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Probiotics and prebiotics for fish culture, at the parting of the ways

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Abstract:

After more than 20 years of research on probiotics for aquaculture, the treatment has become a reality in farms and hatcheries, mainly in those devoted to shrimp production. New insights into gut microbiota have recently offered the opportunity to reconsider the application to fish culture, still lagging behind shrimp industry in the use of probiotics.this regard. Additionally, the concept of prebiotics is emerging as another effective way of disease control in aquaculture. Prebiotics are food ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract. While dietary applications of prebiotics and probiotics may be combined, one or the other may also be independently administered. This commentary highlights the recent developments in our understanding of probiotics and prebiotics and provides pointers to the future.

1. Introduction

The first reported trials of probiotics in aquaculture were conducted in Japan in 1981. Spores of *Bacillus toyoi* were used in the feed to reduce the mortality of elvers of the Japanese eel, *Anguilla japonica*. At that time, the knowledge about gastrointestinal microbiota in fish was scarce. There could have been doubts about the applicability of the same bacteria in land and aquatic animals. But, *Bacillus* strains were chosen for the first attempts, as these soil bacteria were efficient in land animals. In later years, many other strains, including new strains isolated from the aquatic environment and supposedly possessing better ability to colonize the intestine, were tested on fish. So far, few products have passed from the laboratory to the market. One of the main success stories in probiotic applications in aquaculture is *Bacillus*-based products commercialised for shrimp culture. Commercial application of probiotics in fish culture still lags behind. The fast-growing fish industry urgently needs sustainable approaches for disease control including probiotics.

Among the sustainable options available for disease control are immunostimulants, probiotics and prebiotics. The health benefits of immunostimulants and probiotics have been documented relatively better while knowledge on prebiotics is still emerging. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host’s intestinal balance. A number of questions concerning prebiotic applications are currently raised just as probiotic applications were questioned years ago. For example, is it sensible to introduce non-digestible extracts from vegetables in the diet of carnivorous fishes? New understandings of gut microbiota in fish help to answer such questions.

2. New understandings about fish gut microbiota

It is time for a reappraisal of the use of pro- and prebiotics in fish farming, in view of what we have learned recently about the role of gut microbiota in fish. In 2004, Rawls et al. made a decisive contribution to our understanding of the role of gut microbiota , by comparing the intestine of zebrafish larvae reared either conventionally, or in microbe-free conditions. Compared to gene expression in microbe-free fish, 212 genes were significantly up- or downregulated in the digestive tract of fish that were either conventionally-raised, or raised microbe-free, but re-seeded with cultivable microbiota. Many of the genes played roles in immunity, and some were directly involved in fish nutrition. The larvae were fed live *Artemia*. The study demonstrated the essentiality of microbiota in fish larvae fed only animal proteins. The role of microbes in fish nutrition has been recognised for herbivores and omnivores, but this was not obvious with carnivores, which comprise most of the valuable farmed fishes. The authors furthermore found that some responses of the genes to microbiota were conserved across a broad species spectrum: from zebrafish to mouse. For instance, the stimulation of epithelial proliferation in the intestine is highly conserved. Prebiotics could stimulate such epithelial proliferation. However, prebiotic treatment implies the occurrence of a competent beneficial microorganism in fish microbiota. One needs to consider that the bacteria dominant in fish intestine are quite different from those observed in mammals. These bacteria are generally opportunistic, and their occurrence in fish gut is transitory, depending on environmental conditions. The association between microbes and fish host would not last most of the life span, as seen in human microbiota. Recent papers have shown the high variability of the dominant intestinal bacteria among trout farms and cod hatcheries (Figure 1). These findings indicate the need to confirm the benefits of probiotics and prebiotics at in each location.

3. Updating the prerequisites for probiotics

Food safety has become a worldwide concern, and it is no any longer tenable to ignore regulations concerning food safety in many countries. If probiotics is to be considered as a sustainable alternative to antibiotics (Figure 2), their efficacy as well as the lack of pathogenicity must be documented,. Some additional constraints may also arise. For example, the European Food Safety Authority is considering a ban on those microorganisms that harbour acquired antibiotic resistance factors. The cost of undertaking the regulatory approval process may prohibit commercialization of probiotic-based products, particularly those specialty products based on natural fish microbiota. The body of knowledge supporting the use of familiar microorganisms represents a substantial advantage for approvals. Consequently, two types of candidate probiotics may be preferred: those already approved for other animals, and those isolated from fish gut, but belonging to familiar groups, like *Lactobacillus* sp., *Saccharomyces cerevisiae,* etc. The ubiquitous strains of *Bacillus* sp. may also be suitable, on condition that they lack of toxigenic potential.

Probiotics are live feed additives, but some of their effects can be reproduced with dead cells or cell-wall components, particularly those involving the immune response. A key issue is whether the probiotic must be kept alive to be efficient. If viability is not necessary, the simple incorporation of inactive cells or extract in the diet may be more cost-effective. More generally, the elucidation of the mode of action will help in optimizing the administration procedure and schedule.

4. Are prebiotics applicable to fish culture?

The main advantage of prebiotics over probiotics is that they are natural feed ingredients. Their incorporation in the diet does not require particular precautions, and their authorization as feed additives may be more easily obtained, in spite of some concerns about their safety and efficacy. Originally, prebiotics were chosen to stimulate bifidobacteria and lactobacilli in human microbiota. The case is different in fish, where many opportunistic bacteria can utilise a wide range of carbohydrates. Some of these strains may be capable of metabolising the prebiotic, but the effect on the host fish remains uncertain. The pathogenic strains are highly specialised, and unlikely to benefit from the prebiotic. However, the continuous supply of the substrate in the intestine may create the risk that the pathogen could acquire the ability to use either the native compound or its degraded products. It is wise to introduce the prebiotic in the diet with discernment, and many experiments will be necessary with microbial survey, before practical applications in hatcheries and farms. For the present, there are encouraging first trials, and more communications are expected in the forthcoming years (Figure 3).

5. Prebiotics and probiotics: which way to choose?

The immediate answers may be based on probiotics alone, on the condition that these treatments are not subjected to regulatory constraints in the country of application. Hopefully, knowledge gained from such applications will help their authorization elsewhere. In the meanwhile, research on prebiotics can progress, while possibly testing new poly- and oligosaccharides. When Gibson and Roberfroid introduced the concept of prebiotics in 1995, they foretold that synbiotics, combining pre- and probiotics, might be the ultimate solution to control colonic microbiota. This conjecture sounds particularly relevant to fish microbiota, where the introduction of the competent probiotic appears as a precaution against misusing the prebiotic by opportunistic bacteria. Whatever choice may prevail in the end, an integrative effort should put the industry and institutions together in research, and fish farmers should be encouraged in participating as volunteers in broad-scale field trials.

6. Further information and references

There are several reviews on probiotics for aquaculture, notably:

Gatesoupe F.J. 1999. The use of probiotics in aquaculture. Aquaculture 180, 147-165.

Gomez-Gil B. et al. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 191, 259-270.

Irianto A. and Austin B. 2002. Probiotics in aquaculture. J. Fish Dis. 25, 633-642.

Vershuere L. et al. 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev. 64, 655-671.

Wang Xiang-Hong et al. 1998. Application of probiotics in aquaculture. http://www.alken-murray.com/China98.htm, 4 pp.

Rawls' paper is freely available on Internet:

Rawls J.F. et al. 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. PNAS 101, 4596-4601.
http://www.pnas.org/cgi/content/full/0400706101/DC1.

Concerning the regulatory issue, one can refer to:

EFSA 2005. Summary of an Opinion of the Scientific Committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives. EFSA J. 226, 1-12.
http://www.efsa.eu.int/science/sc_commitee/sc_opinions/972/sc_opinion_ej226_qps_en1.pdf
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Sanders M.E. and Huis in't Veld J. 1999. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. Antonie van Leeuwenhoek 76, 293-315.

Sanders M.E. et al. 2005. Weight of evidence needed to substantiate a health effect for probiotics and prebiotics. Eur. J. Nutr. 44, 303-310.

Biographical note

Dr. François-Joël Gatesoupe has worked for three decades in joint research programmes of INRA and Ifremer, in the field of fish nutrition, studying the requirement for fatty acids, live food organisms and compound diets for larviculture. He has paid a particular attention to the interrelationship between intestinal microbiota and the host, striving to improve fish health and larval development with probiotics.

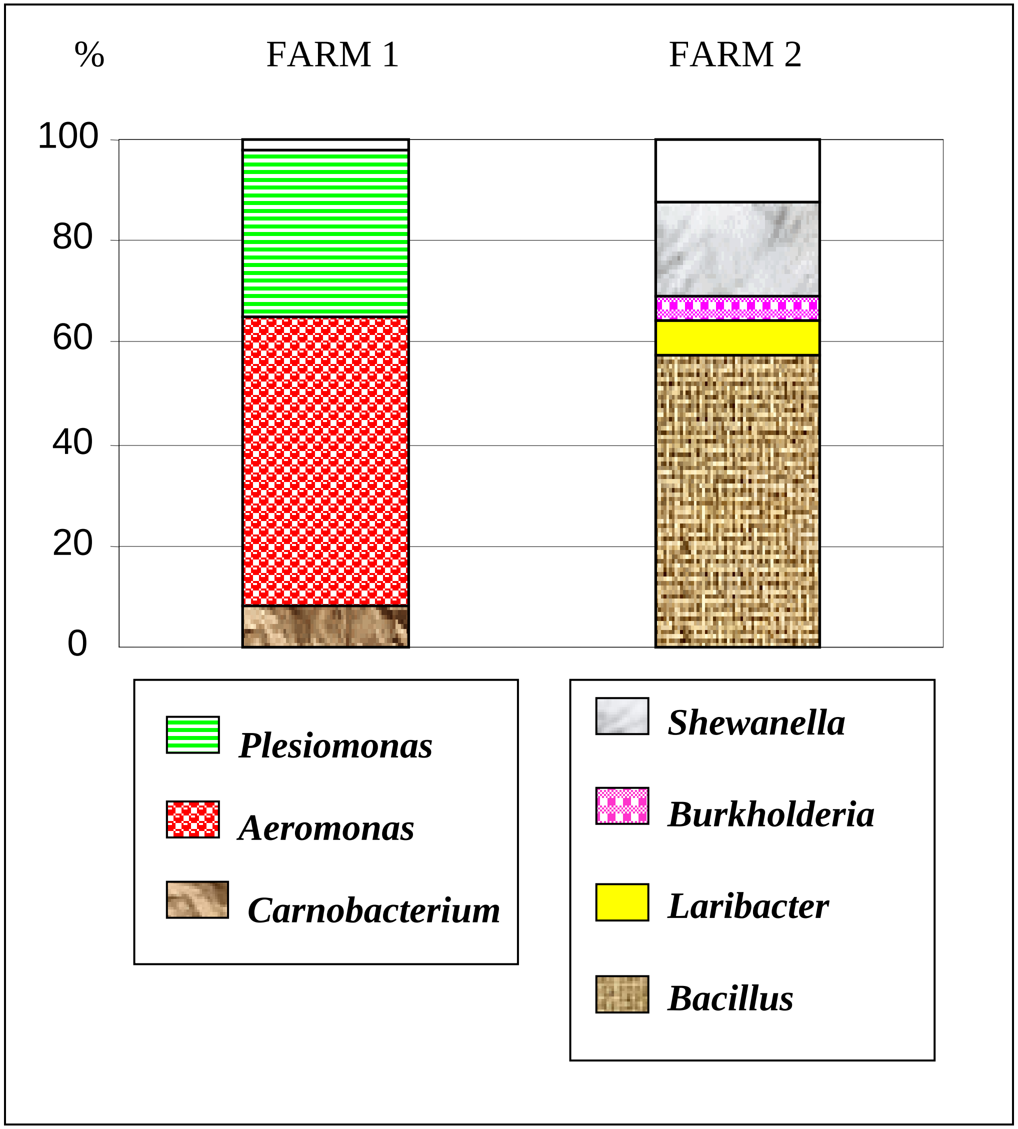

*Fig.1: An example of the variability of bacteria in rainbow trout intestine, 9-10 months post start feeding. None of the genera dominant in one farm were retrieved in the other farm (proportions in % of the total isolates; more details in: Gatesoupe F.J. et al. 2005. Ofimer Probiotic Study on Rainbow Trout. II. Intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*) submitted to probiotic treatment with* Saccharomyces cerevisiae *var.* boulardii. *EAS Special Publication No. 35, pp. 217-218.).*

Fig.2: Probiotics may replace antibiotics in some cases. For instance, it was possible to reduce the incidence of vertebral compression in rainbow trout (arrow) by introducing in the diet either florfenicol for the first ten days of feeding, or Pediococcus acidilactici *for five months post start feeding (Aubin et al. 2005. Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss *Walbaum). Aquac. Res. 36, 758-767).*

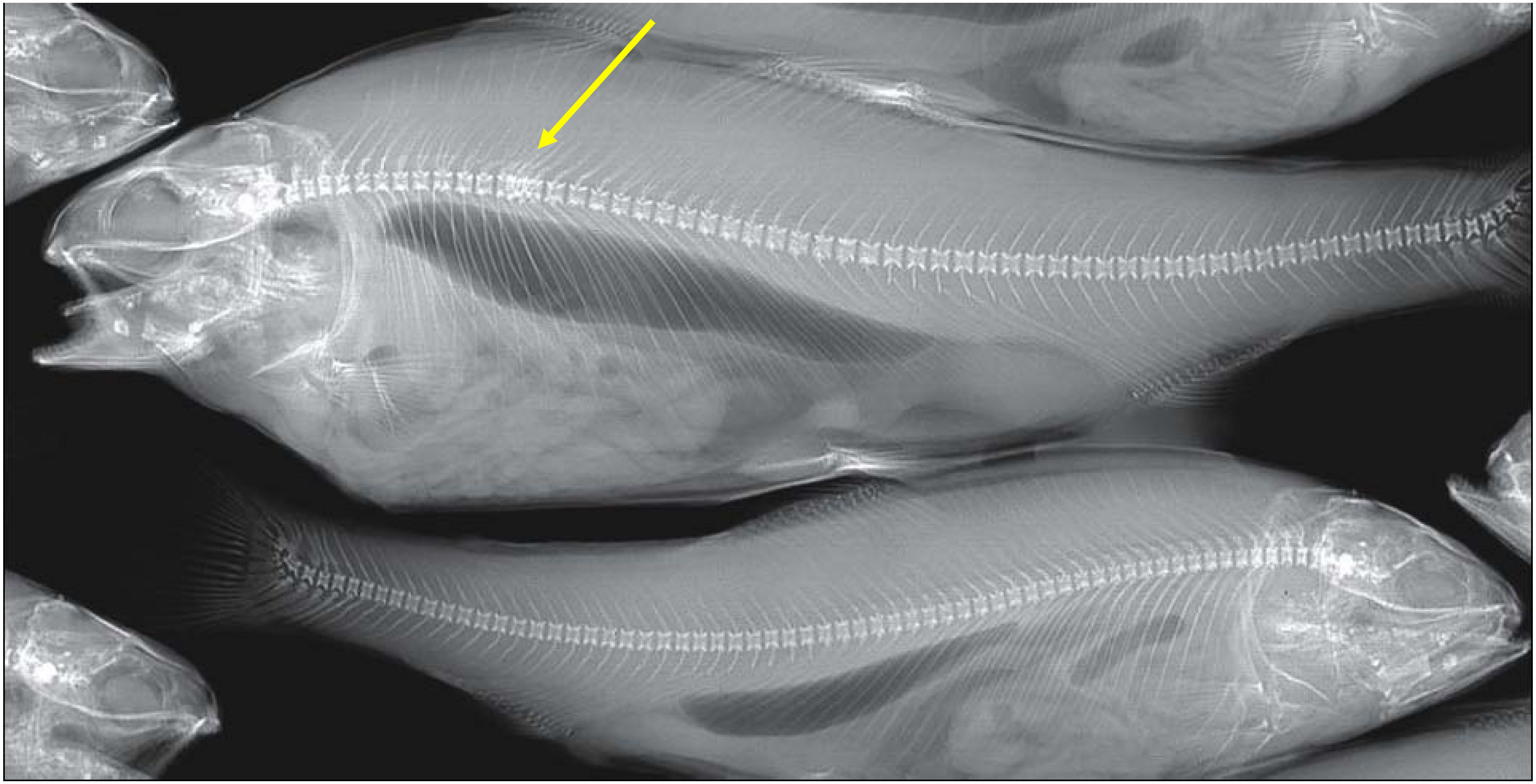

Fig.3: A preliminary experiment with prebiotics for weaning turbot indicated that fructooligosaccharides derived from inulin increased growth, while lactosucrose did not (Mahious et al. 2006. Effect of dietary inulin and oligosaccharides as prebiotics for turbot, Psetta maxima *(Linnaeus, C. 1758). Aquacult. Int. 14, (11 pp., online first).*