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Review

Atlantic cod in the dynamic probiotics research in aquaculture

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ABSTRACT

Probiotics are good bacteria that confer beneficial actions to the host or to their environment through different modes of action. The science of probiotics has evolved through the years and its diversification is an adaptation to the growing number of host species utilizing these beneficial bacteria. The application of probiotics in aquaculture has been regarded as a sustainable and promising strategy not only in the context of disease control but also in nutrition, growth and immunity. Despite commencing almost three decades ago, probiotics research in Atlantic cod still remains an emerging research area. This review provides a comprehensive synthesis of the current knowledge of probiotics research in Atlantic cod and how the present perspectives stand in comparison with the dynamic probiotics research in aquaculture as a whole.

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Contents

1. Introduction

The rapid development of Atlantic cod aquaculture is not spared from questions of sustainability. The major farming bottlenecks in cod include: (*i*) the emergence of viral, bacterial and parasitic diseases; (*ii*) precocious sexual maturation leading to increased production cost and inferior flesh quality; (*iii*) high variation in the egg quality; (*iv*) dependence on wild broodstocks in producing quality larvae and juveniles; (*v*) low growth rates and frequent occurrence of deformities in hatchery-reared fingerlings; and (*vi*) shortage of larvae supply (Hamre, 2006; Karlsen et al., 2006; Kjesbu et al., 2006; Rosenlund and Halldórsson, 2007; Rosenlund and Skretting, 2006). These challenges have hampered the development of the industry both in the economic and biological perspectives. Case in point is the Norwegian cod farming. In 2010, 94% of the total global production is accounted to Norway (FAO, 2012). However 2 years prior to that statistics, the profitability of the cod production in Norway had started decreasing due to the increase in the supply of wild-caught fish and high production cost (FAO, 2012). On top of these, bacterial and viral diseases had become a serious farming bottleneck as the industry geared towards intensive aquaculture.

Fish health control is a pivotal issue in farming, and a range of infections and diseases has already been identified in gadoids (Kjesbu et al., 2006). The impacts of these biological threats in cod aquaculture resulted in several and urgent studies on disease control strategies. The conventional use of antibiotics has been a very popular approach. Antibiotics including flumequine, florfenicol and oxolinic acid are the most commonly used chemotherapeutants in cod aquaculture particularly

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in Norway (Caipang et al., 2009; Grave et al., 1999; Samuelsen and Bergh, 2004; Vik-Mo et al., 2005). The emergence of antibiotic-resistant bacteria (Balcázar et al., 2006) and the inhibition of beneficial gut bacteria by these chemotherapeutants (Sugita et al., 1991) are the reasons why there is a high degree of apprehension on the use of antibiotics in aquaculture. Vaccines are also tapped as prophylactic agents (Caipang et al., 2008; Gudmundsdóttir and Björnsdóttir, 2007; Gudmundsdóttir et al., 2009; Mikkelsen et al., 2011). The use of vaccines in cod had been an intriguing issue during the early years of vaccination trials because the increase in antibody titer was low or negligible after vaccination with either *Listonella anguillarum* or *Vibrio salmonicida* (Espelid et al., 1991; Schrøder et al., 1992). The deficiency of the major histocompatibility class II (MHC II) molecules in Atlantic cod was believed to be the reason of the lack of antibody production (Pilström et al., 2005) and this was supported by the genome sequencing of Atlantic cod that revealed the absence of these immune-related molecules in its immune system (Star et al., 2011). Since viral and bacterial diseases target mostly the immune system of this fish, boosting the immunity through the application of immunostimulants was also explored as an alternative disease control strategy. Several immunostimulants have been tested in cod including alginic acid, fucoidan, phytase, unmethylated CpG oligodeoxynucleotides, lipopolysaccharide, chitosan, levamisol, vitamin C and many others demonstrated that these substances are potent immune modulators (Caipang et al., 2010b, 2011, 2012; Lazado and Caipang, 2012; Lazado et al., 2010b; Magnadottir et al., 2006; Pedersen et al., 2006; Skjermo et al., 2006). However, most of these substances have not progressed into wide scale application as immunostimulants for cod despite encouraging results obtained in earlier studies.

Aside from the mentioned disease control strategies that have already been applied in cod, one area that is acknowledged to be very promising yet generated moderate attention is the use of probiotics. In a review paper on cod diseases that was published in 2006, it was mentioned that probiotics research in cod is scarce (Samuelsen and Bergh, 2004). Even at present, probiotics research in this species can still be qualified as an emerging field even if the first report on the application of "beneficial bacteria" in cod was published almost three decades ago (Hansen and Olafsen, 1989). There have been numerous review papers on the use of probiotics in aquaculture (Balcázar et al., 2006; Irianto and Austin, 2002a; Martinez Cruz et al., 2012; Merrifield et al., 2010; Mohapatra et al., 2013; Nayak, 2010; Newaj-Fyzul et al., in press; Sahu et al., 2008; Sihag and Sharma, 2012; Tinh et al., 2008; Wang et al., 2008), yet none of them have discussed in full detail the status of probiotics application in cod aquaculture. This review paper summarizes the current knowledge and status of probiotics research in Atlantic cod. The first sections of the manuscript discuss three relevant areas in the discussion of probiotics in this species and these are: *i*) the need of a working definition of probiotics; *ii*) the use of commensal bacteria as probiotics; and *iii*) the gut microbiota of cod. These areas provide a solid backbone on the future perspectives provided at the end of this review. This paper relates the major initiatives and breakthroughs in cod probiotics research to the dynamic nature of probiotics research in aquaculture as a whole. It is important to synthesize what had been done not simply to examine the significance of the strategy but most importantly to unravel future possibilities in the advancement of probiotics application towards a sustainable cod aquaculture industry.

2. Probiotics: Diversification of definition

One outstanding feature of probiotics research is the dynamic nature of its science. Through the years, it has evolved into a broader dimension to accommodate the current understanding and to be apt for future applications. The perfect example is the evolution of the definition of "probiotics" — from a human perspective to an aquaculture point of view. The word PROBIOTICS was coined from the Latin word "pro" meaning *for* and the Greek word "bios" meaning *life* (Zivkovic, 1999). From the strictest etymological sense, the term probiotics clearly states that they are beneficial for the life of an organism. The first generally accepted definition of probiotics was proposed by Fuller and it says "… a live microbial feed supplement which beneficially affects the host animal by improving microbial balance" (Fuller, 1989). Though this has been widely used for almost a decade, this definition is one dimensional and very limiting if we apply it to the context of aquaculture. This definition strictly says that the bacteria should be applied as a feed supplement. However, even the addition of probiotics to the rearing water could confer beneficial effects to the host (Makridis et al., 2005; Spanggaard et al., 2001). For example in cod, most of the *in vivo* studies during the early larval development added the probiotic bacteria to the rearing water and only a handful of papers utilized feed supplementation as a mode of application. The definition of Fuller did not take into consideration that the intestinal microbiota of fish is different from humans because the former is constantly interacting with its immediate environment through water exchange. In addition, probiotic action is a multi-faceted mechanism and most probiotic bacteria possess different modes of beneficial action and not just on maintaining the microbial balance of the host. In the early 2000s, a very encompassing definition was proposed by Verschuere and colleagues stating that, "probiotic is live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed and enhancing its nutritional value, by enhancing the host response towards diseases, or by improving the quality of its ambient environment" (Verschuere et al., 2000). However, probiotics may not necessarily be alive to be beneficial to the host as even components of the microbial cells could confer health benefits as well (Salminen et al., 1999). Bacterial viability is not a limiting and crucial factor as several studies have shown that even the heat-inactivated form of probiotics could still provide beneficial effects to fish including Atlantic cod (Díaz-Rosales et al., 2006; Lazado and Caipang, 2013a; Lazado et al., 2010a; Pan et al., 2008; Salinas et al., 2006).

These contrasting definitions present, in partial, the status of probiotics in aquaculture. In particular, the mentioned definitions do not reflect the present knowledge of probiotic applications in cod especially on the mode of application and bacterial viability. The unprecedented results that have been generated through the years should serve as foundation in establishing a unified and concerted effort to define probiotics in an aquaculture perspective. To our knowledge, there is no standing definition of probiotics that is agreed upon by the majority of probiotics researchers in aquaculture. This issue was raised by Merrifield et al. (2010) in their review paper on the status of probiotic application in salmonids. We believe that it is important in the near future to develop a working definition of probiotics in an aquaculture point of view to eliminate ambiguity on the term being used and to acknowledge that physiological differences between organisms exist. The definition should encompass both the industrial and biological concerns of probiotics application in fish. In this paper, probiotics are explicitly defined as live or dead, or even a component of the bacteria that act under different modes of action in conferring beneficial effects to the host or to its environment. This definition is a simplified version of the proposed definition of Merrifield et al. (2010) and reflects the present knowledge of probiotics research in Atlantic cod.

3. Harnessing the commensal bacteria

For several years, the term probiotics has become synonymous with lactic acid bacteria (LAB). The members of LAB such as *Bifidobacterium*, *Lactobacillus* and *Streptococcus* have established an important niche in probiotics research both in humans and animals. The application of human or terrestrial probiotics had become a common practice during the early days of probiotics research in fish for the following obvious reasons: (*i*) they were regarded as safe; (*ii*) their probiotic actions

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