論　文　内　容　要　旨

A novel repressor of the *ica* locus discovered from clinically isolated super biofilm-elaborating *Staphylococcus aureus*

（バイオフィルム超高産生性黄色ブドウ球菌臨床分離株から見出された *ica* locus の新規リプレッサーの機能解析）

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Staphylococcus aureus is among the most common human pathogens, a wide range of infections, from superficial skin and mucosal infections to bone or lung infections, as well as serious systemic diseases. *S. aureus* colonization has been regarded as a risk factor for developing subsequent infections. Some chronic infections, such as endocarditis, osteomyelitis, and those on implanted medical devices, are characteristically associated with biofilm elaboration. Development of biofilms has been divided into at least three physiologically different stages: initial attachment, biofilm maturation, and detachment (or dispersal), which involves specific factors. The matrix of a staphylococcal biofilm is mainly composed of polysaccharides, cell surface and secreted bacterial proteins, and extracellular DNA. Cells encased in the matrix are protected from antibiotic therapy and host immune responses. Dispersal of cells from a biofilm may be important for the dissemination of the bacteria.

The main exopolysaccharide of the *S. aureus* biofilm matrix is poly-*N*-acetylglucosamine (PNAG), which is also known as polysaccharide intercellular adhesion (PIA). The synthesis and accumulation of PIA on the cell surface is carried out by the products of four genes: *icaA*, *icaD*, *icaB*, and *icaC*. These genes are located in one operon and were first identified by Heilmann et al. Recent studies have indicated that the expression of *icaADBC* is affected by a number of regulatory and environmental factors. The *icaR* gene is located adjacent to *icaADBC*, but is divergently transcribed from this operon. The protein encoded by *icaR* belongs to the TetR family of transcriptional regulators and represses *icaADBC* transcription by binding to a region immediately upstream of the *icaA* start codon. Additionally, environmental factors, including glucose, ethanol, high temperatures, and high osmolarity, have been reported to affect biofilm elaboration. Ethanol increases the

expression of *icaA* by repressing *icaR* transcription. In contrast, enhancement of *icaA* expression by high glucose or NaCl levels was found to occur independently of *icaR*.

A 5-nucleotide motif (TATTT) within the *icaR-icaA* intergenic region was previously shown to play a key role in the transcription of the *ica* locus. This study also demonstrated that IcaR binds to a 42-bp sequence within the *ica* promoter region, but not the TATTT sequence. Hence, the effects of the TATTT motif on *icaADBC* expression have been suggested to be controlled by other as yet unidentified repressor(s).

We evaluated the biofilm-elaborating ability of clinical isolates in Japan, and found that TF2758, which was isolated from an atheroma, is an extremely high biofilm producer. Whole-genome sequencing and a microarray analysis of TF2758 discovered a spontaneous mutation in a putative transcriptional regulator gene, one of the 7-gene cluster, which was expressed at markedly higher levels than in a non-biofilm elaborating control strain. We designated this gene as *rob*, regulator of biofilm. In the present study, we demonstrate that Rob is a long-sought repressor that recognizes and binds to the TATTT motif and suggest that Rob is an important regulator of biofilm elaboration through its control of the expression of an as yet uncharacterized hypothetical protein SAOUHSC_2898 (SATF2584) and IcaADBC.