

## Molecular Detection of Enterotoxin B from *Staphylococcus aureus*

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### Abstract

**Background:** Food poisoning due to the bacteria is a big global problem in economically and human's health. This problem refers to an illness which is due to infection or the toxin exists in nature and the food that we use. One of the most important productive factors is *Staphylococcus aureus*. In general different strain of *S. aureus* produces several enterotoxins like: SEA, SEB, SEC. So, the existence of *S. aureus* in foods is a potential risk, especially if the foods are not storage in proper temperature. In this work, by designing of new primer the enterotoxin B (SEB) has been detected using PCR techniques.

**Material and method:** In this study, the primer explorer V4 software was used for designing specific primers. The extracted DNA genomic from *S. aureus* was using for PCR reaction. The PCR product analysed by agarose gel (1.2%) electrophoresis. Also specificity of this method was determined using different strains of *S. aureus* A (SEA), *S. aureus* C (SEC) and *Vibrio cholera* O1. Also, sensitivity was distinguished by serial dilution of *S. aureus* DNA genomic.

**Results and conclusion:** PCR assay showed a 226 bp specific amplified fragment. The sensitivity of this method determined about 200 CFU/ml and also the results showed that according to specific primers, the PCR is very specific. Therefore, this procedure is very sensitive and rapid technique for SEB detection of *S. aureus*. In conclusion, this PCR assay can be used like a simple diagnostic test in clinical laboratories for identification of foodborne disease.

**Keywords:** *Staphylococcus aureus*, Enterotoxins B, PCR, Food poisoning

### Introduction

Food safety and food-borne diseases have attracted extensive attention in the world, and lack of preventative control would causes contamination in the food storage processing and marketing [1,2]. Important factors that might lead to food poisoning consist of 5 categories: uncertain food sources, not enough cooking, improper storage temperature, the polluted equipment and poor personal hygiene. Therefore, the especial anxiety which exist due to the danger of foods mostly includes: microbiology, chemical, remains of pesticide, remains of veterinary medicines and allergic compounds. Food-borne diseases due to the bacteria are a big global problem in 2 aspects: economically and human's health [3,4]. The most important bacteria which cause food pollution and therefore result in food poisoning includes: *Escherichia coli*, *Shigella* spp, *Salmonella* spp, *Bacillus cereus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Vibrio cholera* [5,6]. *S. aureus* as one of the most reasons of illness and mortality in less developed countries is the most common and significant pathogen in both healthy and immune-deficient individuals [7,8]. Accordingly, correct and rapid detection of this bacteria and pyrogenic exotoxins, such as *Staphylococcal* Enterotoxins (SE) is very important. SE is critical factor for food poisonous that produced by some *S. aureus* strains [9,10]. Almost all of the SEs is heat-stable proteins which after ingestion and inhalation to cause gastroenteric syndrome in humans. From 18 SEs that discovery, only a few SEs play a role in food poisoning [11]. The two critical SEs that induce food

poisonous are SEA and SEB [12-14]. On the other hand, traditional detection methods for infection pathogens in clinical laboratories is biochemical techniques and microscopic observation according to enrichment culture which is currently time consuming, laborious and the difficult of quantitative assessment [15,16]. Therefore, for simple and correct detection of several pathogens, we need to a rapid identification assay of food-borne bacterial pathogens using genome-based amplification. For this, molecular detection method such as PCR and Real-Time PCR is recommended for identification of SEB in food material. In this work, we design a new primer for detection of the enterotoxin B (SEB) using PCR techniques.

### Material and Methods

#### Bacterial culture and DNA extraction

At first, *S. aureus* was cultured in (TSB medium) and incubated for overnight in a shaker incubator for 37°C. Then, the bacterial cells were harvested by centrifuge at 5000 g for 5 min. The genomic DNA was extracted using Sina Clone Kit (Iranian Company) and finally, the extracted DNA analysed by agarose gel 1.2%.

#### Primer design

New primers were designed according to the sequence of the enterotoxin B gene (Accession No. KX168628.1). These special primers (Forward primer: GTTCGGGTATTTGAAGATGG and revers primer: CAAATTTATCTCCTGGTGCA) are capable of replicating a 226 bp segment from *SEB* gene.

## PCR reaction

The PCR reaction was performed using specific primers. Mixed PCR was prepared in a volume of 5  $\mu$ l, containing 57 ng DNA, 1  $\mu$ M of each primer, and master mix of Taq DNA polymerase (amplicon PCR kit, Denmark). The PCR program included: Initial denaturation at 95°C for 10 min, 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. Results were analysed by 1.2% agarose gel.

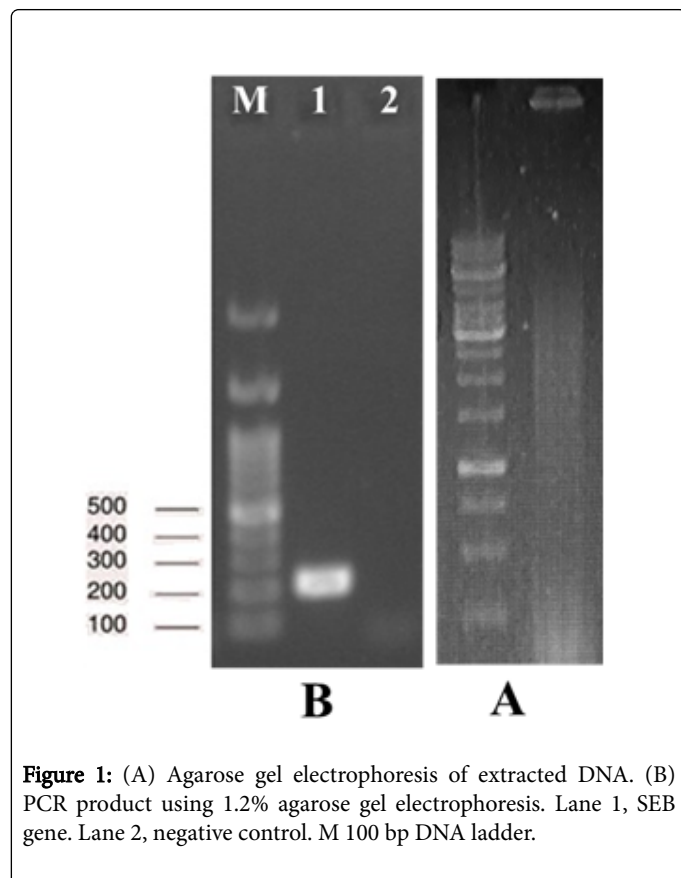
## Sensitivity and specificity

In order to measure the sensitivity of primers, serial dilution of genomic DNA were prepared and used as a template. To evaluate the specificity of primers, a variety of bacteria including *S. aureus* A (SEA), *S. aureus* C (SEC), *Vibrio cholera* O1, were used. The results were analysed by 1.2% agarose gel.

## Results

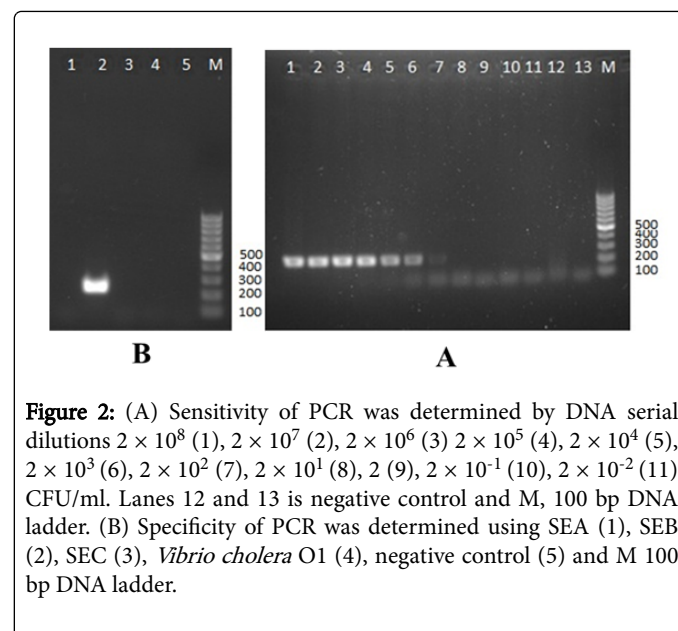
### DNA amplification

DNA was extracted from *S. aureus* B (SEB). The results were analysed by 1.2% agarose gel (Figure 1A). Extracted DNA was used for PCR as template. PCR reaction with pre-prepared primers was then performed according to materials and methods. Amplification of 226 bp fragment confirmed the susceptibility of this assay (Figure 1B).



## Sensitivity and specificity

Serial dilution of Staphylococcal DNA genomic was done from  $2 \times 10^8$  cfu/ml to  $2 \times 10^{-2}$  cfu/ml and was used as a template. PCR products analysis, showed amplification of 226 bp is performed until 200 cfu/ml (Figure 2A). For specificity examination PCR reaction was done for SEA, SEB and SEC genes, *Vibrio cholera* O1 genome. The results showed primers very specific and were only amplified SEB genes (226 bp) (Figure 2B).



**Figure 2:** (A) Sensitivity of PCR was determined by DNA serial dilutions  $2 \times 10^8$  (1),  $2 \times 10^7$  (2),  $2 \times 10^6$  (3)  $2 \times 10^5$  (4),  $2 \times 10^4$  (5),  $2 \times 10^3$  (6),  $2 \times 10^2$  (7),  $2 \times 10^1$  (8), 2 (9),  $2 \times 10^{-1}$  (10),  $2 \times 10^{-2}$  (11) CFU/ml. Lanes 12 and 13 is negative control and M, 100 bp DNA ladder. (B) Specificity of PCR was determined using SEA (1), SEB (2), SEC (3), *Vibrio cholera* O1 (4), negative control (5) and M 100 bp DNA ladder.

## Discussion

*S. aureus* is the most agents for bacterial foodborne diseases that are one of the biggest problems in human health and food safety according to production and secretion of different Staphylococcal Exotoxins (SEs) [7-10]. Therefore, detection and identification of SEs is very important in order to inhibition and control of food poisoning. There are several methods for detection of enterotoxigenic *S. aureus*. Animal and serological test is the conventional SEs detection techniques that are labor-intensive and not the possibility of real-time detection. For this, DNA-based molecular assay such as PCR is a rapid, correct and reliable method with sure results that could be detected SEs genome directly [16-19]. PCR-based detection is simple, inexpensive, sensitive and selective which can even detect each bacterium without genome extraction [20]. In previous investigation, detection of *S. aureus* was carried out using PCR with different rate of sensitivity. In 1992 Brakstad used from PCR for amplification of nuc gene with the detection limit of  $10^3$  CFU [21]. Sowmya in 2012 compared PCR and LAMP method for *S. aureus* detection that sensitivity of PCR reported about  $10^4$  CFU/ml [22]. Cremonesi in 2007 developed detection for enterotoxigenic *S. aureus* that isolates from raw milk and achieved to a detection limit about 100 CFU/g [23]. In this study we designed new primers for the detection of *S. aureus* SEB genes using PCR technique and the specificity and sensitivity of this assay is determined. The result was shown, the sensitivity of this method is about 200 CFU/ml and it was also found that designed primers are highly specific. In conclusion this simple detection could be used for design and development of molecular kit for identification of *S. aureus*.

## References

1. Rao VS, Naveen KR, Polasa K (2012) Foodborne diseases in India-a review. *British Food J* 114: 661-680.
2. Tauxe RV (2002) Emerging foodborne pathogens. *Int J Food Microbiol* 78: 31-41.
3. Food and Drug Administration (2009) Management of food safety practices - achieving active managerial control of foodborne illness risk factors. *Food Code Annex 4*: 501-535.
4. Redman NE (2007) *Food safety: A reference handbook*. ABC-CLIO Inc.
5. Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, et al. (2015) World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal and viral diseases, 2010: a data synthesis. *PLoS Med* 12: 1001921.
6. Jay JM, Loessner MJ, Golden DA (2005) *Modern food microbiology* (7th Ed.). Springer.
7. Dinges MM, Orwin PM, Schlievert PM (2000) Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev* 13: 16-34.
8. Balaban N, Rasooly A (2000) Staphylococcal enterotoxins. *Int J Food Microbiol* 61: 1-10.
9. Novick RP, Schlievert P, Ruzin A (2001) Pathogenicity and resistance islands of staphylococci. *Microbes Infect* 3: 585-594.
10. Pinchuk IV, Beswick EJ, Reyes VE (2010) Staphylococcal enterotoxins. *Toxins* 2: 2177-2197.
11. Letertre C, Perelle S, Dilasser F, Fach P (2003) Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. *J Appl Microbiol* 95: 38-43.
12. Kluytmans JAJW, Wertheim HFL (2005) Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections. *Infection* 33: 3-7.
13. Mao Z, Zheng H, Wang X, Lin S, Sun Y, et al. (2008) DNA microarray for direct identification of bacterial pathogens in human stool samples. *Digestion* 78: 131-138.
14. Ranjbar R, Karami A, Farshad S, Giammanco GM, Mammina C (2014) Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. *New Microbiol* 37: 1-15.
15. Imani FA, Tavakoli H, Naderi A (2010) Detection of enterotoxigenic *Staphylococcus aureus* isolates in domestic dairy products. *Iran J Microbiol* 2: 137-142.
16. Wu S, Duan N, Gu H, Hao L, Ye H, et al. (2016) A review of the methods for detection of *Staphylococcus aureus* enterotoxins. *Toxins* 8: 1-20.
17. Nasrabadi Z, Ranjbar R, Poorali F, Sarshar M (2017) Detection of eight foodborne bacterial pathogens by oligonucleotide array hybridization. *Electron Physician* 9: 4405-4411.
18. Priyanka B, Patil RK, Dwarakanath S (2016) A review on detection methods used for foodborne pathogens. *Indian J Med Res* 144: 327-338.
19. McLauchlin J, Narayanan GL, Mithani V, 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.
20. Stevens KA, Jaykus LA (2004) Direct detection of bacterial pathogens in representative dairy products using a combined bacterial concentration-PCR approach. *J Appl Microbiol* 97: 1115-1122.
21. Brakstad OG, Aasbakk K, Maeland JA (1992) Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J Clin Microbiol* 30: 1654-1660.
22. Sowmya N, Thakur MS, Manonmani HK (2012) Rapid and simple DNA extraction method for the detection of enterotoxigenic *Staphylococcus aureus* directly from food samples: comparison of PCR and LAMP methods. *J Appl Microbiol* 113: 106-113.
23. Cremonesi P, Perez G, Pisoni G, Moroni P, Morandi S, et al. (2007) Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese. *Lett Appl Microbiol* 45: 586-591.