



Encoding Gene Identification in *Staphylococcus aureus* enterotoxin B from Nasal Mucosa of the Dog

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Abstract

This research aimed to know the enterotoxin B in Staphylococcus aureus isolated from the nasal mucosa of the dog. Six of fifteen samples have characteristics like cocci shaped, Gram-positive, mannitol fermented, produce catalase, coagulase and acetyl methyl carbinol. Then to identify enterotoxin B in 6 isolates of Staphylococcus aureus using PCR techniques. Result of the identification of gene that are 50% positive samples had enterotoxin B. Further research is needed on high sensitivity antibiotic for treatment of the caused by Staphylococcus aureus infections, as well as the need cautious to the spread of enterotoxin B in Staphylococcus aureus.

Key words: *Staphylococcus aureus, enterotoxin B., encoding gene identification, PCR*

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Introduction

Staphylococcus aureus is a Gram-positive bacteria of normal flora in the skin and mucosa (Mustak and Esendal, 2010). In most healthy individuals *Staphylococcus aureus* can be found in the nose by 20-30% (Rahimi and Alian, 2013). At the pet animal staphylococci are at 30-70% in the skin and mucosa and more than 14% were *Staphylococcus aureus* (Loeffler *et al.*, 2009). *Staphylococcus aureus* can cause intoxication when contaminated with food or known as *Staphylococcus aureus* Food Poisoning with symptoms of diarrhea (Fooladi *et al.*, 2010). *Staphylococcus aureus* intoxication caused by staphylococcal enterotoxin (SE) (El-Jakee *et al.*, 2013). Stability Staphylococcal enterotoxin (SE) to heat and protease enzymes such as pepsin is one trait that is very important about concerning food safety because the toxins persist despitea foodstuff contaminated SE has been cooked or heated and the toxin if it is worn will be resistant to enzyme in the digestive tract (Salasia *et al.*, 2009; Fooladi *et al.*, 2010).

Staphylococcal enterotoxin(SE) B is the cause of most of the cases of food Poisoning (Fooladi *et al.*, 2010). According Salasia *et al* (2009) in humans have been isolated *Staphylococcus aureus* with varying enterotoxin gene, namely enterotoxin A, B, C, E, G, H and I. From the isolates were taken from the nose or anal swab was reported that over 50% of the isolates produce Staphylococcal enterotoxin B (Ahanotu *et al.*, 2006). Based on the background of the problem, researched the identification of genes encoding *enterotoxin B* in *Staphylococcus*

aureus from the nasal mucosa swab dog in Animal Hospital of the University of Airlangga techniques *Polymerase Chain Reaction* (PCR).

Materials and Methods

The sampling swab the nasal mucosa dog do with a cotton swab and then in streak multilevel media Mannitol Salt Agar then incubated at a temperature of 37°C for 24 hours. Colonies that grew on Mannitol Salt Agar then examined microscopically (Hendrix and Sirois, 2007). *Staphylococcus aureus* bacteria biochemical test using catalase test, coagulase test, test Voges Proskauer (VP) and the haemolysis test (Quinn *et al*, 2002).

DNA extraction using Qiamp tissue kit (QIAGEN, Hilden Germany). Reagents for amplification consisted of 12.5 mL of 2x master mix (introns), 1 mL of Seb primer (sense) and 1 mL of primer (antisense), 0.5 mL of distilled water and 5 mL of DNA template into 0.2 ml eppendorf tube.

The mixture put in a PCR machine and entered the stage of initial denaturation 30 seconds 94°C, 94°C denaturation, annealing and extension 55°C 72°C for 1 minute with 30 times as well as the final cycle extension 72°C for 30 seconds. The final stage, the results of PCR detected by electrophoresis. A total 5µl PCR product of DNA *Staphylococcus aureus* entered in a 2% agarose gel containing 20ml redsafe 2 mL in 1x TBE for running processes. After 30 minutes, the results can be visualized by ultraviolet light with UV transilluminator.

Results and Discussion

Based on the observation of the nasal mucosa swab sample 15 dogs, showed 6 positive bacterial colonies on media ferment mannitol Mannitol Salt Agar and shaped cocci, Gram-positive flocks and microscopic examination. The result of identification with catalase test, coagulase, Voges Proskauer (VP) and the hemolysis test colonies obtained with nature to produce the enzyme catalase and coagulase, producing acetyl-methyl-carbinol and forming β-haemolysis on media Blood Agar. From these results indicate that the bacterial colonies were positive *Staphylococcus aureus* amounted to 6 of 15 swab mucosa, or about 40%.

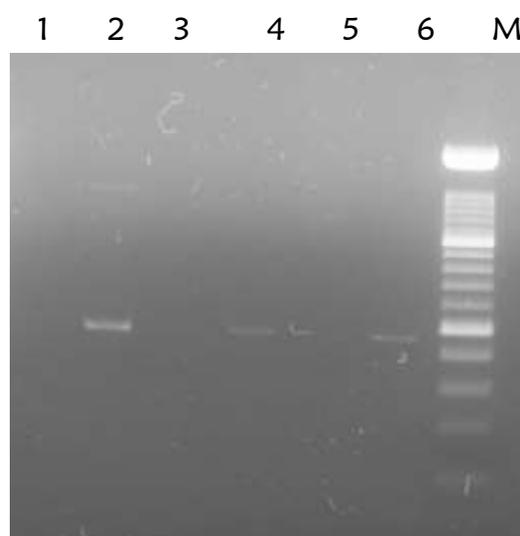


Figure 1. At line 2, 4, 6 SEB gene identified by 478 bp.

Results of the study showed that of the six isolates it contains 50% which produces enterotoxin B. It is probable that these numbers will increase (Bhanderi and Jhala, 2011). *Staphylococcal enterotoxin B (SEB)* is one of Staphylococcal enterotoxin very important (Fooladi *et al*, 2011). Intoxication is one of the cases caused by SEB. Cases of intoxication is a major problem that affects public health. Each year in the United States nearly 6-80 million people affected by intoxication and caused the deaths of approximately 9,000 people. Intoxication due to SEB have been reported in South Batimah Oman, from consuming yogurt is made by a person with chronic infection of the fingernails (Salasia *et al*, 2009). These toxins are not only found in humans, but this toxin also found in mice that caught direstaurant in Tokyo. Of the 910 mice, 18% SEB identified (Taj *et al*, 2014).

According Salasia *et al* (2009) found enterotoxin B has been reported to have the potential to improve the superantigen activity. Important properties are staphylococcal superantigens, namely by showing activity through interaction between antigens and T lymphocytes, in the absence of antigen specificity of the cell. This condition stimulates cell proliferation and an increase in cytokines with high concentrations. Cytokines are produced in large numbers resulting in symptoms of toxic shock syndrome.

Conclusion

Based on the results of research on the identification of genes encoding enterotoxin B of the nasal mucosa swab sample dogs at the Animal Hospital of Education Airlangga University Surabaya on 6 isolates of *Staphylococcus aureus*. Three of 6 isolates of *Staphylococcus aureus* genes enteretoksin B.

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