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論文題目 Studies on the capsular polysaccharide synthesis genes in *Streptococcus suis*

（豚レンサ球菌の莢膜多糖合成遺伝子に関する研究）

This study aimed to verify the loss of capsule, which occurred in many *Streptococcus suis* isolates from pigs with endocarditis. In addition, for better understanding of the unencapsulation mechanism and its biological significance, the genetic backgrounds and potential benefit of the unencapsulation in the pathogenesis of infective endocarditis were investigated using the encapsulated and unencapsulated strains, isolates, and mutants.

S. suis is an important zoonotic pathogen, which can cause various diseases in swine and humans, including meningitis, arthritis, septicemia, and endocarditis. On the basis of antigenicity of the capsular polysaccharides (CPs), *S. suis* strains are classified into different serotypes. Among them, serotype 2 strains were predominantly isolated from clinical cases in both swine and humans. The CP of serotype 2 consists of glucose, galactose, *N*-acetyl glucosamine, rhamnose, and sialic acid and is considered to be synthesized by the Wzx/Wzy-dependent pathway. The CP biosynthesis of *S. suis* is mediated by the genes in capsular polysaccharide synthesis (*cps*) gene cluster. Among the *cps* genes, *cps2J* is found to exist

only in the serotype 2 and 1/2 strains; therefore, *cps2J*-positive strains are suspected to have capsules of serotype 2 or 1/2. However, it was noticed that many *cps2J*-positive isolates from porcine endocarditis were not agglutinated by either anti-serotype 1 or anti-serotype 2 sera, suggesting that the capsular expression of these isolates is repressed due to unknown reasons. Therefore, the following experiments were performed to clarify the mechanism of this phenomenon.

A total of 288 *cps2J*-positive isolates from diseased pigs were examined for the presence of capsule by coagglutination tests and transmission electron microscopy. The results showed that all 32 (100 %) isolates from pigs with meningitis were encapsulated, whereas 86 (34 %) of 256 isolates from pigs with endocarditis were unencapsulated, indicating that capsule loss often occurred in the isolates from endocarditis. Subsequently, 43 unencapsulated isolates were randomly selected to verify mutations in the genes of their *cps* gene clusters. PCR analysis demonstrated that 10 and 8 of the 43 isolates had insertions and deletions, respectively, in their *cps* gene clusters, particularly in *cps2A-2G* and *cps2P-2S* (*neuBCDA*) regions; however, no apparent mutation was detected in the remaining 25 isolates by the analysis. Further analysis of their *cps* gene clusters by sequencing and complementation demonstrated that most of the remaining isolates had nucleotide alterations of a single or a few base pairs in the *cps2E-2F* region, causing frameshift, missense or nonsense mutation(s) of the genes. Consequently, at least 32 of 43 representative unencapsulated isolates analyzed in this study have lost their CPs due to the mutations involving two glycosyltransferase genes (*cps2E* and *cps2F*).

In contrast to *cps2E-2F*, no inactivating mutation was found in *cps2I*, *cps2J* and *cps2O*, and only one missense mutation was found in *cps2N* among the unencapsulated endocarditis isolates analyzed, implying that mutations in these regions may affect the viability of *S. suis*. The attempts to construct various *cps* deletion mutants suggested that

mutations in *cps2I-2J* and *cps2N-2O* regions are lethal for *S. suis*; however, additional mutations in *cps2E-2F* can alleviate the fatal effect induced by the mutations in these two regions. According to the CP structure and proposed functions of *cps* genes of *S. suis* serotype 2, *cps2E* and *cps2F* are considered to encode the initial and second glycosyltransferases, respectively, of the Wzx/Wzy-dependent pathway, whereas *cps2J* and *cps2N* are thought to be involved in side chain formation of the repeat unit. In addition, *cps2I* (*wzy*) and *cps2O* (*wzx*) were proposed to be responsible for capsular polysaccharide polymerase and flippase, respectively. Therefore, in *S. suis* isolates from porcine endocarditis, mutations of the genes involved in CP backbone formation seem to cause their capsule loss, while loss of function of the genes responsible for the side chain formation, polymerase, and flippase are likely to be deleterious for *S. suis*.

CP of *S. suis* possesses a potential role to inhibit phagocytosis and is believed to be an essential virulence factor of *S. suis*; therefore, the capsule loss of many porcine endocarditis isolates found in this study was surprising and implied some role of unencapsulation in the pathogenesis of endocarditis. Since bacteria-platelet attachment is thought to be a major mechanism initiating infective endocarditis. For understanding of the biological significance of unencapsulation, binding ability of *S. suis* to platelets was examined using encapsulated and unencapsulated strains, isolate, and mutant. The binding ability of unencapsulated isolate NL194 to porcine platelets was significantly higher degree than that of encapsulated strain P1/7. In addition, the unencapsulated mutant 89/1591CPS2B adhered to the platelets greater degree than its encapsulated parent strain 89/1591 ($P < 0.05$). Similar results were also obtained when human platelets were used, suggesting advantageous property of individual unencapsulated cells to cause infective endocarditis.

The loss of capsule was reported to promote biofilm formation of *S. suis*. In accordance with the previous reports, the biofilm formation ability of the unencapsulated

Δ*cps2A-2S* mutant was significantly higher degree than its encapsulated parent strain P1/7. Although encapsulated *S. suis* per se cannot adhere to host cells efficiently, adherence assays using plastic wells coated with and without *S. suis* biofilms showed that the biofilm produced by unencapsulated *S. suis* cells promotes the adherence of encapsulated cells to surfaces. Therefore, in the host, unencapsulated subpopulations generated by *cps* mutations may contribute to the establishment of the infection not only by colonizing on the host surfaces using its increased adherence, but also by forming thick biofilms and working as a scaffold for further colonization of the remaining encapsulated population.

In conclusion, this study demonstrated that approximately one third of the endocarditis isolates from swine lost the ability to produce capsules and that more than 70% of representative unencapsulated isolates carried the inactivating mutations in the two glycosyltransferase genes (*cps2E* and *cps2F*). The unencapsulation was further demonstrated to enhance bacterial adherence to both porcine and human platelets, biofilm formation, and adherence of encapsulated population by coating surfaces with biofilms of unencapsulated cells. Although the capsule is considered to be an important virulence factor in *S. suis*, the results from this study suggest that loss of capsule is beneficial, not only to individual cell of *S. suis* but also to its entire population, in the course of infective endocarditis.