting center of the involuntary nervous system, causing nausea, vomiting, abdominal cramps and diarrhea [4, 5]. Staphylococcal enterotoxin type A (SEA) and staphylococcal enterotoxin type B (SEB) are a major cause of gastroenteritis. There are different methods for the detection of *S. aureus* toxins in food samples such as latex agglutination, ELISA, radioimmunoassay and etc that require four to five days to obtain results after initiation of sample analysis [6, 7]. The polymerase chain reaction (PCR), which is a technique for the in vitro amplification of specific segments of DNA, offers a rapid, sensitive and specific identification method for the genes responsible for toxins produced by *S. aureus* [8, 9].

These bacteria, because easily grow in different conditions, can be separated from a variety of foods, including milk and dairy products, meat products, fish, vegetables, salads [10]. *Schizothorax zarudnyi* is a specific fish in the world that belongs to Sistan and Baluchistan province in Iran and this fish is one of the food sources among the people of Sistan and Balouchistan. The aim of this study was to detect *S. aureus* enterotoxin type A and B in *Schizothorax zarudnyi* fish by PCR technique.

Materials and Methods

In this cross-sectional study, 35 *Schizothorax zarudnyi* were taken from lake (Chahnime lake). Trials lasted from February 2012 until June 2012 and all experiments were performed in the Laboratory of Food Hygiene, Veterinary Faculty in University of Zabol. In this study *S. aureus* PTCC 1112 and *S. epidermidis* PTCC 1435 were obtained from the Iranian Science and Technology Research Organization.

Isolation and identification of Staphylococcus aureus: twenty five grams of muscle of per fish was homogenized in 225 ml of Peptone Water in a stomacher (Seaward model, Germany). Then these were incubated in Brain Heart Infusion broth (BHI) (Merck) at 37°C for 24 h. Then 0.1 ml of it were cultured on Baird parker (Merck) and incubated for 48 h. In samples that bacteria were grown, after seeing black colonies with clear zone in Baird Parker, other biochemical tests were performed that including coagulase test: using citrated rabbit plasma and were performed both slides and tubes methods. DNAse test: was performed by using DNAse agar (Merck, Germany) and HCL 10%. Mannitol fermentation test: was performed by using mannitol salt agar (Merck, Germany) in anaerobic conditions *S. aureus* is the only bacterium that able to ferment mannitol in anaerobic conditions. VP test was also performed. Indeed *S, aureus* is the only coagulase positive and VP positive bacterium.

Extraction of DNA: DNA was extracted by Cinna Pure DNA extraction kit following manufacturer's instructions then extracted DNA was checked for the quality by means of 1% agarose gel electrophoresis. The concentration of DNA was determined by UV spectrophotometer. Finally, the extracted genomic DNA was stored until use at -20°C freezer.

Polymerase Chain Reaction (PCR): First the sequence of sea and seb gene was obtained from Gen Bank, and then we designed two pairs of primers for these genes. After check the specificity of the primers in NCBI

BLAST site, primer pair for each of these genes was confirmed. Then primers were ordered to build to the Takapozist Company (Table 1). The quality of primers was examined before PCR reactions by electrophoresis on 2% agarose gel.

Table 1. Primers used for the detection of Staphylococcus aureus SEA and SEB genes

Gene	Primer	Sequence Base pair (5'-3')	Gene location	PCR product size
SEA	SEA-1	ttggaaacggttaaaacgaa	490-509	
	SEA-2	gaaccttcccatcaaaaaca	591-610	120
SEB	SEB-1	tcgcatcaaactgacaaacg	634-653	
	SEB-2	gcaggtactctataagtgcc	1091-1110	478

The amplification reactions containing 1 µl of template DNA, 2.5 units of Taq DNA polymerase, 2 µl each of 10 mMdATP, dTTP, dCTP and dGTP, 2.5 µl of 10 X reaction buffer, 1.5 µl of $MgCl_2$ (50 mM) and Final volume was raised to 25 microliter with distilled water. The reaction was performed at 30 cycles (Table 2).

Table 2. Time and temperature used in PCR

Number	Steps	Temperature(°c)	Time	Number of cycles
1	First denaturation	94	30 sec	1
2	Denaturation	94	1 minute	30
	Annealing	55	1 minute	
	Extension	72	1 minute	
3	Final extension	72	30 sec	1

To check the product of reactions, 5 microliters of them were transferred to 1% agarose gel for electrophoresis, then stained with ethidium bromide and evaluated. All products of PCR of the studied genes had expected size and there were no nonspecific band. In all reactions *S. epidermidis* PTCC 1435 was used as a negative control. Frequency and the proportion of fish with *S. aureus* were calculated. Also the proportions of specimens containing SEA and SEB genes were determined. 95% confidence interval for the each of the ratios using the binomial distribution was calculated. Stata statistical software was used in the calculations.

Results

Considering enterotoxin stype A and B are heat-stable so foods that contaminated with these two types enterotoxin are toxic, and in a short time can cause gastroenteritis. Detection of *S. aureus* enterotoxin genes by PCR is a specific, sensitive, inexpensive and fast method which can replace traditional immunological assays.

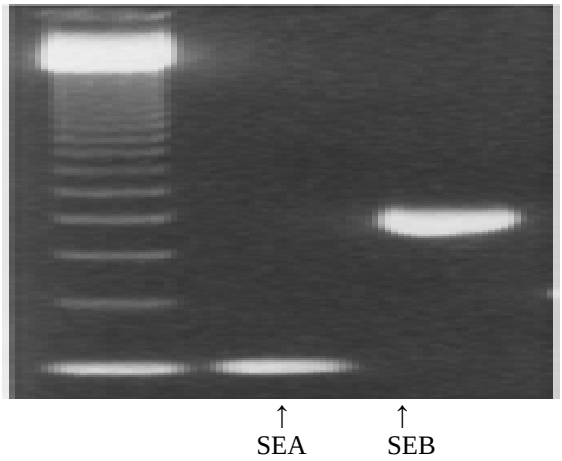

Figure1. Electrophoresis of PCR products of Staphylococcus aureus isolated from Schizothorax zarudnyi fish using primer for SEA gene (lane b), the SEB gene(lane c), DNA ladder 123 bp (lane a).

Discussion

Several studies have reported that a high proportion of isolates from outbreaks of staphylococcal food poisoning occurring in South Korea, France, Japan and United Kingdom could produce SEA, either alone or with another toxin [13]. Bystron et al. had tested 65 food samples that made from half-baked chickens, by multiplex PCR technique for detection of enterotoxin-producing Staphylococcus aureus, 11 strains of *S. aureus* was isolated and identified in Turkey [14]. Beata Holeckova et al. examined different food samples (sausage and a variety of soups) and identified and isolated 43 strains of *S. aureus* in Slovak Republic, that 15 strains (34.88%) of *S.* aureus bacteria were known enterotoxigenic by multiplex PCR. Seven of the 15 bacterial strains (16.28%) were eligible enterotoxin genes [15]. In the present study, the size of fragment was 120 bp for SEA gene corresponding to the amplification of the gene SEA, indicating the presence of gene that encoding enterotoxin A in *S. aureus* (Fig. 1, column 2) and the size of fragment was 478 bp for SEB gene that confirmed presence of gene that encoding enterotoxin B (Fig. 1, column 3).

Table 3. Comparison between staphylococcus aureus groups in terms of containing or lacking SEA and SEB genes

Groups (%)		Frequency (%)	Proportion (%)	Limits of confidence (95%)	
				Upper limit (%)	Under limit (%)
Without Staphylococcus aureus		22	62.9	78.5	44.9
With Staphylococcus aureus	Had no SEA and SEB genes	3	8.6	23.1	1.8
	Had SEA gene	5	14.3	30.3	4.8
	Had SEB gene	3	8.6	23.1	1.8
	Had both SEA and SEB	2	5.7	19.2	0.7
	Total	35	100	_	_

Thirty seven point two percent (13 cases) of 35 samples of *Schizothorax zarudnyi* fish were positive for the presence of *S. aureus*. PCR results showed the isolated *S. aureus* that 14.3% (5 samples) contains SEA gene, 8.5% (3 cases) have SEB gene and 5.7% (2 cases) have both SEA and SEB genes and 2 cases did not have any SEA and SEB genes.Statistically, the frequency of sea and seb genes is not significantly different (Table 3).

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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