（学位第３号様式）

学　位　論　文　要　旨

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題　目	Infection mechanism of *Edwardsiella piscicida* via glycoconjugates remodeling (糖鎖リモデリングを介した *Edwardsiella piscicida* の感染メカニズムの解明)

Edwardsiella piscicida, a gram-negative bacterium, belong to the family Enterobacteriaceae causing serious economic losses to aquaculture. *E. piscicida* produces NanA sialidase which cleaves sialic acids from α(2,3) sia-linked glycoprotein on host cell surface. The cleavage of terminal sialic acids by bacterial sialidase is the most crucial step for the sequential degradation of host glycoconjugates and reveals binding sites for pathogens that seem plausible as a critical factor in bacterial infection. However, the significance of cleavage sialic acid by NanA sialidase on *E. piscicida* pathogenicity remains unclear. In addition, the roles of the resultant released sialic acid and its catabolism in *E. piscicida* pathogenicity remain to be elucidated. Firstly, we studied the involvement of sialidase activity and bacterial invasiveness using several *Edwardsiella sp.* strains. We found that the pathogenic strains showed higher sialidase activity as well as NanA expression level than non-pathogenic strain. The host cell invasion was significantly enhanced by NanA-overexpression *E. piscicida* and suppressed in the presence of a sialidase inhibitor DANA. In addition, sialidase cleaved the terminal sialic acids on host glycoconjugates to unmask the glycoprotein containing *N*-acetylglucosamine and mannose that can be used as an adhesion anchorage. Secondly, we demonstrated that free sialic acid significantly increased cellular infection toward GAKS cells via sialic acids metabolic pathways. Three enzymes involve in the metabolic pathway of sialic acid in *E. piscicida* have been identified including *N*-acetylneuraminate lyase (NAL), dihydrodipicolinate synthase (DHDPS) which removes a pyruvate group from sialic acid to yield *N*-acetylmannosamine, and one *N*-acetylneuraminate cytidyltransferase (CMP-Neu5Ac synthetase) which combined sialic acid with CMP to form CMP-Neu5Ac. Among these enzymes, we focused on two enzymes belong to *N*-acetylneuraminate lyase family: NAL and DHDPS, which play a critical function in regulating of sialic acid catabolism in bacteria. We found that NAL significantly enhanced infection *in vitro* as well as the mortality of zebrafish larvae in bath-infection *in vivo,* whereas DHDPS did not. Moreover, NAL exhibited greater the expression of *E. piscicida* phenotypes including biofilm formation and motility ability, presumably due to the up-regulation of sialic acid catabolism-related genes, such as NanE, and the increment free and conjugated-GlcNAc contents. In summary, this study provides the evidence to elucidate the significance of desialylation by sialidase as well as free sialic acid and its catabolism regulated by *N*-acetylneuraminase lyase in *E. piscicida* pathogenicity.