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Review

The use of probiotic bacteria against *Aeromonas* infections in salmonid aquaculture

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ABSTRACT

Aeromonas species are ubiquitous bacteria in terrestrial and aquatic milieus. In salmonids, they are renowned as enteric pathogens causing haemorrhagic septicaemia, fin rot, soft tissue rot and furunculosis resulting in major die-offs and fish kills. In recent years, there has been a growing interest in controlling disease problems through alternative methods since the use of chemotherapeutic agents may lead to occurrence of resistant bacteria. Lactic acid bacteria may provide protection to create a hostile environment for pathogens. This review summarizes the current understanding of *Aeromonas* infection in salmonids and the use of probiotics in aquaculture for the purpose to prevent these pathogenic bacteria, including the definition and mechanism of probiotics action, and describes their application, prospects and difficulties associated with their use in aquaculture.

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1. Introduction

Global fish production continues to outpace world population growth, and aquaculture remains one of the fastest-growing food producing sectors. In 2012, aquaculture set another all-time production high and now provides almost half of all fish for human food (FAO, 2014). Continuing expansion of aquaculture is viewed as a key strategy to ensure global food and nutrition security and close the "fish-gap", i.e. the disparity between sea food supply and demand (Ellis et al., 2016). Moreover, under these conditions of intensive production, aquatic species are subjected to high-stress conditions, increasing the incidence of diseases and causing a decrease in productivity (Lara-Flores, 2011; Cruz et al., 2012). Bacterial agents are among the highly encountered causes of diseases in aquaculture and also stressful conditions play important role in establishing and aggravation of the bacterial diseases in fish farms (Saranu et al., 2014; Musefiu and Olasunkanmi, 2015). Diseases caused by *Aeromonas* spp. are commonly implicated in episodes of mortality (Ariole and Oha, 2013).

The genus *Aeromonas* encompasses a diverse group of straight coccobacillary to bacillary Gram-negative bacteria that commonly occur in the aquatic environment and are also isolated from food

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products (Hatje et al., 2014). Initially, aeromonads were recognized only as pathogens that cause systemic illnesses in poikilothermic animals. Today, the genus *Aeromonas* is regarded not only as an important disease-causing pathogen of fish and other cold-blooded species but also as the etiologic agent responsible for a variety of infectious complications in both immunocompetent and immunocompromised persons (Janda and Abbott, 2010).

During the last decades, antibiotics have been used as traditional strategy for fish diseases management (Krishnan, 2014). The public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial resistant bacteria and resistance genes, and the occurrence of antimicrobial residues in products of aquaculture. The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in bacteria in aquatic environments from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens (WHO, 2006; Heuer et al., 2009). Considering these factors, as well as the negative effect of residual antibiotics of aquaculture products on human health, the European Union and the USA implemented bans on, or restricted the use of antibiotics. The norms are stringent and there are many events of returning consignments to the exporting countries for not maintaining the prescribed standards (Lara-Flores, 2011).

In addition, the global demand for safe food has prompted the search for natural alternative growth promoters to be used in aquatic feeds. There has been heightened research in developing new dietary supplementation strategies in which various health and growth promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated (Denev et al., 2009). Hence the use of probiotics i.e., a live microbial feed supplement which benefits the host by modifying the host-associated or ambient microbial community, by enhancing the host response towards disease, by ensuring improved use of feed or enhancing its nutritional value, or by improving the quality of its ambient environment, in aquaculture is being encouraged (Panigrahia et al., 2004). Sugita et al. (2002) suggested that about 1–10% of intestinal bacteria isolated from both marine and freshwater fish exhibit antibacterial activity against fish pathogenic bacteria and can play a role in probiotic treatment of fish. The dietary introduction of probiotic bacteria could reduce mortality of fish challenged with a virulent *Aeromonas* sp. (Cruz et al., 2012). The purpose of this study was to summarize a potential of using probiotic bacteria in the prevention of *Aeromonas* infections in salmonid aquaculture.

2. Characteristics of *Aeromonas* spp.

The taxonomy of the genus *Aeromonas* has been dogged by confusion and controversy (Saavedra et al., 2006). In Bergey's Manual of Systematic Bacteriology the genus was divided into three mesophilic and motile species (*Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* biovar *sobria*) and the psychrophilic non-motile species (*Aeromonas salmonicida* subsp. *salmonicida*, *Aeromonas salmonicida* subsp. *masoucida* and *Aeromonas salmonicida* subsp. *smithia*) (Yánez et al., 2003). The psychrophilic species grow best at temperatures between 22 °C and 28 °C. In contrast, mesophilic strains grow optimally between 35 °C and 37 °C, although many strains can also grow at 2–41 °C. Motility of the mesophilic species is facilitated through presence of a single polar flagellum (Percival et al., 2014).

The phylogenetic position of *Aeromonas,* as determined by 16S rRNA gene sequences analysis, is in the class *Gammaproteobacteria*, order *Aeromonadales*, and the family Aeromonadaceae (Tomás, 2012; PHE, 2015). The aeromonads are Gram-negative, rod-shaped, non-spore forming bacteria, facultative anaerobic, catalase and oxidase positive, as well as chemoorganotrophic. They produce diverse kinds of extracellular hydrolytic enzymes such as arylamidases, esterases, amylase, elastase, deoxyribonuclease, chitinase, peptidases, and lipase. Their optimum pH range is between 5.5 and 9 and optimum sodium chloride concentration range is 0–4% (Igbinosa et al., 2012; PHE, 2015).

2.1. Aeromonas Salmonicida

The bacterium *Aeromonas salmonicida* subsp. *salmonicida* is the causative agent of classical furunculosis, a systemic disease of salmonid fish (salmon, trout, etc.) characterized by high morbidity and mortality (Dallaire-Dufresne et al., 2014). *Aeromonas salmonicida* subsp. *salmonicida* is often referred to as typical *Aeromonas salmonicida*, whereas the other strains have been referred to as atypical (Gudmundsdóttir and Björnsdóttir, 2007). Typical and atypical *A. salmonicida* infections have been reported worldwide, with the exception of New Zealand and South America (Hirvelä-Koski, 2005). Australia has managed to keep free of the disease thanks to strict import regulations. In Denmark furunculosis is probably the most important bacterial pathogen in marine rainbow trout farming, while it is less important in freshwater (Buchmann et al., 2012).

Typical furunculosis affects both salmonids and non-salmonid fish and although the route of entry of this bacterium is still debated, there are some reports describing intestinal presence (Bøgwald and Dalmo, 2014). Any age salmonid is susceptible. It has also become a serious problem in marine fish, especially Atlantic salmon culture (Noga, 2010). Furunculosis is a complex disease that takes different forms depending on the health, age, and species of fish as well as the environmental conditions, especially temperature (Dallaire-Dufresne et al., 2014). Furunculosis derives its name from the lesions resembling boils, i.e. furuncles that develop on the skin and musculature of fish (affected by the sub-acute or chronic form of the disease) (Austin and Austin, 2012). It may occur in a peracute, acute, subacute or chronic form, but the distinction and transition between the different forms is not sharp (Buchmann et al., 2012). Peracute disease, which is the least common presentation, has been seen in salmonid fry (Noga, 2010). Furuncles develop from localization of haematogenous bacteria in the muscle or skin, not from an external skin infection (Noga, 2010). These furuncles may rupture exposing open deep ulcers on the surface. A large number of bacteria are released from these lesions and contribute to the spread of the infection (Bruno et al., 2013). During outbreaks, all moribund fish, especially those with skin ulcers, should be promptly removed and disposed of properly (i.e., do not allow contagion to reenter the system). Mortalities are usually low (Noga, 2010). Some fish may recover from disease but the damaged muscle tissue is replaced by scar tissue and the quality of such fish is poor (Buchmann et al., 2012).

Atypical furunculosis forms a very heterogeneous group of bacteria affecting both non-salmonids as well as salmonids. Examples include *A. salmonicida* subsp. *achromogenes*, subsp. *masoucida*, subsp. *pectinolytica* and subsp. *smithia* (Bøgwald and Dalmo, 2014). Atypical *A. salmonicida* display high diversity in biochemical and physiological characters (Buchmann et al., 2012).

Atypical *A. salmonicida* infections associated with disease outbreaks in fish can be manifested, similar to furunculosis. The course of the disease can be peracute, acute, subacute or chronic as described for classical furunculosis. Bacterial isolation should be done from recently developed skin ulcerations as well as from internal organs (Gudmundsdóttir, 1998).

2.2. Aeromonas Hydrophila

Motile aeromonad infection (MAI) is probably the most common bacterial disease of freshwater fish. All freshwater fish are probably susceptible. MAI has been associated with several members of the genus *Aeromonas*, which are ubiquitous in freshwater environments. By far the most important fish pathogen is *A. hydrophila* (syn. *A. liquefaciens*, *A. formicans*), and members of this group are often referred to as the *A.hydrophila* complex. Many other *Aeromonas* species have been taxonomically identified, but only a few aeromonads have been considered