Elucidation of the gene transfer mechanism of staphylococcal enterotoxin A

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Research aims

Staphylococcus aureus produces enterotoxin A (SEA), causing not only food poisoning but also a zoonosis whose toxin gene is transferred by bacteriophage. In order to prevent food poisoning and disease caused by *S. aureus*, we used another strain of *S. aureus* to inhibit the transfer of SEA gene by phage. We demonstrated that the SEA-producing strain (No.29) was lysed and the SEA gene was transferred to the non-SEA producing strain (No.77) when No. 29 was in contact with colonies of No.77 (**Fig. 1**). However, the mechanisms of lysis induction and the adsorption of the phage to host bacteria have not been clarified. In this study, we aimed to examine the factors affecting the lysis of the SEA-producing strain and the mechanism of SEA gene transfer.

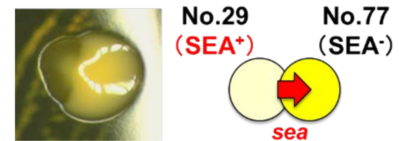

Fig. 1: Lysis of *S. aureus* No.29

Methods

S. aureus No.29 (SEA$^+$; No.29) and *S. aureus* No. 77 (SEA$^-$; No.77) were used in this study. Growth conditions including growth stage, temperature, medium, $MgCl_2$ and NaCl concentrations, and pH were examined to assess if they affected the transfer of the SEA gene. Thereafter, metabolome analysis using LC-MS/MS was performed on No.29 cells when they were co-cultured with No.77 in a dialysis culture flask. Furthermore, based on the reference genome base sequence of the known *S. aureus* NCTC8325 (accession number NC-0077795)[1], genomic resequencing of No.29, No.77, and No.77-L22 (strain receiving SEA gene of No.29) was performed using a next-generation sequencer.

Results

Factors promoting lysis colony formation of *S. aureus* No.29 were examined. Optimal temperature and incubation of seed-culture was at 37 °C for 6 h. When the NaCl concentration and pH value in the medium were high, No.29 lysed easily. In contrast, No.29 did not lyse when $MgCl_2$ was added. These results suggested that formation of lysis colonies tends to occur in an environment suitable for the growth of bacteria. Principal component analysis (PCA) showed that flavin adenine dinucleotide (FAD) levels were significantly decreased in No.29 co-cultured with No.77 in a dialysis culture flask for 10 h compared to levels in No.29 without a co-culture (**Fig. 2**). In contrast, *N*-acetylglucosamine 6-phosphate (GlcNAc-6-P) and phosphatidylglycerol (PG) levels significantly increased in No.29 co-cultured with No.77 (**Fig. 3**). There was no difference in the content of *N*-acetylmuramic acid (MurNAc) which is a component of the *S. aureus* cell wall. These results suggest that the self-lytic enzyme of No.29 was activated by co-culture with No.77. Single Nucleotide Polymorphism (SNP) analysis was performed by mapping to the reference genome sequence *S. aureus* NCTC 8325 (accession number NC-0077795). The transfer of the SEA phage derived from No.29 into No.77 caused the addition of 1,103 SNPs. In addition, the insertion position of the SEA gene of No.77-L22 was between 2,037,774 bases and 2,038,315 bases.

Conclusion

In this study, we examined the mechanisms of lysis of an SEA producing strain (strain No.29) and the transfer of the SEA gene. We observed that the formation of lysis colonies occurred in an environment suitable for the growth of bacteria, and activated the self-lytic enzyme of No.29 by co-culture with No.77. Strains No.29, No.77. and No.77-L22 were resequenced using a next-generation sequencer. We are currently analyzing the insertion position of the SEA gene phage cassette of No.77-L22. Based on the obtained results, we plan to identify genes involved in SEA gene transfer. Further research is needed to clarify the mechanisms of lysis of the SEA-producing strain as well as SEA gene transfer.

References

1) Bæk, K. T., Frees, D., Renzoni, A., Barras, C., Rodriguez, N., Manzano, C., and Kelley, W. L. (2013). Genetic variation in the *Staphylococcus aureus* 8325 strain lineage revealed by whole-genome sequencing. *PLoS One*, **8(9)**: e77122.

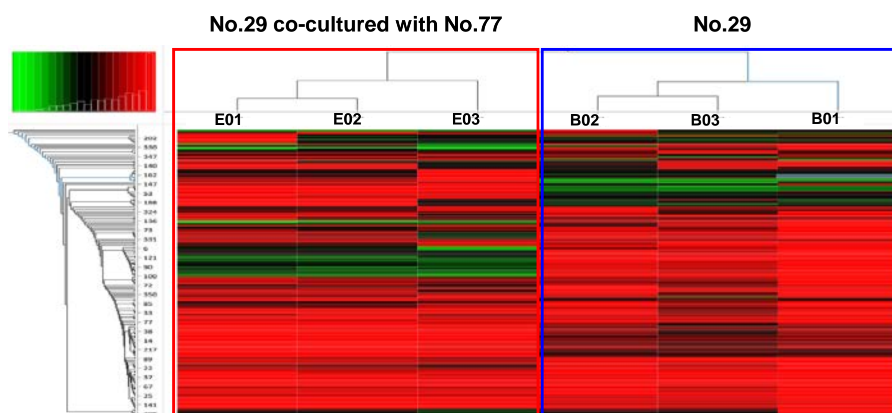

Fig. 2: Clustering analysis of co-cultivation-resolved intracellular metabolite values. Co-cultivation-resolved intracellular metabolite values were visualized using a heat map. Green colorations display lower than average concentrations whereas red colorations represent higher than average concentrations of intracellular metabolites. E01-E03: Co-culture with *S. aureus* No.29 and *S. aureus* No.77 in dialysis culture flask, B01-03: A single cultured *S. aureus* No.29.

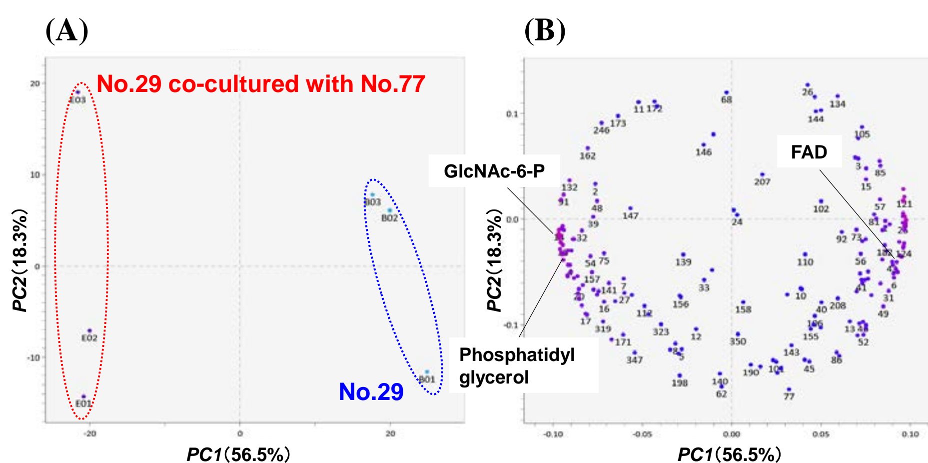

Fig. 3: (A) Score and (B) loading plots of the two component principal component analysis model developed using identified intracellular metabolites from *S. aureus* No.29. (A) B01-B03: intracellular metabolites of *S. aureus* No.29, which increased by co-cultivation, E01-E03: intracellular metabolites of *S. aureus* No.29, which decreased by co-cultivation.