# TRACING *Listonella anguillarum* BY IMMUNOGOLD LABELLING TECHNIQUE IN THE GUT OF GNOTOBIOTIC SEA BASS (*Dicentrarchus labrax*) LARVAE

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## Introduction

Recently, Rekecki et al. (submitted) demonstrated *Listonella anguillarum* within the gut of gnotobiotic sea bass larvae (*Dicentrarchus labrax*) following immersion challenge, suggesting that the gastrointestinal (GI) tract is a portal of entry of the pathogen. However, adhesion and translocation through gut enterocytes of *L. anguillarum* are poorly understood and merit further elucidation.

The aim of the present study was therefore to further evaluate the previous findings with respect to adhesion to gut enterocytes, putative bacterial endocytosis and presence of intraepithelial macrophages and dendritic cells by using transmission electron microscopy (TEM) immunogold labelling technique.

## Materials and methods

Fertilized sea bass eggs were disinfected according to the standard protocol in order to obtain germ-free conditions. On day 4 after hatching (DAH4) *L. anguillarum* HI610 ($10^8$ colony forming units / ml) was administered via immersion to germ free sea bass larvae. Larvae were killed with an overdose of benzocain at 2-6-12-24-48-72-120-168 hrs post exposure (p.e.). For TEM evaluation, larvae were fixed in Karnovsky fixative and further processed according to Rekecki et al. (submitted). Immunogold labelling technique using antibodies raised against *L. anguillarum* was carried out and adjusted according to the protocol described by Ringø et al. (2006).

## Results

Shed intestinal cells with disintegrated microvilli were regularly visualized in the gut lumen in late stages of exposure (168 hrs p.e.). Invagination of some of the luminal cells and putative engulfment of bacterial structures by pseudopode-like formations were demonstrated. The engulfed structures were positive for protein A colloidal gold indicating that these structures were of bacterial origin (Fig. 1A). Similar structures were absent within the lining epithelium. Intact bacteria were also observed in close contact with the apical brush border in the gut lumen, but microvilli of the epithelial cells were intact. The apical portions of the enterocytes contained lysosome-like structures positive for protein A (Fig. 1B). Filamentous structures within the gut lumen were visualized after 120 hrs p.e. in close association or in direct contact with bacteria (Fig. 1C-D).

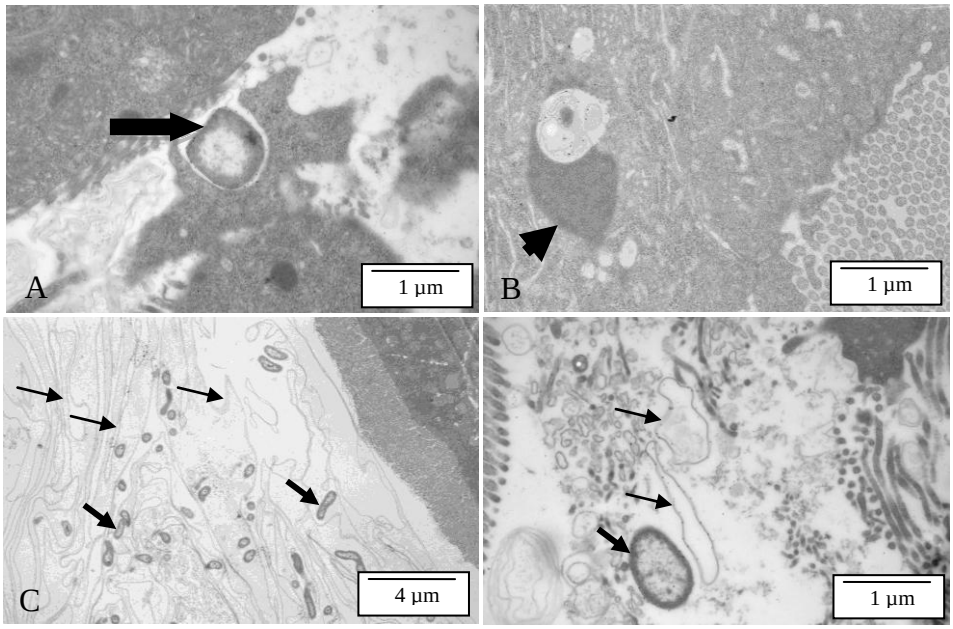

Fig.1 Transmission electron microscopy (immunogold labelling) of DAH11 (168 hrs p.e., A-B) and DAH9 (120 hrs p.e., C-D) sea bass larvae exposed since DAH4 to *Listonella anguillarum*. A) An immunogold positive bacterial-like cell (arrow) engulfed by shed host cell in midgut lumen. B) Immunogold positive lysosome (arrowhead) situated in the apical portion of a hindgut enterocyte. C-D) Filamentous structures (thin arrow) positive for protein A colloidal gold in close association with bacteria (thick arrow) in the midgut.

**Discussion**

The present study detected exfoliation of enterocytes into the gut lumen without causing any damage to the epithelial lining. However, these dying, shed enterocytes where still actively taking up the pathogenic agents. This phenomenon could be attributed to anoikis, a type of apoptosis of luminal detached enterocytes triggered by the loss of anchorage as suggested by Frisch et al. (1994). According to Kim et al. (2010), next to natural epithelial shedding, enterocyte exfoliation is an inherent host defence mechanism against invading pathogens, leading to a quick elimination of damaged host cells colonised by pathogens. Lysosome-like structures were visualized in the  indicating intracellular digestion of bacteria. This observation, together with the presence of microcolonies and surrounding filamentous structures within the gut lumen, provide indications for biofilm formation in the monognotobiotic host and are important to understand whether the GI tract acts as an important infection route for pathogenic bacteria and merits further evaluation.

**References**

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