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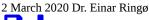
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Use of probiotics in shellfish aquaculture

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An alternative approach to promote animal health during production



The use of probiotics in shellfish culture is an alternative approach to immunoprophylactic controls. Photo by Darryl Jory.

In the 1970s and 1980s, antibiotics were commonly used in disease control in finfish and shellfish aquaculture, but their indiscriminate use for treating infectious diseases led to selective pressure of antibiotic resistance, a property that may be transferred to other organisms.

Moreover, it is generally accepted that antibiotics administration in finfish and shellfish modulate the gut microbiota, which in turn exerts negative effects on humans. Based on this fact, the European Union in 2003 banned the use of antibiotics in shellfish production. The use of probiotics is one of the alternative approaches to immunoprophylactic control in aquaculture and is considered as a supplementary strategy or alternative to vaccines and chemicals.

There are several definitions of probiotics but the most widely used is the definition by the World Health Organisation (WHO): "Live microorganisms that, when administrated in adequate amounts, confer a health benefit to the host." There is a long history of claims for health-promoting microorganisms. It has been reported that the Roman historian Plinius in 76 B.C. recommended the administration of fermented milk products for treating gastroenteritis.

During the last decades, several reviews have discussed probiotics and their impacts on shellfish aquaculture as growth promoters; on nutrition and environmental capacity; as immunostimulants; and as a prophylactic against infectious diseases. Several probiotics species are currently used in shellfish aquaculture, including from the genera *Lactobacillus*, *Enterococcus*, *Bacillus*, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Microbacterium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces* and *Vibrio*.

This article – adapted and summarized from the <u>original</u> [Ringø, E. 2020. Probiotics in Shellfish Aquaculture. *Aquaculture and Fisheries* 5(1): 1-27.] – reviews probiotics use in sustainable shellfish aquaculture.

Methods of administration

To my knowledge, the first application of probiotics in aquaculture was carried out by Kozasa in 1986, but since then their use has increased rapidly and several comprehensive aquaculture reviews have discussed the topic.

Regarding their use, it is essential to investigate the best way of administration, their optimal dose, and some technical aspects like keeping the probiotics alive in dry pellets. Additionally, some important questions regarding probiotics include the use of species isolated from the host, host specificity, and the

use of strains from other species or commercial probiotics.

Probiotic administration depends on several factors, including the probiotic or probiont, the supplementation form, the vector of administration, dosage level and duration of application. Several different administration modes have been used, including:

Oral administration via diet or water/bath

Supplementation of the diet is the most common administration method. Generally, probiotics and cell wall components (parabiotics) are applied in the feed as freeze-dried cultures, which are sometimes mixed with lipids to be added as top dressing. Probiotics can also be added to the entire tank or pond water. And for fish and shellfish larvae, live food (e.g. artemia) has proved to be an efficient carrier of probiotics.

Administration of several probiotics in combination

Since the early 1990s, most probiotic studies in aquaculture used single administration, but during the last decade, supplementation of multiple probiotics in the diets to aquatic animals has gained interest. The advantage of multiple-strain preparations is that they are active against a wider range of conditions and species.

Use of inactivated bacteria, or spores

For example, oral administration of heat-inactivated *Lactobacillus delbrueckii* ssp. *lactis* and *Bacillus subtilis*, individually or combined. Spores are structures produced by a few bacteria genera and are resistant to many environmental or induced factors that the bacteria may be subjected to. The spores help the bacteria to survive by being resistant to extreme changes in the bacteria's habitat, including extreme temperatures, lack of moisture/drought, or being exposed to chemicals and radiation. Bacterial spores can also survive at low nutrient levels, and according to some authors, spore-forming probiotic bacteria have received increased scientific and commercial interest.

Modes of action

The selection of potential probiotic strains is based on many different criteria, such as growth in mucus, acid and bile tolerance, survival in gastric juice, production of extracellular enzymes, production of antimicrobial substances which inhibit in vitro growth of pathogens and biosafety (hemolytic activity and antibiotic susceptibility).

Adhesion to the intestinal mucosa is considered an important selection criterion and a prerequisite for persistent beneficial effects of probiotics. Also, competitive exclusion, where the probiotic organism colonizes the gut, thereby inhibiting adherence and colonization of pathogenic bacteria by producing inhibitory substances which impede pathogenic organisms from adhering and colonizing the gastrointestinal (GI) tract. Additionally, substances produced by probiotics can act as antagonists for a quorum sensing (process where single-celled organisms monitor their population density by detecting the concentration of diffusible, small signal molecules produced by the cells themselves) mechanism. Or by enzymatic contribution to digestion, as shown in several studies.

Competition for iron is another mode of action: for pathogenic bacteria, the ability to acquire iron is vital to survival in the host. In consequence, many genes involved in iron acquisition are associated with bacterial virulence. Siderophores – low molecular weight substances produced by probiotic candidates or beneficial gut endosymbionts – reduce the availability of iron for pathogenic bacteria, as siderophores has high affinity for ferric ion.

Another mode includes by improved immunity, and increased macrophage activity and antibody level. Probiotics which can enhance host immunity and disease resistance of finfish and shrimp have received much interest during the last decade. Among probiotic bacteria, lactic acid bacteria (LAB) and *Bacillus* species are most frequently used, and they have shown to promotes the health of the host animal by stimulating the innate immune response and improving resistance towards pathogenic microbial infection. Finally, by antiviral effects and by improvement of water quality in ponds through modulation of the water microbiota, improved water physicochemical parameters and by disease control.

Perspectives

The importance of probiotic administration and their beneficial health effects have been discussed in several reviews, and there is much information available on the use of probiotics in aquaculture, including in shellfish culture. Although we cannot conclude that probiotics are better than immunostimulants or vaccines, their beneficial effects on the host and their environment are one of the most promising approaches used to control diseases and modify the culture environment.

In future studies on probiotics in shellfish aquaculture, biofloc culture systems using probiotics should be investigated regarding growth performance, immune response, gut microbiome and disease resistance, as only limited information is currently available. In addition to probiotics, paraprobiotics (cell wall components) may also serve as an alternative to the use of antibiotics in the prevention and treatment of infections caused by pathogens. In this regard, it is of interest to notice that both probiotics and paraprobiotics can bind directly to pathogenic bacteria, which limits adherence and colonization of the pathogen to gut cells.

The functionality of gut microbiota depends on the ability of microorganisms to interact within the GI tract, which benefits the host through their effect on inflammation, metabolism, immunity and even behavior. It is crucial to increase our knowledge on probiotics adhering and colonizing the GI tract of shellfish, in the context of their improved growth performance and health. However, in shellfish aquaculture it is not clear whether host-associated probiotics are more effective than probiotics from other origins, and this merits further investigations.

References available in original publications.

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