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Review

Microbial manipulations to improve fish health and production – A Mediterranean perspective

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ABSTRACT

The interactions between the endogenous gut microbiota and the fish host are integral in mediating the development, maintenance and effective functionality of the intestinal mucosa and gut associated lymphoid tissues (GALTs). These microbial populations also provide a level of protection against pathogenic visitors to the gastrointestinal (GI) tract and aid host digestive function via the production of exogenous digestive enzymes and vitamins. Manipulation of these endogenous populations may provide an alternative method to antibiotics to control disease and promote health management. Applications of probiotics for Mediterranean teleosts can stimulate immune responses, enhance growth performance, feed utilisation, digestive enzyme activities, antioxidant enzyme activities, gene expression, disease resistance, larval survival, gut morphology, modulate GI microbiota and mediate stress responses. Although considerably less information is available regarding prebiotic applications for Mediterranean teleosts, prebiotics also offer benefits with regards to improving immune status and fish production.

Despite the promising potential benefits demonstrated in current literature, obtaining consistent and reliable results is often difficult due to our incomplete understanding of indigenous fish GI microbiota and their subsequent host interactions which mediate and drive both localised and systemic host immunological responses. Additionally, the probiotic and prebiotic (biotics) mechanisms which mediate host benefits at the mucosal interface are poorly understood. Future studies focused on these interactions utilising gnotobiotic techniques should provide a better understanding of how to extract the full potential of biotic applications to promote immune function of Mediterranean teleosts.

1. Introduction

Since the EU moratorium on the banning of antibiotic growth promoters in animal feeds [1], including fish, research for alternative nutraceutical products has been a major objective for both Mediterranean and global fish culture practices. Alternative methods of disease prevention have been sought where manipulation of microbial populations in the rearing environment, and associated with the fish host, have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immunological responses. In this respect, microbial manipulation of the microbiota within the gastrointestinal tract (GI) of the fish host and live feed microbial assemblages have received great attention due to recent studies which have given us a more broad understanding of the importance of the endogenous microbiota of fish in mediating immunological development and functionality, particularly at the mucosal interface within the GI tract.

The present review is focused on Mediterranean fish species, which have been extensively studied over the past 10 years, and continue to constitute a key source of quality seafood in southern Europe, Northern Africa and several Middle Eastern countries [2,3]. Despite the progress in hatchery and culture techniques, intensive

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Abbreviations: CFU, colony forming units; DGGE, denaturing gradient gel electrophoresis; dph, days post-hatching; EM, electron microscopy; FISH, fluorescent *in situ* hybridisation; GALT, gut associated lymphoid tissue; GI, gastrointestinal; HK, head–kidney; HSP, heat shock protein; MOS, mannan oligosaccharides; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

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production of marine fish in the Mediterranean remains difficult, especially at the larval stage, with one of the most serious production constraints being the control of infectious diseases.

2. The role of gut microbiota in fish health and development

The gut of small marine yolk sac larvae upon hatching is straight, often with ciliated cells [4–6], sterile [7], with no open mouth present immediately following hatching of some species such as common dentex [8], solea [9], sea bass [10] and sea bream [11]. The opening of the mouth, in dentex and sea bream larvae, occurs 4 days post-hatching (dph) [8,11] and the mouth of sea bass yolk sac larvae opens after 2–3 days (at 15–16 °C) when the larvae are about 4.5 mm in length and the digestive system appears to be functional [12].

From this moment onwards the GI tract is thought to be one of the most important and intimate sites of interaction with the external world and is considered one of the major portals for pathogenic invasion in fish [13–15]. However, larval fish have a poorly developed immune system, relying primarily on their innate immune response [16]. In this sense the commensal microbiota colonizing the larval stage of fish, which reflects the microbiota of the eggs, the rearing water, and the microbiota of live food organisms during the first-feeding stage [17], can be considered the first line of defence, providing an effective barrier through competitive exclusion of chemicals, nutrients and attachment space. Furthermore, certain bacterial groups, such as lactic acid bacteria (LAB), which are often identified as indigenous components of the gut microbiota of fish [18,19], provide antagonism to potential pathogens via the production of a combination of extracellular products (e.g. lactic acid, hydrogen peroxide, carbon dioxide, siderophores, antibiotic peptides/proteins, organic acids, ammonia and diacetyl). However, the endogenous bacterial populations of fish do not merely provide a defensive barrier against enteric pathogens; as is the case with terrestrial animals, the interactions between the gut microbiota and the fish host are integral in mediating the development, maintenance and effective functionality of the intestinal mucosa. This has been demonstrated by comparing gut differentiation and gene expression of germ free and conventionally reared zebrafish (*Danio rerio*) larvae [20–23]. For a review of the effects of endogenous fish gut microbiota on host health, disease resistance and digestive function readers are referred to the reviews of Gomez & Balcázar [24], Denev et al. [25] and Merrifield et al. [26].

At the early larval stage, commensal and favourable bacteria in the GI tract stimulate immune activities, localised morphological development and are fundamental in maintaining mucosal tolerance (i.e. identifying and differentiating pathogenic insults from feed particles/commensal bacteria). The GI microbiota continue to play a role in maintaining the effective functionality after GI differentiation and disturbance in these immune regulatory functions by an imbalanced microbiota may contribute to the development of diseases or reduced functionality [27]. It is believed that probiotics and/or their components/products interact with the gut associated lymphoid tissue (GALT) to induce immune responses. Unlike mammals, fish lack Peyer's patches, lymph nodes, secretory IgA and antigen-transporting M cells in the gut [28–32], although a recent lectin binding experiment demonstrated the presence of cells with mammalian M-cell characteristics in the intestinal epithelium of the salmonid mid gut [33]. In teleosts, the level of GALT organization is lower than in mammals but many diffusely organized lymphoid cells, macrophages, granulocytes and mucus IgM found in the intestine of fish constitutes the immune function [29,32,34–36]. More recently it has been suggested that IgT is specialized in mucosal immunity in rainbow trout (*Oncorhynchus mykiss*) [37]. Data obtained in sea bass [30], carp (*Cyprinus carpio*) [38] and rainbow trout [39] suggest that the lymphocytes located in the intestinal mucosa are mostly T cells, as in some mammalian species, although Ig^+ cells have also been described in the teleost intestinal mucosa [29,30,32].

Immunopurified intestinal lymphocytes from sea bass expressed T cell receptor-β transcripts (*TCR*-β) [40] and intraepithelial lymphocytes (IELs) in rainbow trout, which do not seem to home specifically to the gut mucosa, express transcripts of various T-cell marker genes (homologs of *CD3*-ε, *CD4*, *CD8*, *CD28*, *TCR*-β, *TCR*-γ and *TCR*-ζ) [39]. Also the expression of *TCR*-γ homologs has been recently reported in the intestine of Atlantic salmon (*Salmo salar*) [41]. In addition, Danilova et al. [42] detected TCR α positive cells in the oesophagus and intestine of 9 day-old zebrafish by *in situ* hybridisation. On the contrary, Trede and collegues [43] were unable to detect T cells in the periphery of zebrafish in the first week of life. In teleosts, the immune function of intestinal T cells is still largely unknown, although the rainbow trout IELs isolated from the intestine were shown to be spontaneously cytotoxic against a mouse tumour cell line [44]. Additionally in this species, Fischer et al. [45] demonstrated that the major effector cells that exhibit specific cytotoxicity against allogeneic cells are $CD8^+$ lymphocytes with a phenotype and gene expression pattern similar to those in higher vertebrates. Cytotoxic activity against allogeneic cells has also been reported in channel catfish (*Ictalurus punctatus*) [46] and carp [47].

Functional differences have been reported in the different segments of the teleost intestine. In fact, the second gut segment of fish possesses strong antigen uptake capacity, and the uptake and transport of antigens followed by their processing by intraepithelial macrophages, is reported in different teleost species [34,48]. In sea bass, the uptake of particulate antigens (*Vibrio anguillarum* bacterin) has been detected at this anatomical site after oral or anal administration [49] and recent investigations in brown trout (*Salmo trutta* L.) and Atlantic salmon have shown that the anterior intestine is also able to absorb macromolecules and deliver the absorbed substance into circulation [50,51].

This brief description of the morphological and functional organization of the teleost GI tract and its immune system, clearly introduces the importance of finding strategies to modulate the composition of the gut microbiota to benefit the host. Methods of modulating these populations primarily include probiotics and prebiotics (collectively referred to as “biotics” within this review). Other methods, such as bioremediation and bioaugmentation (microbial applications to improve water quality), have also been explored in a range of aquatic species [52]; however applications with regards to Mediterranean teleosts are somewhat limited.

2.1. Probiotics

The definition of probiotics for aquatic animals is somewhat unclear; probiotics are often defined as applications of entire or component(s) of a micro-organism which are beneficial to the health of the host [53] but other probiotic definitions are more encompassing. For example, Vershuere et al. [54] defined probiotic applications as live microbial adjuncts which beneficially affect the host by modifying the host-associated or ambient microbial community resulting in improved feed utilisation or nutritional value, by improving disease resistance or the quality of the environment. The usual defining points that are debated include whether the microbe is applied alive or dead, whether the application is administered via the feed or rearing water and whether the host benefits are solely restricted to immunological parameters. It has been suggested from previous variations of definitions that a probiotic for aquatic animals may fall under the following broad definition: any microbial cell provided via the diet or rearing water which subsequently benefits