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Review Article

The advancement of probiotics research and its application in fish farming industries

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ABSTRACT

Fish are always susceptible to a variety of lethal diseases caused by different types of bacterial, fungal, viral and parasitic agents. The unscientific management practises such as, over feeding, high stock densities and destructive fishing techniques increase the probability of disease symptoms in aquaculture industries. According to Food and Agriculture Association (FAO), each and every year several countries such as China, India, Norway, Indonesia, etc. face a huge loss in aquaculture production due to mainly bacterial and viral diseases. The use of antibiotics is a common practise in fish farming sectors to control the disease outbreak. However, the antibiotics are not long term friend because it creates selective pressure for emergence of drug resistant bacteria. Probiotics are live microorganisms that confer several beneficial effects to host (enhances immunity, helps in digestion, protects from pathogens, improves water quality, promotes growth and reproduction) and can be used as an alternative of antibiotics. In recent year, a wide range of bacteria have reported as potential probiotics candidates in fish farming sectors, however, *Lactobacillus* sp. and *Bacillus* sp. gain special attention due to their high antagonistic activities, extracellular enzyme production and availability. In this present review, we have summarized the recent advancement in aquaculture probiotics research and its impact on fish health, nutrition, immunity, reproduction and water quality.

Contents

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1. Introduction

The intensive fish culture enhances disease probability (that reduce production rate and flesh quality) and ultimately hampers the economy of the country. Till date, antibiotics are more popular to farmers due to its rapid action and availability; however, it badly affects the water ecosystem. The replacement of antibiotics with probiotics might be an alternative way to control the pond health, as well as the diseases in fish. The word probiotic is constructed from the Latin word “pro” (for) and “bios” (life). The probiotics are live microorganisms (bacteria, yeast and fungi), which when administered in adequate amounts confers a health benefit to the host (Havenaar and Huis, 1992; Kechagia et al., 2013; Steenbergen et al., 2015). Gatesoupe (1999) has stated that the first use of probiotics in aquaculture was started in the mid-1980s (Kozasa, 1986) and since then interest in such environment-friendly treatment has been increased rapidly. Later on, Irianto and Austin (2002) have reported the scientific use of probiotics in aquaculture sectors and claimed as the alternative of antibiotics in near future.

The water qualities (pH, dissolved oxygen, dissolved carbon dioxide and organic load and minerals) are the major determinants for enhancing production rate and maintaining good health in fish. Alteration of such parameters foster the growth of several obligate or facultative pathogenic bacterial strains such as *Aeromonas*, *Pseudomonas*, *Citrobacter*, *Proteus*, *Streptococcous*, *Edwardsiella*, *Staphlycoccous* and different species of *Vibrio*, which cause huge mortality in both freshwater and brackish water fish (Welker et al., 2005; Verma et al., 2006; Cruz et al., 2012; Sihag and Sharma, 2012). Among several pathogens, *Aeromonas hydrophila* and *Aeromonas salmonicida* are considered to be the most common pathogens in freshwater fish, while *Vibrio anguillarum* and *Vibrio Parahaemolyticus* are the most familiar bacterial pathogens in marine environment, which cause different types of fish disease like ulcer disease, carp erythrodermatitis, motile *Aeromonas* septicemia etc. (Lightner et al., 1992; Cruz et al., 2012; Silva et al., 2014; Bartkova et al., 2016; Ghomrassi et al., 2016).

The use of antibiotics is common practise in aquaculture, however, it creates a selective pressure for emerging drug resistant bacteria, which might be transmitted through food chain from fish to human (SCAN, 2003; Kim et al., 2004; Cabello, 2006; Sørum, 2006; Da Costa et al., 2013; Tanwar et al., 2014). Furthermore, antibiotics inhibit or kill beneficial microbial flora in gut and disturb the natural ecosystem that affects fish nutrition, physiology and immunity (Rekecki et al., 2009; Rawls et al., 2007; Maynard et al., 2012). To avoid such adverse situation, probiotics candidates have been introduced in fish farming industries for better health management practise (Cruz et al., 2012). Along with diseases control, probiotics strains are also responsible for other beneficial purposes such as, extracellular enzyme production (Ray et al., 2012; Banerjee et al., 2013a,b), growth promotion (Cruz et al. 2012), maintaining water quality and immune modulation (Nayak, 2010; Montalban-Arques et al., 2015). In recent year, several microorganisms such as bacteria (Rengpipat et al., 2000; Patil et al., 2007; Vijayabaskar and Somasundaram, 2008; Nouri et al., 2010; Ibrahem, 2015; Banerjee et al., 2016a,b) and yeast (Chiu et al., 2010; Pinpimai et al., 2015; Caruffo et al., 2015) have been used randomly as promising probiotic candidates in fish industries. However, in this present review, we have focused only on bacterial probiotics and their vast applications in aquaculture sectors. Until now, this area is not so explored and a few probiotics are commercially available to market. In this present review, we have summarized the progress of probiotic research in fisheries science.

2. Selection criteria of probiotics

The successful probiotic candidate has to possess several important criteria (Fig. 1). In last few years, plenty of reports have been published regarding the screening, selection and characterization of fish probiotic bacterial strains (Vázquez et al., 1996; Gatesoupe, 1999; Vijayabaskar and Somasundaram, 2008; Harikrishnan et al., 2010; Liu et al., 2012), however, few are available for commercial uses. The selection procedures (both *in vivo* and *in vitro*) of probiotic bacteria are laborious and differ slightly from one organism to another, as the mode of action of probiotic candidate varies from aquatic to terrestrial animals (Huis in't Veld et al., 1994; Hou et al., 2015; Song et al., 2015). However, the general selection parameters are almost same and have been discussed below.

2.1. Non-pathogenic

The selected bacterial strain must be non pathogenic to fish (Zokaeifar et al., 2012). The degree of pathogenicity depends on toxin producing capability and it varies from one strain to another strain. For example, *Aeromonas hydrophila* is considered to be a deadly pathogen in fish (Mohideen and Haniffa, 2015), however few strains of *Aeromonas hydrophila* are used as probiotic candidates in fish (Gunasekara et al., 2010). Several *in vitro* techniques such as haemolytic activity, manitol utilization ability and other biochemical tests have been introduced to check the bio-safety of the selected bacterial strains. *In vivo* tests (fish fed with probiotic bacteria) also should be performed to confirm the non-pathogenic activity of the selected candidates. Pathogenicity of any bacterium is determined on the basis of disease symptoms (both internal and external) and mortality rate.

2.2. Drug resistant gene

The emergence of multi-drug resistant bacteria is a big treat for animals, including fish. In general, the drug resistant property of bacteria comes from the plasmid encoded genes (Jackson et al., 2011). The successful probiotic strain must not possess any plasmid-encoded antibiotic resistance gene or gene cluster (Gueimonde et al., 2013). In stress condition (presence of antibiotics), bacteria evolve very fast due to their high mutation rate and this unique property can be transferred from one species to another through lateral gene transfer mechanism. Thus, before selecting a bacterial isolate as promising probiotic candidate, few experiments (broad spectrum antibiotic sensitivity and PCR detection of multi drug resistant gene) should be performed.

2.3. Tolerance to pH

Probiotic candidate is administrated along with food and thus, it has to face a changing environment in gut in term of pH level. The environment of gut provides a favourable ecological niche for endosymbionts (Ray et al., 2012); however, at different physiological conditions such as during metabolism the pH level of the gut varies greatly. Even, during metabolism, different types of bile salts are also secreted (Buchinger et al., 2014). So, the probiotic candidates must have the ability to tolerate a wide range of pH (low acidic to high alkaline) and high concentration (>2.5%) of bile salts.