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Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry

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Abstract

Two strains of *Saccharomyces cerevisiae* were tested as probiotics for rainbow trout fry, during the first month of feeding. Each strain was introduced into separate diets, at the rate of 10^6 CFU g^{-1} and their effects were compared with those of a control diet. Two rearing conditions were simultaneously compared, to test the adaptability of the probiotic treatment. From start feeding onwards, the water supply came from either spring or river, resulting in two different temperature ranges, 11–11.5 and 7–8 °C respectively. Growth and development were optimal in spring water, while some delay was observed with colder river water. A slight but significant increase in mortality was also observed in the river group. In all groups, the counts of bacteria associated with trout intestine were maximum 10 days post start feeding (dpsf; 10^7 CFU g^{-1}). The counts of probiotic yeast were also maximum at 10 dpsf (10^4–10^5 CFU g^{-1}), but the decrease was slower in river than in spring water. An autochthonous yeast, *Debaryomyces hansenii*, was also retrieved associated to the intestine of the control group in high numbers after 240 degree days of experiment (10^4–10^5 CFU g^{-1}), while the colonization level was significantly less in trout fed the probiotic diets. The effect of the dietary yeast was observed by assaying the activity of three enzymes in the brush border membrane of the enterocytes: alkaline phosphatase (AP), γ-glutamyl-transpeptidase (GGT), and leucine-amino-peptidase N (LAP). At 10 and 20 dpsf, the trout reared in spring water had higher activities of the three enzymes when they were fed the strain *S. cerevisiae* var. *boulardii*, suggesting an earlier maturation of the digestive system in this group, compared with trout fed either the other strain of *S. cerevisiae* or the control diet. The effect was not observed in trout reared in river water with slower growth. Both *S. boulardii* and *D. hansenii* seemed to stimulate digestive maturation in fish, but the natural colonization by *D. hansenii* was likely too late for trout reared at optimal temperature. The supplementation of trout starter diet with *S. boulardii* may be particularly useful in fast growing conditions.

Keywords: Start feeding; Larval development; Gut maturation; Brush border membrane; Yeast; *Debaryomyces hansenii*

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1. Introduction

Many probiotic bacteria have been proposed to improve health in rainbow trout. The strains were generally antagonistic to pathogens (Jöborn et al., 1997; Gram et al., 1999; Robertson et al., 2000; Spanggaard et al., 2001; Irianto and Austin, 2002; Aubin et al., 2005a), and an important feature was the ability to colonise fish gut (Jöborn et al., 1997; Nikoskelainen et al., 2001a,b). The immune system was stimulated in rainbow trout by several probiotics (Irianto and Austin, 2002; Nikoskelainen et al., 2003; Raida et al., 2003; Panigrahi et al., 2004). Inactivated bacterial cells might be also efficient to control furunculosis, but the viability of the probiotics influenced the immune response (Irianto and Austin, 2003; Panigrahi et al., 2005).

Andlid et al. (1995) suggested that yeast isolated from rainbow trout might also improve health, with a particular attention to their colonisation potential. Like probiotic bacteria, *Saccharomyces cerevisiae* var. *boulardii* acted as pathogen antagonist and immunomodulator in mammals (McFarland and Bernasconi, 1993), but the yeast increased also the specific and total activities of digestive enzymes in the brush-border membrane (BBM; Buts et al., 1986, 1999). *S. cerevisiae* var. *boulardii* had some effect on rainbow trout metabolism, since the dietary supply of the yeast increased muscle lipids and red pigmentation, while improving the resistance of trout to *Yersinia ruckeri* (Aubin et al., 2005b; Quentel et al., 2005). It seems that the probiotic efficiency of *S. cerevisiae* is dependent on the strain (Fietto et al., 2004; Van der Aa Kühle et al., 2005).

Considering the presence of autochthonous yeast with probiotic potential in trout intestine, a dietary supply of allochthonous strains might seem worthless. Aubin et al. (2005a) hypothesised that autochthonous *Debaryomyces hansenii* could stimulate the mucosal metabolism in rainbow trout intestine, while interfering with the dietary yeast. However, the occurrence of autochthonous yeast was different in several locations (Gatesoupe et al., 2005a). More generally, the intestinal microbiota of rainbow trout was highly variable in time, and between farms (Spanggaard et al., 2000; Huber et al., 2004; Gatesoupe et al., 2005a,b). Some environmental conditions may account for this variability, for instance the rearing temperature (Lésel, 1990). Consequently, probiotics may serve as a precaution in front of this variability, but at the same time, the efficiency of the treatments should be validated in several rearing conditions.

The present experiment was conducted on rainbow trout fry at start feeding, to compare the effects of two probiotic strains of *S. cerevisiae*, in combination with the cross effects of two rearing conditions, with particular attention to intestinal microbiota, and to the activity of BBM enzymes in trout enterocytes. The effects of the strain already tested on rainbow trout (Aubin et al., 2005a,b) were compared to those of another strain, which was recommended by Lara-Flores et al. (2003) as growth promoter for Nile tilapia.

2. Materials and methods

2.1. Rearing conditions and diets

The strain of rainbow trout (*Oncorhynchus mykiss*), and the general rearing conditions were as described by Aubin et al. (2005a), but two water qualities were compared in the present experiment. All the eggs were incubated and hatched in UV-treated spring water (11.54±0.02 °C, mean±standard error). Ten days post hatching, the fry were dispatched in 18 tanks. Nine tanks were kept receiving UV-treated spring water, while in the nine other tanks, the water supply was shifted to filtered river water within 5 h, resulting in a temperature decrease from 11.4 to 7.2 °C. Then the two conditions differed in their temperature ranges, 11–11.5 and 7–8 °C for the spring and the river, respectively.

The diets were prepared with Ecostart® 15, crumble size '01'. The control diet was obtained by coating the pellets with cod liver oil (32 ml kg^{-1}). Two experimental diets SC and SB were prepared with the probiotic yeast *Saccharomyces cerevisiae* strain NCYC Sc 47/g (National Collection of Yeast Culture, Norwich, UK), or *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 (*S. boulardii*, Institut Pasteur, Paris, France), respectively. Both strains were obtained as commercial preparations, Biosaf® Sc 47 and Levucell® SB20, respectively. The active dried yeast preparations were powdered by grinding and sifting through 100 μm, then suspended in cod liver oil. The amounts of powder were adjusted in the oily suspensions to obtain a final concentration of ca. 10^6 Colony Forming Units (CFU) of yeast per gram of experimental diet, after the pellets had been coated with the shaken suspensions (32 ml kg^{-1}). After coating, the actual counts of yeast on Sabouraud agar with antibiotics (Aubin et al., 2005a) were $(7\pm3)\times10^5$ and $(9\pm1)\times10^5$ CFU g^{-1} of *S. cerevisiae* (diet SC) and *S. boulardii* (diet SB), respectively (mean±standard error).

One day before start feeding, the fry weighed 60 mg, and they were dispatched in three tanks per treatment, i.e. the three diets crossed with the two water qualities. At start feeding, 423±2 fish were counted per tank, without