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Expert Commentary

DIVERSITY AND FUNCTIONALITY OF GASTROINTESTINAL MICROBIOTA IN FARMED FISH: A COMMENTARY ON WHAT WE HAVE LEARNED AND FUTURE DIRECTIONS

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Based on the knowledge acquired from land animals, the contribution of intestinal microbiota to fish nutrition was hypothesized, in view of the ability of some bacterial strains to produce either nutrients or enzymes, possibly active in the digestive process of the host. These early studies were reviewed by Ringø et al. (1995), for instance. However, these findings were moderated due to the relatively low level of culturable bacteria that were generally recovered from the gut of fish farmed in cold and temperate waters, in comparison with homeotherms, and with some warm water herbivorous fishes, in which intense intestinal fermentation was detected (Clements, 1997). The threshold for contributing significantly to the catabolic process of digestion was estimated to 10^7 bacterial bodies per g of digestive tract by Lésel (1993). This author wondered about possible anabolic function by releasing micronutrients, even at low population density. In most aquacultured species, the nutritional role of gut microbiota was generally considered as secondary, with few exceptions. That may change with what we have learned from new studies applying molecular methods. It is now admitted that culture-independent methods are required to account for the real complexity of gastrointestinal biota in fish (Pond et al., 2006). The proportion of culturable bacteria was estimated between 10 and 50% in human colonic biota (Zoetendal et al., 1998), and the same range was generally found in rainbow trout intestine by Huber et al. (2004), but this rate was only 2% in one fish. Such extremely low culturability was also noted in samples from Atlantic and coho salmons (Holben et al., 2002; Romero and Navarette, 2006).

This variability in fish gut microbiota is an important feature, which requires further investigations to understand its nature. The different characterization methods may account partly for discrepancies observed among data available in the literature (Pond et al., 2006). If

culture-dependent methods are obviously biased, there is no ideal solution, and molecular methods are also subjected to PCR biases (Zoetendal et al., 2004). Verner-Jeffreys et al. (2003) noted some difficulty in amplifying genomic DNA from *Vibrio alginolyticus*, and Romero and Navarette (2006) suggested that the combination of molecular and culture-based analyses may provide a better understanding of microbiota. Beyond methodological bias, bacteria appeared to vary with sampling season (Al-Harbi and Uddin, 2004) or location (Skrodenyte-Arbaciauskiene et al., 2006), and more generally, with environmental conditions. Unlike humans, omnivorous and carnivorous fish does not seem to harbour specific and resilient intestinal biota. The frequent dominance of opportunistic proteobacteria suggests the formation of temporary consortia of strains coming from surrounding microbiota, while able to survive and to multiply in the digestive tract (Huber et al., 2004). The effects of deterministic factors from feed and environment were investigated by comparing the data collected in different experimental conditions, especially in farmed species, but stochastic factors seemed also important for the colonization of aquatic niches by bacteria (Verschuere et al., 1997). The functional niches may thus be occupied by a variety of different taxons, whereas the selection process is not fully predictable.

This unpredictability challenges the weight of characterising microbiota in particular experimental conditions, while attempting to draw conclusions of general import (Pond et al., 2006). Besides taxonomical characterization of the main components of fish gut microbiota, further research should address the relevance of general tools used to described diversity when they are applied to this particular ecosystem. Notwithstanding normal circumspection, the culture-independent approach enlarged radically the field of view on microbiota. That opened the way to abstain from tedious species naming, while overcoming the uncertainty inherent in species composition. Classical ecological indices seemed suitable to describe microbial diversity after fingerprints from partial amplicons of 16S rRNA gene, without need for sequencing (Eichner et al., 1999; Hill et al., 2003; Hewson et al., 2006), but these indices may vary with the methods. For instance, Moeseneder et al. (1999) found with Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis more taxonomic units than when they used Denaturing Gradient Gel Electrophoresis (DGGE), which seemed thus to underestimate richness. Bias may be also expected in abundance assessment, due to different numbers of copies of the 16S rRNA gene in bacterial species (Fogel et al., 1999). Instead of ribosomal RNA genes, Giacomazzi et al. (2004) proposed to use *rpoB* gene, a single copy of which seemed always present.

Buckling et al. (2003) demonstrated that bacterial populations lose their potential to diversify, as they are adapting to a specific niche. Nelson et al. (2003) observed high diversity in bacteria from gut of wild herbivorous mammals, which are exposed to environmental changes. These authors took the example of drought -against which wildlife is more resistant than domestic animals - to hypothesize that microbial diversity could play a part in health and endurance. This aspect has not been yet explored in farmed fish, which are under process of domestication. A first instance was not in favour of this hypothesis, since Holben et al. (2002) found strong dominance of two species of *Mycoplasma* in wild salmon, whereas further richness and evenness appeared in farmed salmon. However, this single observation cannot preclude from investigating the possible impact of aquaculture on microbial diversity, and its consequences on fish health.

There has been recently a growing interest in axenic and gnotobiotic rearing of fish (Marques et al., 2006). It was demonstrated in gnotobiotic zebrafish that gut microbiota

stimulated epithelial differentiation and proliferation, gut motility, protein uptake, nutrient metabolism, and innate immunity (Rawls et al., 2004; 2006; Bates et al., 2006). These functional roles are likely not limited to early ontogeny, but the information is missing for juveniles and on-growing fish. The progresses in metagenomics and metaproteomics have raised great hopes for a better understanding of metabolic activities in microbiota (Gill et al., 2006; Wilmes and Bond, 2006), but it will likely take time to apply to aquaculture, in front of the huge diversity of microbiomes. A first coarse estimate of metabolic activity may be obtained by using 16S rRNA as template for RT-PCR (Moeseneder et al., 1999), though sequences from 16S rRNA genes may correspond to highly diverse genomes (Jaspers and Overmann, 2004). The diversity indices based on the RT-PCR products from 16S rRNA cannot generally depict the actual activities expressed in the microbiome. It may be worth using in strict experimental conditions, where everything should be constant except for the tested variable. The functional response of microbiota to one environmental parameter, or to one dietary compound, may be thus described by possible changes from evenness in metabolic activity. Ward (2006) emphasized the interest to combine classical culturing and enrichment techniques to the metagenomic approach, when possible. For example, Panas et al. (2007) selected bacteria able to degrade phosphonoacetate by enrichment, then by discrimination with RT-PCR, Temperature Gradient Gel Electrophoresis (TGGE), cloning, and sequencing. Such methods may find applications for aqua feeds, like fibrous plant-based protein sources, or dietary prebiotics.

These recent advances have brought valuable ways of investigation, which can be applied to aquaculture nutrition with reasonable effort. If we cannot delineate yet the import of gut microbiota in fish nutrition, future research plans should address this issue.

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