Staphylococcal Enterotoxin A (SEA) Reteined in Cells and Its Degradation in a Model Gastric Juice

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I. INTRODUCTION

Staphylococcus aureus contaminates foods such as meat products, dairy products, and other processed foods (Tsutsuura and Murata, 2017). *S. aureus* has various virulence factors such as toxins, adhesins, superantigens, and proteases. Staphylococcal enterotoxin A (SEA) which causes staphylococcal food poisoning in humans is one of the bacterial exotoxins produced by the bacteria. The potency of *S. aureus* to produce SEA differs among strains, and the SEA produced in food is very stable (Tsutsuura et al., 2013). In this study, we examined the change in the amount of SEA retained in the bacteria after SEA production in detail and whether digestive enzymes degrade it in gastric juice.

II. MATERIALS AND METHODS

After pre-cultivations, each BHI broth was inoculated with SEA-producing *S. aureus* suspension containing each of the eleven strains at 10^2 and 10^6 CFU/mL and incubated with shaking at 10, 15, and 37ºC (Tsutsuura and Murata, 2017). The culture medium was centrifuged at 10,000 g for 15 min at 4°C to collect cells. The precipitate was washed with PBS, resuspended in SDS-PAGE sample buffer, and then boiled for 5 min. After centrifugation, the supernatant was used as the bacterial internal extract. This and the culture supernatant were subjected to western blot analysis. Simulated gastric juice (pH 1.5) was made following Bornhorst and Singh (2013). The gastric juice was added to the culture supernatant or precipitate obtained by centrifuging the culture medium and then incubated with shaking at 37°C for 0.5-1 hr. After centrifugation, 1.5 M Tris-HCl was added to the supernatant and then subjected to western blot analysis. The staphylococcal counts were determined by colony-counting on mannitol salt agar plates. SEA was determined with western blot analysis. The detection limit was about 0.5 ng/mL.

III. RESULTS AND DISCUSSION

Figure 1 shows the growth of *S. aureus* (C-77-L22) and its SEA production inside and outside the cells when the bacteria were inoculated with inoculum sizes of 10^2 and 10^6 CFU/mL and incubated at 37°C. With both inoculum sizes, SEA was not detected inside the bacterial cells at the early stage of growth, but only detected outside the cells. It was suggested that the SEA produced in the early exponential phase was not retained inside the cells but was rapidly released outside the cells. The time required for SEA production was longer at 10°C and 15°C than at 37°C but the rate of SEA production inside and outside the bacteria was similar to that at 37°C. From these results, the SEA in the bacteria may retain a little or temporarily, or may be eluted from inside to outside due to the death of bacteria.

Figure 2 shows the appearance of SEA bands on SDS-PAGE before and after gastric juice treatment which were detected by western blot. The SEA bands disappeared after 0.5-1 hr of incubation at 37°C with artificial gastric juice added to purified SEA or culture medium containing SEA.

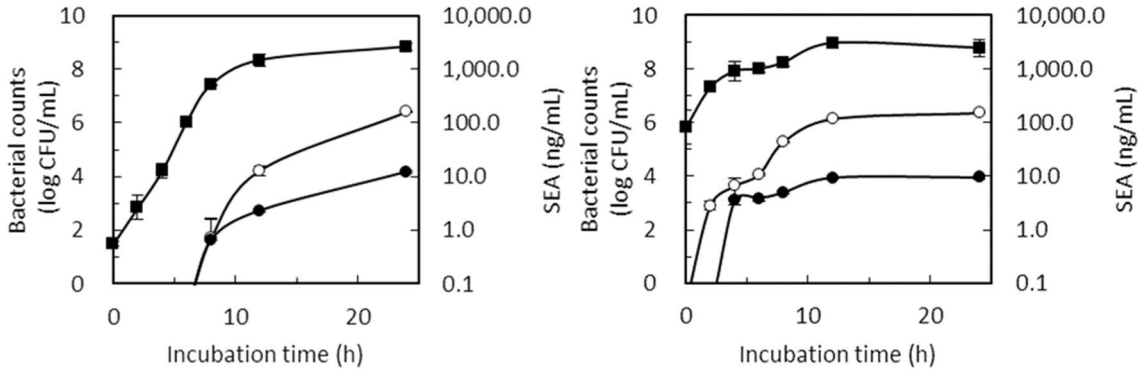

Figure 1. Growth (■) and intracellular and extracellular SEA (● and ○) of *S. aureus* with inoculum sizes of 10^2 CFU/mL (Left) and 10^6 CFU/mL (Right) in BHI broth at an incubation temperature of 37°C (n = 3).

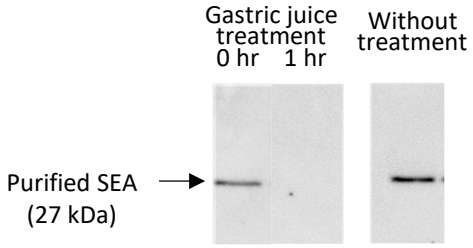

Figure 2. SEA bands before and after gastric juice treatment on western blot analysis

IV. CONCLUSION

In this study, the amounts of extracellular and intracellular SEA of *S. aureus* were quantified. The results showed that the intracellular SEA was as much or less than the extracellular SEA, suggesting that SEA was temporarily retained in the bacteria, and was quickly released after production. We also clearly showed that SEA was digested in a model gastric juice. Further study will be needed to evaluate SEA digestion under various conditions imitating digestion in the human stomach.

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