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Review: Developments of Lactic Acid Bacteria as Probiotic for Bacterial Diseases control in Aquaculture

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Abstract – In the recent decades, lactic acid bacteria (LAB) in aquatic organism have been one of the major interesting research subjects due to their various existence strains in fish microbiota. Moreover, LABs are sometimes abundant in the intestine of several fish species. Many recent papers indicated that several LAB strains are harmless and have been reported for beneficial effects on fish health. There was also converging evidence that led us for more research and findings regarding LAB as a promising probiotics in aquaculture. This article provides an overview of the variability of LAB in gastrointestinal (GI) tract of fish and the development of this species as probiotics. LAB was known able to colonise the gut, and has antagonistic activity against some fish pathogens. This harmless bactreriocin-producing strain may confer benefits in increasing disease resistance, improving nutrient digestibility and growth of the host animals. In addition, these strains may reduce the need of antibiotics usage in future aquaculture industry.

Keywords: Fish, Gastrointestinal microbiota, Lactic acid bacteria, Probiotic

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Introduction

Aquaculture has grown rapidly and represent one third of the world fisheries production (FAO, 2016). Global aquaculture production in 2014 was recorded at 73.8 million tonnes with Asian region as a whole has been produced more farmed fish than wild catch fish since 2008, with total production reached to 44.1 percent in 2014, increase from 42.1 percent in 2012 and 31.1 percent in 2004. Unfortunately, the development of commercial aquaculture and intensive fish production worldwide has increased the risk of the infectious disease. It have been stated that, bacterial infections are considered to be a major cause of mortality in fish hatcheries (Grisez and Ollevier, 1995). Frequent usage of drugs and antibiotics for treatment and prevention led to development of drug-resistant microorganisms with antibiotic residues retained in fish flesh and environment (Aly et *al.*, 2008; Zapata *et al.*, 2013). Replacing drugs and antibiotics with effective and inexpensive probiotics have gained acceptance to avoid resistance in fish farming and antibiotic residues in fish flesh for human consumption (Verschuere *et al.*, 2000; Balzacar *et al.*, 2007; Rengpipat *et al.*, 2008). This approach would be in accordance with the Ecosystem Approach for the sustainable growth and expansion of aquaculture, promoted by Food and Agriculture Organization, (FAO) in The State of the World Fisheries and Aquaculture (FAO, 2007). Furthermore, probiotics can control pathogens through a variety of mechanisms. The interest in lactic acid bacteria (LAB) was highlighted as groups of candidate probiotics that could improve fish health (Ringø and Gatesoupe, 1998; Ringø, 2004; Gatesoupe, 2007). Uses of LAB as a probiotic organism have gained acceptance for human and terrestrial animal purposes. In addition, LAB have been well documented as part of the indigenous gut microbiota of several fish species (Ringø and Gatesoupe, 1998; Ringø, 2004; Ringø *et al.*, 2005; Ringø, 2014) and known to be present in the intestine of healthy fish (Ringø and Gatesoupe, 1998; Hagi *et al.*, 2004; Pandiyan *et al.*, 2013). This review discusses and evaluates the broader knowledge about the developments of using LAB as a probiotic in controlling diseases in aquaculture.

Disease Outbreaks in Aquaculture: An Overview

With the rapid developments of commercial aquaculture farming, disease was reported to become the major constraint of the development of aquaculture globally. According to Wei and Wee (2014), most of the pathogens are opportunist to infect cultured fish once the environment is deteriorated. Meanwhile bacterial diseases frequently attacked cultured fish followed by parasites, virus and fungi (Toranzo *et al.*, 1995). Among the bacterial species, vibriosis and aeromonad disease poses a threat to most aquaculture farms either marine or freshwater species.

Early disease in fish farming was reported on 1932 in Indonesia where some of the fish have been infected with white spot disease. In the year 1983, *Lernaea cyprinacea* was attacked almost 30% of hatchery in Indonesia that led to loss merely 1.5 billion of fish fry (Djajadireja, 1983). In Malaysia, disease outbreaks have been documented in 1989 which affected the freshwater fish cultured with total loss of USD 1.3 millions (Wee and Wee, 2014). By the year 1990, vibriosis attacked marine fish in Malaysia and caused USD 7.4 million loss (Shariff, 1995). Moreover in China, bacterial disease due to *Aeromonas hydrophila* and *Vibrio fluvialis* were reported to infect fish farms from 1990 to 1993 (Wei, 2002).

Since the disease outbreak have been frequently occurred along with the increasing of aquaculture farming, prevention methods have been proposed and one of it is by introducing the antibiotic in the culture system. However, abuse usages of antibiotics have been resulted in the development of drug-resistant microorganisms and leave the residues in the fish and in the environment (Gomez-Gil *et al.*, 2000). Alternatively, the use of LAB as probiotics which proven to have beneficial effects on fish health (Gatesoupe, 2007; Ringø *et al.*, 2010; Pandiyan *et al.*, 2013) is getting a demand..

LAB and Their Variability in Microbiota of Fish

Lactic acid bacteria have been described as Gram-positive, facultative anaerobic, catalase-lacking organism, cocci or rods, usually non-motile, non-sporulating bacteria that produce lactic acid as a major or sole product for fermentative metabolism (Ringø and Gatesoupe, 1998; Pfeiler and Klaenhammer, 2007). LAB is generally recognized as safe (GRAS) organism (Holzapfel *et al.*, 1995; Silva *et al.*, 2002; Klaenhammer *et al.*, 2005) as it have been found to be non pathogenic and can't cause disease. Classifications of LAB are based on their morphological, diverse metabolic capacity, and physiological criteria. Taxonomically, six families of Lactobacillales have been described in the stomach, and/or intestine of fish (Ringø, 2004). Additionally, Gram-positive lactic acid bacteria are divided into two groups that depending on their DNA with high or low G+C content. GC content will show the specific and strain- dependent transcriptional host responses that are dependent on the physiological state of the consumed cells. Thus, provide support for the functionality of probiotics (Saxelin *et al*., 2005). First group contain above 50% of G+C includes the genera such as *Atopobium*, *Bifidobacterium*, *Corynebacterium*, and *Propionibacterium*. While the second group contain below 50% G+C contain and includes the typical LAB genera which are *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (Stiles and Hozapfel, 1997; Klaenhammer et al., 2005; Pfeiler and Klaenhammer, 2007).

Lactic acid bacteria (LAB) is widespread in most of the ecosystems and can be found in soil, water, plants and also animals and generally associated with plant and animal raw materials, and some of it occurs in the respiratory, intestinal and genital tracts of human and animals. In aquatic environment, LAB are not considered as their belonging, however certain genera such as *Carnobacterium*, *Lactobacillus*, *Enterococcus* and *Lactococcus* have been found in fish and their surrounding environment (Stiles and Hozapfel, 1997; González *et al.*, 2000; Ringø *et al.*, 2000; Calo-mata *et al.*, 2008).

Certain strain of LAB have been observed from the indigenous gut microbiota of several fish species (Ringø and Gatesoupe, 1998; Ringø, 2004; Ringø *et al.*, 2005; Balzacar *et al.*, 2007; Ringø *et al.*, 2010). In fish microbiota, LAB is sometimes abundant in the intestine, notably in freshwater fish (Gatesoupe, 2007). The abundant of LAB species are different in every fish and environment. Cai *et al.*, (1999) reported the present of *Lactococcus garvieae*, *Pediococcus acidilactici* and *Enterococcus faecium* isolated from the intestine of common carp and estimated counts up to 10^7-10^8 colony-forming units (CFU/g).

The diversity of the LAB have been well documented in freshwater fish (Cai *et al.*, 1999; González *et al.*, 2000; Hagi *et al.*, 2004; Bucio *et al.*, 2006) and also in marine fish. Buntin *et al.*, (2008) have been successfully isolated 160 strains of LAB from different marine species including shellfish and shrimp with the total population of 4-5x 10^4 to 10^5 (CFU/g) wet weights of GI tracts. Table 1 compiles the finding of successfully isolated LAB from fish gastrointestinal tract (GI).

Role of LAB as Probiotics in Controlling the Bacterial Disease of Aquatic Animals

Probiotics in Greek word can be defined as “for life” (Gismondo *et al.*, 1999) and can be referred as “friendly”, “good”, and “healthy” bacteria that confer a health benefit to the host.. Lactic acid bacteria are known to have probiotic properties and colonize the intestine of fishes as normal microflora (Ringo and Gatesoupe, 1998). Ringø (2004) and Ringø *et al.* (2005) suggested that LAB might be involved in the primary defense system against pathogenic colonization and also adherence of pathogenic bacteria in the GI tract. Several LABs isolated from fish and aquatic animals were reported play a role in antagonistic activity against fish pathogenic agents (Ringø *et al.*, 2005; Ringø, 2008).

Balzacar *et al.* (2007) observed that rainbow trout (*Oncorhynchus mykiss*) had a high number (how much?) of LABs especially *Lactococcus lactis*, *Lactobacillus plantarum* and *Lactobacillus* *fermentum*. Few studies agreed that bacteria such as LAB that able to colonize the gut as well as remain to the epithelial surface would disturb the adhesion of pathogens (Lazado *et al.*, 2012). The ability to attach the intestinal mucus could prevent pathogen from colonize by blocking the intestinal infection route thus reduce the risk of infection. For instance, an indigenous of probiotic strain of *Lactobacillus fructivorans* was able to colonize the gut of gilthead sea bream but only after weaning (Carnevali *et al.,* 2004).

Ringø *et al.* (2005) in their review paper have discussed the antagonistic ability of LAB, *Carnobacterium* isolated from fish towards pathogen in *in vitro*. Other studies reported *C. maltaromaticum* isolated from the gut of Atlantic salmon and Arthic charr inhibited the growth of *Aeromonas salmonicida* (Ringø *et al.,* 2000, 2001).

Generally, biofilm could be permanent reservoirs of bacteria through their formation on the surface consequently act as barrier (Gomez *et al.*, 2016) as well as being pivotal factor in the disease cycle of pathogenic bacteria (Bogino *et al.,* 2013). Terraf *et al.*, (2012) reported that *Lactobacillus* species able to produce biofilm and could promote colonization of the host by avoiding colonization of pathogenic bacteria. Additionally, *E. faecium* and *E. faecalis* have been reported able to produce high biofilm (Baldassarri *et al.,* 2001) with supplemented of glucose in their culture medium (Kristich *et al.,* 2004).

LAB as a health and growth promoter in aquaculture

Probiotic demonstrated its beneficial role as the stimulation of growth for aquatic animals through the production of enzymes that aided in digestion process. Irianto *et al.* (2000) proposed that hydrolytic enzymes (amylase and protease) were able to improve nutrients absorption by detoxify potential harmful compound that exist in feed. Suzer *et al.* (2008) showed that, treatment of *Lactobacillus* in larvae resulting in increasing of specific activity of digestive enzymes as well as promote better growth rate. Other than that, Askarian *et al.* (2011) proved that, feeding the Beluga (*Huso huso*) and Persian sturgeon (*Acipenser persius*) with two LABs (*Lactococcus curvatus* and *Leuconostoc mesenteroides*) able to produce extracellular enzymes which able to enhance specific growth rate, survival and improved digestion.

Despite able to promote growth rate, probiotic was able to modulate the immune system of fish. Several probiotics either individually or in combination can enhance both systemic as well as local immunity in fish (Nayak, 2010). For instance, *Lactobacillus rhamnosus*, *Lactobacillus lactis* and *Lactobacillus acidophilus* observed in fish are able to trigger the phagocytic cells in host that responsible for early activation of the inflammatory response and play a role in antibacterial defenses (Hughes *et al.,* 2004). In addition, these three strains also able to enhance the lysozyme in the host, which become indispensable tool for fish to fight against infectious agents (Lindsay, 1986). Among LAB species, *L. rhamnosus* and *E. faecium* were found able to regulate the cytokines in the spleen and kidney of *O. mykiss* (Panigrahi *et al.,* 2007). Cytokines are protein mediators that produced by immune cells which contribute to cell growth as well as a defense mechanism of the host against disease (Peddie and Secombes, 2002). Table 2 provides a list of studies of LABs as some of the LABs that have been proven by the researcher to be promising probiotic for the aquatic organism.

Table 1: Lactic acid bacteria (LAB) in gastrointestinal (GI) tract of fish

LAB genus/ species	Fish species/ family	Lactic acid bacteria isolated from				Reference
		Whole intestinal tract	Stomach	Small intestine	Large intestine	
Lactobacillus sp.	Artic charr		/			Ringø (1993)
	Atlantic cod	/				Strøm and Olafsen (1990)
Lactobacillus plantarum	Artic charr		/	/	/	Ringo *et al.*, (1998)
	Rainbow trout	/				Balzacar *et al.*, (2007)
	Nile tilapia	/				Zapata *et al.*, (2012)
Lactobacillus fermentum	Rainbow trout	/				Balzacar *et al.*, (2007)
	Nile tilapia	/				Zapata *et al.*, (2012)
Lactococcus lactis	Rainbow trout	/				Balzacar *et al.*, (2007)
Lactococcus garvieae	Common carp	/				Cai *et al.*, (1999)
Carnobacterium sp.	Arctic charr		/	/	/	Ringø *et al.*, (1998)
	Atlantic salmon			/	/	Ringø *et al.*, (1997)
	Rainbow trout	/				Wallbanks *et al.*, (1990)
Carnobacterium divergens	Artic charr				/	Ringø *et al.*, (1997)
	Atlantic cod			/	/	Strøm (1988)
	Atlantic salmon			/	/	Strøm (1988)
Carnobacterium piscicola	Artic charr		/	/	/	Ringø *et al.*, (1998)
	Rainbow trout	/				Starliper *et al.*, (1992)
Enterococcus faecium	Nile tilapia	/				Zapata *et al.*, (2012)
	Common carp	/				Cai *et al.*, (1999)
	Sea bass	/				Bourouni *et al.*, (2012)
	Sea bream	/				Bourouni *et al.*, (2012)
Enterococcus faecalis	Snakehead	/				Allameh *et al.*, (2014)

Table 1 (continue)

LAB genus/ species	**Fish species/ family**	**Lactic acid bacteria isolated from**				**Reference**
		Whole intestinal tract	**Stomach**	**Small intestine**	**Large intestine**	
Enterococcus durans	Common carp	/				Cai *et al.*, (1999)
Enterococcus sanguinicola	Sea bass	/				Bourouni *et al.*, (2012)
	Sea bream	/				Bourouni *et al.*, (2012)
Streptococcus sp.	Salmonids	/		/	/	Trust and Sparrow (1974)
	European eel	/				Esteve and Garay (1991)
Leuconostoc mesenteroides	Artic charr		/	/	/	Ringø *et al.*, (1998)
	Snakehead	/				Allameh *et al.*, (2012)
Weissella confusa	Sea bass	/				Rengpipat *et al.*, (2008)

Table 2: Lactic acid bacteria (LAB) used as probiotics in aquatic animal

LAB genus or species	**Target host(s)**	**Beneficial effect**	**Reference**
Lactobacillus acidophilus	African catfish	Growth performance, immunoglobulin concentration and hematological parameters.	Fernandez *et al.*, (2011)
	Nile tilapia	Immunity increased and protected against *Sterptococcus iniae*	Aly *et al.*, (2008)
Lactobacillus rhamnosus ATCC 53101	Rainbow trout	Reduction in mortality caused by *A.hydrophila*	Nikoskelainen *et al.*, (2001)
	Tilapia	Protect against *Edwarsilla tarda* infection	Pirarat *et al.*, (2006)
Enterococcus faecium SF 68	European eel	Prevent against *Edwardsiellosis*	Chang and Liu, (2002)
Enterococcus faecium MC13	Larval shrimp	Protect against *V.harveyi* and *V. parahaemolyticus*	Swain *et al.*, (2009)
Vagococcus fluvialis	Sea bass	Protection against *V. anguillarum* infection	Sorroza *et al.*, (2012)
Weissella confusa	Sea bass	Protect against several fish pathogens	Rengpipat *et al.*, (2008)

Future Prospects

Through several years, LABs have been using widely and researched for human and terrestrial animal purposes. There is a growing number of studies reporting the LAB as the probiotics candidate, for example; improving fish health by colonizing the epithelial surface of the fish host in the stomach and intestine (Ringø and Birkbeck, 1999), antagonistic ability against pathogen *in vitro* (Ringø *et al.*, 2000, 2001), improve water quality in the culture systems (Verschuere *et al.*, 2000) and also able to stimulate immune system in fish (Gatesoupe, 2008).

In vitro inhibition does not necessarily mean that the candidate probiotic will work *in vivo* (Balzacar *et al.*, 2007; Gatesoupe, 2008). Hence, in order to prove the effectiveness of the potential LAB strains, direct method needs to be applied in culture systems as it is important to provide larvae with a healthy environment that includes a beneficial microbial community (Gomiz-Gil *et al.*, 2000). However, many questions have been pointed out regarding their long term effects as dietary supplement, optimum duration of the treatment should be estimated, such as; Are they acting as a food or are they competing with potentially harmful bacteria? How can a probiotic strains be differentiated from a pathogenic one? These questions were often come out yet the answers are still questionable. Marteau and Shanahan (2003) proposed a further study on the risk of translocation and permanent colonization of the probiotics as one of the safety issues concern, and these points should be taken into consideration before LAB are use commercially for fish. The selection and source of probiotics also play an important role while the optimization of the probiotic for commercial application is required to avoid unnecessary expense (Tuan *et al.*, 2013). A number of probiotic products have been researched as evidenced by their efficacy in aquaculture. Further, new analysis methods, including molecular and *in-vivo* validation, is expected be done to provide significant result for both the quality and functional properties of LAB as probiotics.

Conclusion

The increasing demand for safe and effective aquaculture products as alternatives to antibiotics on growth promotion and health maintenance will open the opportunity for LAB to be explored as a potential probiotics in aqualculture industry.

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