

# Evidence for the facultative intracellular behaviour of the fish pathogen *Vibrio ordalii*

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*Vibrio ordalii* is an extracellular, Gram-negative bacterium that produces vibriosis in salmonids. While pathogenesis is not fully understood, this bacterium has numerous likely genes for adhesion, colonization, invasion factors and, as recently suggested, intracellular behaviour. Therefore, this study aimed to clarify possible intracellular behaviour for *V. ordalii* Vo-LM-18 and ATCC 33509T in the fish-cell lines SHK-1 and CHSE-214. Confocal microscopy revealed Vo-LM-18 and ATCC 33509T inside cytoplasm in both fish-cell lines at 4 hr post-inoculation (hpi). At 8 and 16 hpi, the proportion of fish cells invaded by both strains increased. Moreover, intracellular *V. ordalii* were observed after 8 hpi inside mouse embryonic fibroblasts (MEF), demonstrating that entry was not due to a cellular phagocytosis process. Flow cytometry confirmed immunocytochemistry results, with both *V. ordalii* evidencing statistically significant differences in the number of infected cells between 8 and 16 hpi. Interestingly, *V. ordalii* infection did not significantly damage fish cells, as determined by LDH liberation. Viable counts at 8 hpi detected, on average for both lines,  $176 \pm 47$  CFU/ml of culturable intracellular Vo-LM-18 and ATCC 33509T cells. These in vitro findings support the facultative intracellular behaviour of *V. ordalii* and may be of importance for understanding pathogenicity and survival in aquatic environments. © 2019 John Wiley & Sons Ltd

intracellular facultative

Vibriosis

virulence mechanism

animal

cell line

confocal microscopy

fish disease

flow cytometry

indirect fluorescent antibody technique

microbiology

physiology

salmonine

veterinary medicine

Vibrio

vibriosis

Animals

Cell Line

Fish Diseases

Flow Cytometry

Fluorescent Antibody Technique, Indirect

Microscopy, Confocal

Salmon

Vibrio

Vibrio Infections