論 文 要 旨

C55 bacteriocin produced by ETB-plasmid positive *Staphylococcus aureus* strains is a key factor for competition with *S. aureus* strains.

（ETB プラスミドを保有する黄色ブドウ球菌の産生する C55 バクテリオシンは他の黄色ブドウ球菌との拮抗に重要な因子である）

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【序論及び目的】

S. aureus is a major pathogen to humans. *S. aureus* causes various diseases such as suppurative diseases, pneumonia, food poisoning and toxic shock syndrome because this organism produces many toxins and exoenzymes. Among toxins, exfoliative toxin (ET) is closely related with the onset of bullous impetigo in mostly children. Up to date, three ETs named as ETA, ETB and ETD have been identified in *S. aureus*. The eta gene coding for ETA, etb and etd are located in the bacteriophage, the plasmid (pETB) and pathogenecity island in chromosomal DNA, respectively. Previously, the nucleotide sequence of pETB plasmid was completely determined. In pETB plasmid, bacteriocin synthesis genes are also identified, and previous report indicated that ETB-positive *S. aureus* strains produced lantibiotics, known as C55. Many bacteria produce antibacterial peptides known as bacteriocin to interfere with other bacteria. In gram positive bacteria, bacteriocins are generally classified with two groups, class I and class II bacteriocins. class I bacteriocin is also called as lantibiotics. Lantibiotics contain an unusual amino acid, lanthionine, which is post-transcriptionally modified to methyllanthionine. C55 bacteriocin produced by *S. aureus* belongs to class I. It contains lanthionine and consist of two peptides, C55 alpha and C55 beta. It is reported that the antibacterial activity of C55 was effective to *M. luteus, L. lactis* and *S. aureus* but not *S. epidermidis.* C55 showed high homology with lacticin3147 produced by *L. lactis*.

In this study, we tried to evaluate the two points listed below:

1. To identify the immunity factors against C55 bacteriocin in ETB-positive strain
2. To investigate the effect of C55 bacteriocin on the competition between ETB-positive and ETB-negative strains

【材料及び方法】

1. Bacterial strains and growth conditions.

S. aureus was aerobically grown in 5 ml trypticase soy broth (TSB) (Becton Dickinson Microbiology Systems, Cockeysville, MD) in a glass tube at 37°C with shaking (100 rpm/min). *Escherichia coli* XL-II was aerobically grown in 5 ml lysogeny broth (LB) at 37°C with shaking (100 rpm).

2. Bacteriocin susceptibility test.

Susceptibility to bacteriocin produced by *S. aureus* TY4 or TY825 was evaluated by a direct method. Briefly, an overnight culture of each bacteriocin-producing strain was stab-inoculated onto TS agar (TSA) plate and cultivated 16 h at 37°C. After confirming that the diameter of the growth zone of the bacteriocin-producing strain was uniformly 2 mm, 6 ml of pre-warmed half-strength TS soft agar (0.75%) containing *S. aureus* cells (10^6 cells/ml) was poured over the TSA plate. The plates were incubated for 20 h at 30°C. The diameters (mm) of the clearing zones surrounding the bacteriocin-producing strains were measured in three directions. Three independent experiments were performed, and the average diameter was calculated. We found a clear zone of inhibition surround ETB-positive strain but not ETB-negative

strain suggest that ETB-positve strains has the ability to produced bacteriocin.

3. Identification of immune factors.

The genes *orf* 46 and *orf* 47, which encode ABC transporters, are located among the genes that flank the bacteriocin biosynthesis genes in pETB plasmid. We attempted to determine whether these genes together with their flanking open reading frames (ORFs) (*orf* 45 and *orf* 48) are associated with resistance to bacteriocin. We obtained various DNA fragments via PCR amplification using the specific primers, and then cloned into the pCL15 vector. The plasmid was transduced into the pETB-negative strain. Strains obtained were evaluated the susceptibility against C55 bacteriocin. We found that immunity factors *orf46-47* showed resistant and *orf48* showed partial resistant against C55 bacteriocin suggested that immunity against C55 bacteriocin is located on pETB plasmid.

4. Co- culture of *S. aureus* with another *S. aureus*.

Overnight cultures of *S. aureus* strains were diluted to an OD_{660} of 1.0 (10^9 cells/ml). The culture was further diluted 100-fold to reach 10^7 cells/ml. Then, 100 µl of each of two *S. aureus* strains was mixed well. A 10-µl aliquot of the mixed culture was spotted on TSA plates. After 8 h of incubation at 37°C, the section of agar forming the bacterial colonies was excised and suspended vigorously in 500 µl of TSB. The appropriate dilutions were plated on TSA as well as TSA containing erythromycin (10 µg/ml), chloramphenicol (10 µg/ml) or tetracycline (10 µg/ml) given the differential susceptibility to antibiotics of the *S. aureus* strains used in this study. After 1 day, colony-forming units (CFUs) grown on TSA and on TSA containing antibiotics were determined, and the population percentage of each *S. aureus* strain was calculated. We found that the proportion of ETB-positive strain is higher than that of ETB-negative strain suggested that C55 bacteriocin produced by pETB-positve strains affects the proportion of each strain when pETB-positive and pETB-negative strain co-exist.

【結論及び考察】

The susceptibility test of C55 like bacteriocin against various S. aureus strains were evaluated and found that ETB-negative strains were susceptible against C55 bacteriocin. Also, pETB-deleted strains in ETB-positive strains were susceptible to C55-like bacteriocin. Then, we looked for immunity factor in the pETB plasmid and found the genes (*orf* 46 and 47) which showed homology with immunity factor of several bacterial strains. The gene of *orf* 46 and *orf* 47 encodes ATP binding proteins of ABC transporters and permease, respectively. The *orf* 45 and *orf* 48 encode uncharacterized protein. The strain harboring *orf* 46 and *orf* 47 showed the full resistance against C55 bacteriocin. The strain harboring *orf* 48 showed partial resistance to the bacteriocin.

In co-culture assay, ETB-positive *S. aureus* TY4 or plasmid-deleted TY4 (TY4-) and ETB-negative 209P (high susceptibility to C55) were used. The percentage of the 209P strain showed increase when co-cultured with TY4 compared to the percentage when co-cultured with TY4-. Then, co-culture assay using TY4- containing immunity genes (*orf46-48*), TY4 or TY4- was performed. When co-cultured with TY4, the proportion of TY4- containing immunity genes (*orf46-48*) was higher than that ofTY4-.

Our results imply that pETB-positive strains may replace pETB-negative strains *in vivo*. ETB-producing strains sometimes cause bullous impetigo, a common skin disease that largely occurs in children. Given that *S. aureus* is a commensal bacterium of the skin, infection of human skin by ETB-producing strains may cause the elimination of the non-ETB-producing *S. aureus* strain, which first colonizes the skin.

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