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**A flounder gill cell line as a model to study the invasion process of *Edwardsiella tarda* into marine fish**

B Wang\*, L Song, T Yu, Y Han, X-H Zhang  
*Ocean University of China, China*

As an important intracellular bacterial pathogen in aquaculture, *Edwardsiella tarda* causes diseases in a wide variety of marine and freshwater cultured fish. However, the process of its entry into its natural host, fish cells, and its intracellular life style remain obscure. Here, a flounder gill cell line FG-9307 and various strains of *E. tarda* including the whole-genome sequenced strain EIB202 were used to study the interactions between the pathogen and the host cells. Internalization and intracellular replication of *E. tarda* were observed in the host cells under light microscopy using Giemsa stain and confirmed by transmission electron microscopy. Increased formation of structures resembling cellular protrusions were found in the proximity of the site of bacterial entry. Concurrently observed was the membrane absence at the point of contact in some cases, implicating an entry process through a “triggering” mechanism associated with a presumable contact hemolytic activity. Large amounts of replicated bacteria were found free in the cytosol after gentamicin resistance assay. Extensive electron-lucent areas with destroyed organelles in the cytoplasm and impaired plasma membrane with escaping bacteria were also observed following extended incubation. Apoptosis analysis using a flow cytometry system showed that the apoptosis rate of *E. tarda* infected cells was not significantly different from those uninfected. Meanwhile, no evidence of direct cell-to-cell spread of *E. tarda* was observed, integrated with the fact of undetected apoptosis and plasma membrane lysis of infected host cells after bacterial intracellular replication, implicated a spreading strategy in the fish host distinct from other well-studied cytosolic bacterial pathogens such as *Shigella flexneri* and *Listeria monocytogenes*. In addition, after *E. tarda* was incubated with the phagocytes from the head kidney of flounder, the pathogen exhibited shell structures resembling bacterial capsule, which may provide a special survival advantage upon infection of the host.

Keywords: *Edwardsiella tarda*, flounder, gill cell line, invasion