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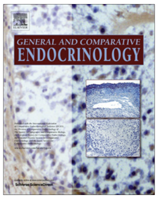

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Effects of probiotic administration on zebrafish development and reproduction

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ABSTRACT

As the consumption of probiotics increases worldwide, scientists focus on identifying bacterial strains able to improve human life quality and evidence the biological pathways affected by probiotic treatment. In this review, some recent observations on the effects of changes of microbiota on zebrafish metabolism were discussed.

In addition, the effects of *Lactobacillus rhamnosus* – a component of the human gut microflora – as a diet supplement on *Danio rerio* were presented. When administered chronically, *L. rhamnosus* may affect larval development and the physiology of reproductive system in the zebrafish model. It was hypothesized exogenous *L. rhamnosus* accelerates larval growth and backbone development by acting on insulin-like growth factors-I (igfI) and -II (igfII), peroxisome proliferator activated receptors-α and -β, (pparα,β) vitamin D receptor-α (vdrα) and retinoic acid receptor-γ (rarγ). Gonadal differentiation was anticipated at 6 weeks together with a higher expression of gnrh3 at the larval stage when *L. rhamnosus* was administered throughout development. Moreover, brood stock alimented with a *L. rhamnosus*-supplemented diet showed better reproductive performances as per follicles development, ovulated oocytes quantification and embryos quality. A plausible involvement of factors such as leptin, and kiss1 and 2 in the improvements was concluded. The observations made on the physiology of female reproduction were correlated with the gene expression of a gigantic number of factors as the aromatase cytochrome p 19 (cyp19a), the vitellogenin (vtg) and the α isoform of the E2 receptor (erα), luteinizing hormone receptor (lhr), 20-β hydroxysteroid dehydrogenase (20β-hsd), membrane progesterone receptors α and β, cyclin B, activinβA1, smad2, transforming growth factor β1 (tgfβ1), growth differentiation factor9 (gdf9) and bone morphogenetic protein15 (bmp15.) A model in which the exogenous *L. rhamnosus* in the digestive tract of zebrafish from the first days of life through sexual maturation positively influences the physiological performances of zebrafish was evidenced and a number of pathways that might be influenced by the presence of this human probiotic strain were proposed.

1. Introduction

Over the last past 20 years, the zebrafish (*Danio rerio*) has attracted considerable attention as a pre-eminent vertebrate model for studying genetics and development (Grunwald and Eisen, 2002), and more recently, for understanding human diseases and for the screening of therapeutic drugs (Keller and Murtha, 2004; Mathias et al., 2012; Penberthy et al., 2002; Sumanas and Lin, 2004). The characteristics that have contributed to its popularity as a model of human disease and development are: ease to produce and maintain in laboratory conditions, small size, rapid development and generation time, optical transparency during early development. In addition, zebrafish and human genomes have been shown to share extensive conserved syntenic fragments and many zebrafish genes and their human homolog display structural and functional genetic similarities to humans (Barbazuk et al., 2000) and finally, its genome sequence are further refined and available on web (http://www.ncbi.nlm.nih.gov/genome/guide/zebrafish/).

Given the importance of this experimental model, zebrafish was used to study the effects of the *Lactobacillus rhamnosus*, a probiotic registered for human consumption, on the intestinal microbiota, gonadal development and reproduction of zebrafish.

The genus *Lactobacillus* produce and secretes numerous substances; these may be grouped in substances anchored to the lipid membrane, to cell wall, cell membrane by N- or C-terminus, released into the extracellular medium or be part of the cellular membrane appendance (Lebeer et al., 2008). Transcriptomic and proteomic studies evidenced a large number of neo secreted protein by *Lactobacillus*, and these effector molecules influence the

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host organism at different levels, including the inhibition of intestinal pathogens (Eijsink et al., 2002), mucosal integrity (Seth and Yan, 2008; Yan et al., 2007), immune system modulation (Smits et al., 2005), metabolic interactions (Marco et al., 2006; Semova et al., 2012), cell growth (Schlee et al., 2008) and reproduction (Gioacchini et al., 2010a,b,c; Gioacchini et al., 2011; Gioacchini et al., 2012; Gioacchini et al., 2013; Giorgini et al., 2010).

2. Zebrafish gut microbiota

The knowledge of how gut microbial communities gather and cooperate with vertebrate hosts is largely derived from a few laboratory model species including zebrafish (Camp et al., 2009; Cheesman and Guillemin, 2007). In fact, zebrafish has been extensively used to study vertebrate development and physiology; only recently it has been recognized as a model for studying host–microbiota interactions. At this purpose, lately a method for zebrafish gnotobiotic husbandry, to study host responses to the gut microbiota was optimized (Rawls et al., 2004).

The first insights into the association of gut microbiota from zebrafish reared in laboratory were obtained by sequencing libraries of bacterial 16S rRNA genes (Bates et al., 2006; Brugman et al., 2009; Rawls et al., 2006). These authors evidenced that zebrafish gut microbiota was rich in the bacterial phylum Proteobacteria, with the phyla Firmicutes and Fusobacteria during both larval and adult stages. Recently, Roeselers et al. (2011) evidenced that the gut microbiota of laboratory-reared zebrafish has similar composition to that of zebrafish collected from their natural habitat. The presence of bacterial members shared in caught and domesticated zebrafish in different locations, suggested a selection of taxa by intestinal habitat with a realization of a core gut microbiota (Roeselers et al., 2011). The genera present in the gastrointestinal tract seem to be those which can survive and multiply in the intestinal tract (Ring and Strm, 1995).

The bacterial genera display day-to-day fluctuations; in fact, the bacterial composition may vary with salinity, antibiotics and chromic oxide (Ring and Strm, 1995). However, the overall diversity of bacterial communities may vary as a function of diet and environment (Sullam et al., 2012; Wong and Rawls, 2012). Using gnotobiotic zebrafish, Semova et al. (2012) evidenced that bacterial diversity and richness were markedly higher in the intestine of fed fish in respect to starved ones, indicating that feeding results in gut-specific enrichment of bacterial species in zebrafish. Semova et al. (2012) also evidenced that microbiota composition might influence energy balance of the host by the up-regulation of fatty acid uptake and lipid droplets formation.

Zhou et al. (2012) applied 10 *Lactobacillus* strains via the food to zebrafish to test the adhesive capabilities on the intestine walls using partial rpoB gene DGGE analysis, clone libraries and scanning electron microscopy. Changes of *Lactobacilli* levels in the gastrointestinal (GI) tract of probiotic-treated fish revealed that the adhesive capabilities of *Lactobacilli* varied greatly; the *L. buchneri*, *L. plantarum*, *L. brevis* and *L. rhamnosus* were identified as highly adhesive gut strains. Microbial changes in the GI were also observed when *L. rhamnosus*$^{501\text{TM}}$ was administered, the same species was present in the intestine of all probiotic-treated group, while it was absent in all replicates from the control group. The PCR-DGGE fingerprints analysis revealed that the impact of the administration of the probiotic had a clear and typical effect on the microbial communities, causing a shift in microbial fingerprints, and low similarity of approximately 50% between the groups (Gioacchini et al., 2012).

Although the large dissimilarity between the groups, no significant differences were observed in terms of microbial community and ecological parameters. However, trends toward differences were observed in respect to microbial richness (P¼ 0.105), diversity (P¼ 0.112) and evenness (P¼ 0.138).

Bacterial populations on the gastrointestine affected individual phylotypes either by suppressing them or by stimulating the presence of certain phylotypes that were absent or no detectable in the control. In zebrafish, the treatment with *L. rhamnosus* increased the presence of *Streptococcus thermophilus* causing a clear shift in microbial communities (Gioacchini et al., 2012).

The PCR-DGGE analysis in the ovaries showed the absence of *L. rhamnosus* and any detectable bacterial rRNA in the gonad indicating that the probiotic does not affect directly the ovary but operate by systemic way (Gioacchini et al., 2012).

3. Effects of probiotic on growth and calcification

Zebrafish raised with probiotic-supplemented diet showed faster development, with earlier onset of backbone calcification (Avella et al., 2012).

Previous studies (Hooper and Gordon, 2001; Hooper et al., 2002) showed that beneficial microbes led an organism to more efficiently use diet-derived energy sources, leading to growth improvement (Dimitroglou et al., 2011). The administration of *L. rhamnosus* to zebrafish larvae induced the presence of this bacterial in the intestine and a plausible increased of *Lactobacillus*' metabolites in the intestine that, by a systemic diffusion, may exert its influence on the expression of specific genes (Avella et al., 2012).

Rawls et al. (2004) evidenced the ability of the microbiota to modulate the transcription of 212 genes, 8 of which were implicated in the promotion of nutrient metabolism. Avella et al. (2012) recently correlated the presence of *L. rhamnosus* in the zebrafish digestive tract with a modulation of the expression of several genes involved in larval development. The growth-related factors, the insulin-like growth factors I and II (igfI and igfII) showed higher gene expression in treated fish, while myostatin (mstn) levels were significantly reduced by probiotic administration. Changes of igfs and mstn were correlated with higher zebrafish growth. The igfI and II up-regulation (Avella et al., 2012) found in zebrafish administered with *L. rhamnosus* was found concomitant with an anticipated backbone development. Together with these changes, an increase of vdrα and rarγ gene expression was observed. The ligands of these two receptors (vitamin D and retinoic acid) are key factors for morphogenesis and chondrogenesis process (Mendelsohn et al., 1994a,b,c). The vitamin D stimulates calcium absorption and retention (Saggese et al., 2002), while the igfs plays a role as main determinants of backbone calcification and bone mass accretion, controlling muscle and skeletal cell growth and division. A number of vitamins and fatty acids are naturally released by *Lactobacillus* spp., these products are able to bind different nuclear receptors including the vdr, rar (Chawla et al., 2001; Narva et al., 2004; Teusink and Smid, 2006) and on pparα and β both involved in skeletal development (Burdick et al., 2006). The study on calcification evidenced a more extensive calcification of centra in group treated with probiotics, indicating a faster backbone calcification (Avella et al., 2012).

4. Effects of *L. rhamnous* on gonadal differentiation and reproduction

Study performed in the zebrafish intestine evidenced that, in the developing intestine, the resident intestinal bacteria influence epithelial cells by the enhancing β-catenin stability and promoting cell proliferation (van der Flier and Clevers, 2009). β-catenin is under the control of wnt signaling, its protein is a transcription factor that once translocated into the nucleus interacts with co-activators, such