

## Effects of administration of probiotic strains on GALT of larval gilthead seabream: Immunohistochemical and ultrastructural studies

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Received 12 December 2005; revised 16 January 2006; accepted 14 March 2006

Available online 28 March 2006

### Abstract

Two bacterial strains *Lactobacillus fructivorans* (AS17B), isolated from adult seabream (*Sparus aurata* L.) gut, and *Lactobacillus plantarum* (906), isolated from human faeces, were administered contemporaneously during seabream development using *Brachionus plicatilis* and/or *Artemia salina* and dry feed as vectors.

Experimental group A received the probiotic strains already via rotifers from day 5 post-hatch (ph), whereas treatment of group B began with *Artemia* feeding from day 27 ph. Fish were sampled at day 28 ph (group A and control) and day 99 ph (groups A, B and control) for electron microscopy, histology and immunohistochemistry with the polyclonal antiserum ORa against homologous serum Ig and the mAb G7 specific for seabream acidophilic granulocytes. In all groups, timing and pattern of differentiation of the digestive tract did not differ. Furthermore, neither tissue damage nor manifest inflammation was provoked by probiotic administration.

At day 28 ph, the developing GALT already housed mucosal leucocytes, including Ig<sup>+</sup> cells but no acidophilic granulocytes. No differences were seen between experimental groups.

At day 99 ph, the density of Ig<sup>+</sup> cells (+51%) and acidophilic granulocytes (+284%) was significantly higher ( $p < 0.05$ ) in group A than in controls. Also group B had a higher density of Ig<sup>+</sup> cells (+17%) and acidophilic granulocytes (+130%) compared with controls, although less pronounced. Light and electron microscopy observations detailed the occurrence of heterogeneous populations of lymphocytes and granulocytes in the developing intestinal mucosa, and highlighted the net expansion of G7<sup>+</sup> acidophilic granulocytes (A +536%, B +292% vs. control) due to probiotic administration.

**Abbreviations:** GALT, gut-associated lymphoid tissue; IEL, intraepithelial lymphocyte; IHC, immunohistochemistry; IR, immunoreactive; mAb, monoclonal antibody; MGG, May-Grünwald-Giemsa; Lp, lamina propria; PBS, phosphate-buffered saline.

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