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学 位 論 文 要 旨

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題目: Studies on the relationship between quorum sensing and biofilm formation of *Eikenella corrodens*
(*Eikenella corrodens* のクオラムセンシングとバイオフィーム形成の関連性に関する研究)

In this study, we investigated the relationship between quorum sensing and pathogenicity of *E. corrodens*. To identify the factor responsible for decreasing AI-2 activity in stationary phase, we purified AI-2 inactivating enzyme from *E. corrodens* partially and characterized it. Moreover, to clarify the LuxS dependent signal's role in pathogenicity including biofilm formation, we investigated the effect of purified AI-2 on biofilm formation of *E. corrodens*. Furthermore, we compared the biofilm formation between wild type strain and *luxS* mutant strain using flow cell system and various microscopic techniques.

In chapter 1, we investigated the mechanism underlying decrease in AI-2 activity in stationary phase of *E. corrodens*. To analyze the mechanism, we extracted and purified AI-2 from the supernatant of mid-log-phase culture. Simultaneously, the stationary-phase culture supernatant was fractionated by ammonium sulfate precipitation. On incubating purified AI-2 and 4-hydroxy-5-methyl-3(2*H*)-furanone (MHF) with each fraction, the 30% fraction decreased both AI-2 and MHF activities. The data suggest that AI-2 and MHF were rendered inactive in the same manner. Heat and/or trypsin treatment of the 30% fraction did not completely arrest AI-2-inactivation,

suggesting that partially heat-stable proteins are involved in AI-2 inactivation. We observed that an enzyme converted MHF to another form. This suggests that *E. corrodens* produce an AI-2 inactivating enzyme, and that AI-2 can be degraded or modified by it.

In chapter 2, we investigated the role of AI-2 in biofilm formation of *E. corrodens*. To determine whether the AI-2 molecule affects biofilm formation directly, we added purified AI-2 to *luxS* mutant and wild-type *E. corrodens* and compared biofilm formations by using a static assay. Results indicated that biofilm formation in *E. corrodens* was enhanced by the addition of AI-2. We also compared the biofilms formed by flow cell system for the *luxS* mutant and the wild type by using scanning electron microscopy and confocal laser scanning microscopy. The number of viable bacteria in the *luxS* mutant biofilm was dramatically reduced and more sparsely distributed than that of the wild type, which suggested that AI-2 might enhance the mature biofilm. Conversely, further analysis by modified confocal reflection microscopy indicated that the wild-type biofilm was matured earlier than that of the *luxS* mutant, and became thinner and more sparsely distributed with time. These data suggest that LuxS may facilitate the maturation and detachment of biofilm in *E. corrodens*.