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Role and mode of action of using probiotic (Lactic Acid Bacteria) and prebiotic (Mannan Oligosaccharide) in aquaculture

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ABSTRACT

The principal function of a diet is not only to offena sufficient amount of nutrients to accomplish metabolic desires of the body but also to amend various functions of the body. Probiotics and prebiotics are either beneficial microorganisms or substrates that help the growth of these microorganisms which can be suitably harnessed by the food manufacturers and holds considerable promise for health care industry. When these dietary supplements provided in feeds, even in small amounts increase growth and survival during culture, improve feed conversion, enhance development and improve immune status and /or stress of fishes and crustaceans. All of these benefits are occurred due to modulation of gastrointestinal (GI) microbes and improved physical GI structures. Lactic acid bacteria (LAB), particularly *Lactobacillus acidophilus*, are considered to be one of the most utilized probiotic strains. This group of bacteria produces lactic acid as their main metabolic product during carbohydrate fermentation benefits the host animal by modifying the growth of beneficial microorganisms which ultimately outcompete potentially hazardous bacteria in the intestinal ecosystem and reinforce the natural defence mechanisms. Among the established prebiotics, mannan oligosaccharide (MOS) is one of the preferable prebiotics used as the dietary supplement for fish and crustacean species. The most promising prospects of using probiotics and prebiotics are sketched out, but considerable efforts of research will be necessary to elucidate various factors in order to develop their applications in aquaculture, as it is clear that probiotic and prebiotic efficiency is dependent on a number of independent and interacting factors related to the culture conditions and host species. This article gives an overview of probiotic (LAB) and prebiotic (MOS), their health effects, mode of action and safety issues including future prospects.

Keywords: Lactic acid bacteria, Mannan oligosaccharide, Mode of action, Probiotics, Prebiotics

INTRODUCTION

The intensification process of the culture practices increases the risk of prevalence of stress related disease outbreaks (Bondad-Reantaso et al. 2005), which are responsible for huge fish losses. Therefore, aquaculture industry considers the disease occurrence as the state of restriction to aquaculture production at which adversely affects economical development (Ibrahem et al. 2010). For a long time, administration of antibiotics was used as the most common method for dealing with the occurrence of bacterial infections in aquaculture (Li and Gatlin 2005). The indiscriminate application of these antibiotics as a remedial method for controlling

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bacterial pathogens has been responsible for the development of resistance bacteria, which has greatly reduced the effectiveness of the treatment options and may be responsible for long term adverse effects in the aquaculture environment (Defoirdt et al. 2007,Villamilet al.. 2014) including accumulation in fish body tissue, immuno suppression and destruction of beneficial microbial flora (Smith et al. 2003, Sapkota et al. 2008). Vaccination can be used as a method for controlling many fish diseases instead of antibiotic treatments, but for many diseases vaccines are unavailable or are in the early stages of development (Yousefian and Amiri 2009). Furthermore, the use of antibiotics or vaccines in fish culture is laborious and expensive. Disease prevention is thus preferable as it is more beneficial than treatment.

In recent years, therefore considerable attention has been given to the modification of intestinal microflora through the use of non-nutrient dietary components, particularly probiotics and prebiotics (termed biotics), to boost the growth, immune system and disease resistance capability of fish (Gatesoupe 2005, Wang et al. 2008, Merrifield et al. 2010). Though the objectives of using these dietary supplements are similar, the manner in which they modify the intestinal microbial community differ. Probiotic is regarded as "one or more microorganisms which have beneficial effects for the host, and is able to exist in the digestive tract due to its tolerance to acid and bile salts" (Irianto and Austin 2002).Though using probiotics in aquaculture is relatively new, the interest in them has increased tremendously due to their potential in disease control (Wang et al. 2008).

Another comparatively new but effective approach to disease control in aquaculture is the use of prebiotics. Prebiotics are primarily defined as those non digestible food ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's intestinal balance (Gibson and Roberfroid 1995). Modes of action of MOS differ from other oligosaccharide candidates, such as fructo oligosaccharides and transgalacto oligosaccharides as it functions in an indirect manner within the gastrointestinal tract as opposed to changing the natural intestinal microbiota directly (Flickinger et al. 2003). MOS has some important characteristics which make it more attractive as an animal feed additive. Earlier research has stated that heat treatment does not change the ability of MOS to perform normally, which make it possible to include MOS in pelleted diets (Hooge 2004).

Probiotic: "Probiotic" term originate from the Greek words '*pro;* and '*bios'* which means "for life" (Schrezenmeir and de Vrese 2001) and this term carries different meanings over the years. Dr. Elie Metchnikoff was the first person who clarified the useful role played by some bacteria as observed among farmers who consumed milk containing pathogen and that "reliance on intestinal microbes for food makes it possible to take steps to change the flora of our bodies and to replace harmful microbes by beneficial microbes" (Metchnikof 1907). However, the term probiotic was first introduced by Lilly and Stillwell (1965) after modification of the original word "probiotika." This term was used to illustrate the substances secreted by a microorganism which stimulates the growth of another. It was also described as an agent which functions as a contrary to antibiotics. In 1974, Parker (Parker 1974) defined it as "organisms and substances which have a beneficial effect on the host of animal by contributing to its intestinal microbial balance." Later in 1989, Fuller attempted to improve Parker's definition by clarifying that it is "a live microbial feed supplement which beneficially affects the host animals by improving its intestinal microbial balance" and mentioned that it would be useful in a range of extreme temperatures and salinity changes (Fuller 1989). Subsequently, it was recommended that probiotics were "monocultures or mixed cultures of microorganisms which can be applied to humans or animals, that do good to the host by improving the properties of indigenous microflora" (Havenaar and Huis 1992). Because of high interest of probiotics used in aquaculture, Moriarty (1998) suggested to extend the definition of probiotics to "living microbial additives that benefit the health of hydrobionts and therefore increase productivity." The following year, Gatesoupe (1999) defined probiotics as "in a certain way of microbial cells administered, so that they can reach the gastrointestinal tract and be alive with the aim of improving health". The inhibition of pathogens by using probiotics was carried out in the same year and this led the definition to be expanded to"… live microbial supplementation can give benefits to the host by improving its microbial balance" (Gram et al. 1999). An expert with the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) stated that probiotics are live microorganisms which when consumed in adequate amounts confer a health benefits for the host (FAO 2001).

Recently, it has been established that probiotic organisms have shown antimicrobial activity by altering the intestinal microflora thereby secreting a wide range of antibacterial substances such as bacteriocins and organic acids and competing with pathogens for adhesion to the intestine, competing for nutrients which are necessary for pathogen to survive. They are also capable of modulating the immune system. Because of these criteria, when viable probiotics are administered at certain concentrations, they will favorably affect the health of the host (Myers 2007). In addition, nowadays probiotics are defined commonly as “friendly or healthy bacteria,” (Wang et al. 2008).

Characteristics of Probiotic: According to Fuller (1989) a good probiotic should have the following characteristics:

➢ Should be a strain which is capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease.

➢ Should not have any side effect; should neither be pathogenic nor toxic, not only with regard to the host species but also with regard to aquatic animals in general and human consumers.

➢ Should be viable under normal storage conditions and able to survive during industrial process.

➢ Should be capable of surviving and metabolizing in the gut environment, e.g. resistant to bile and low pH due to organic acids enrichment.

➢ Possess high ability to multiply in the intestine.

➢ Should have strong adhesive capability with the digestive tract of the host.

➢ Should have antagonistic characteristics against one or more pathogenic microorganisms.

Types of Probiotics: There are various types of microorganisms that have been shown probiotic characteristics and commonly used for both human and animal consumption is presented in Table 2.1. Among them two most important types of probiotics are *Lactobacilli* and *Bifidobacteri* (Robertson et al. 2000). Besides, various types of yeast species, particularly, under the genus of *Saccharomyces* have been considered as significant probiotic candidate for human and animal uses (Holzapfel et al. 1998).

Table 1. Common probiotics used for human and animal consumption

***Lactobacillus* species**	***Bifidobacterium* species**	**Other species**
Lactobacillus acidophilus	*Bifidobacterium bifidum*	*Bacillus cereus*
L. rhamnosus	*B. breve*	*Enterococcus faecium*
L. casei	*B. infantis*	*Lactococcus lactis*
L. bulgaricus	*B. longum*	*L. cremoris*
L. gasseri	*B. adolescentis*	*Streptococcus thermophilus*
L. crispatus	*B. lacis*	*Saccharomyces cerevisiae*
L. plantarum	*B. animalis*	*S. boulardii*
L. salivarius		
L. buchneri		
L. johnsonii		
L. reuteri		
L. fermentum		

Lactic Acid Bacteria: Considering the above probiotic characteristics, lactic acid bacteria are claimed to be the most utilized probiotic (Robertson et al. 2000). These probiotic bacteria are characterized as gram positive, non-sporulatingcocci or rod shaped and produced lactic acid as their main metabolic product during carbohydrate fermentation. Previous study claimed, lactic acid bacteria as the normal microflora in the gastrointestinal (GI) tract of healthy animals such as mammals and aquaculture species (Ringø 2008, Aly et al. 2008, Wang 2011) and have been considered as being safe for feeding fish (El-Ezabi et al. 2011).These probiotic bacteria are provided benefit to the host animal by modifying the growth of beneficial microorganism that can contribute inhibitory compounds such as lactic acid, hydrogen peroxide, diacetyl, acetaldehyde and bacteriocin, which would ultimately outcompete the potentially hazardous bacteria from the intestinal ecosystem and would reinforce the natural defence mechanisms of organisms (Ringø and Gatesoupe 1998, Gatesoupe 1999, Balcázaretal. 2007). Among the lactic acid bacteria, *Lactobacillus acidophilus* has been considered one of the important probiotic because of its inhibitory effect against numerous pathogenic microorganism (Al-Dohail 2010, Talpur et al. 2014, Akter et al. 2018).

Modes of Action: Probiotics can influence the decline of the prevalence and reduce the duration of diseases by stimulating the resistance to colonization and inhibitory effects against pathogens. Both *in-vitro* and *in-vivo* tests after using several probiotic strains, exhibited the inhibition of pathogenic bacteria through various mechanisms. For a few years, many researches were conducted on the beneficial effects of probiotics. It is difficult to understand the real modes of action of probiotics, and only some explanations are available.

The possible modes of action of using probiotic include:

i) Competition for adhesion site/competitive exclusion of pathogenic bacteria (Gomez-Gil et al. 2000, Vine et al. 2004, Bagheri et al. 2008).

ii) Colonization (Bagheriet al. 2008).

iii) Production of antimicrobial substances/antibacterial activity (De Keersmaeckeret al. 2006, Alyet al. 2008, Vila et al. 2010, Enanyet al. 2012, Akteret al. 2020).

iv) Sources and competition for nutrients (Prieur et al. 1990, Verschuere et al. 2000, Bagheri et al. 2008).

Competition for adhesion site/competitive exclusion of pathogenic bacteria: Competition for adhesion sites and colonization on the intestine and other tissue surfaces are the most common and significant mechanism of probiotics action to combat against harmful pathogens (Ringø et al. 2007). This is referred to as competitive exclusion of pathogenic bacteria (Ohashi and Ushida 2009). The appropriate adhesion of pathogenic bacteria to the enteric mucus and intestinal wall surface is very important for any pathogen to cause damage to the host animal (Olsson et al. 1992, Vine et al. 2004). The physical blocking of pathogenic bacteria colonization by probiotic bacteria from their favorite adhesion site, such as intestinal villus, goblet cells and colonic crypts is referred to as competitive exclusion (Chichlowski et al. 2007). The adhesion of the probiotics on the epithelial cells is normally performed in two ways. One is non-specific depending on the physico-chemical factors and another is specific which is based on the adhesion of the probiotics on the surface of the adherent bacteria and receptor molecules (Salminen et al. 1996). One of the most important mechanisms of the intestinal microflora is to resist the adhesion of pathogenic bacteria to the host intestine, thereby creating of a physiologically negative environment, with regard to pH, redox potential, and hydrogen sulfide enrichment (Fons et al. 2000). However, by maintaining good husbandry practices and environmental conditions, the composition of microbial communities can be altered, which ultimately will be able to excite the proliferation of selected bacterial species. It is well established that the presence/existence of microflora in the intestinal tract of aquatic animals can be altered by supplying beneficial microorganisms through feeding, which ultimately will reduce the availability of opportunist pathogens (Deven et al. 2009). In fact, there are some evidence that lactic acid bacteria, particularly*L. lactis* and *L. plantarum* can successfully reduce the adhesion of pathogenic bacteria *A. hydrophila*, *A. salmonicida* and *V. anguillarum*in intestinal mucus of fish (Balcazar et al. 2008).

Colonization: Probiotics execute their activity through colonization and the secretion of many growth-promoting substrates in the host intestine (Bagheri et al. 2008). Probiotics colonization of the gastrointestinal tract of host animals is probable only after birth, and prior to a very competitive indigenous microflora that has been installed. After being installed, only the supplement of high levels of probiotic can activate its temporary and artificial dominance. After the intake had stopped, the population of probiotics in the gastrointestinal tract of mature animals show a rapid decrease within days (Fuller 1992). A microorganism is capable to colonize in the gastrointestinal tract whenever it can stay there for a long time by possessing a higher multiplication rate than its expulsion rate (Conway 1997). The attraction of bacteria to the mucosal surface association within the mucous gel or attachment to epithelial cells is the main reason of this colonization. The adhesion and colonization of the mucosal surfaces by probiotics are possible protective mechanisms against pathogenic organisms through a competition for nutrients and the binding sites (Westerdahl et al. 1991), or modulation of immune response (Salminenet al. 1998, Al-Dohail et al. 2009, 2011, Talpur et al. 2014, Akter 2015, Munir et al. 2018). Several factors responsible for the colonization of microorganisms are as follows**:**

(i) Host related factors: These include body temperature, redox potential levels, enzymes, and genetic resistance. For instance, bacteria can enter the host body through the mouth, either being mixed with water or food particles, and while passing along the alimentary tract, some of them are able to adhere as part of a resident micro flora. Among the rest of the micro flora some are destroyed throughout the digestive process while passing through the intestine, and the rest is eliminated via the faeces. Another factor which may be able to suppress bacterial growth is the production of any antimicrobial substances by the host.

ii) Microbe-related factors: These are the effects of antagonistic microorganims, bacteriocins, lysozymes, proteases, formation of hydrogen peroxide, ammonia, and sudden changes of pH values due to production of organic acids (Gram et al. 1999, De Vrese and Marteau 2007, Vila et al. 2010, Akter 2015). It is well recognized that lactic acid bacteria produce substances, for example bacteriocins, that are capable to inhibit the growth of some pathogenic microorganisms.

Production of antimicrobial substances / antibacterial activity: Bacterial antagonism is a familiar event in nature; therefore, the interactions of microbes play a significant role to maintain the equilibrium between useful and potentially pathogenic micro-organisms (Balcazar et al. 2004). Probiotics secret certain inhibitory substances such as bacteriocins, organic acids (lactic acetic and butyric acid) and H_2O_2 (De Keersmaecker et al. 2006, Vila et al. 2010, Akter 2015, Akter et al. 2018), which are able to show antagonistic activity against the pathogenic microorganisms, and consequently will prevent their propagation in the host bodies. It was previously reported that bacteriocinsacidophilin, lactocidin and acidolin are secreted by one of the most important probiotic strain *L. acidophilus,* whereas *L. plantarum* produces lactolin (Vila et al. 2010). They are also able to show antimicrobial activity by lowering the pH of the intestine (De Keersmaecker et al. 2006), the agglutination of pathogenic microorganisms, binds with toxic metabolites (Fonden et al. 2000, Oatley et al. 2000, Haskard et al. 2001), and the production of mucus (Mattar et al. 2002, De Vrese and Marteau 2007). Several researches have reported that probiotics especially *Lactobacillus* sp., *Bifidobacterium* sp. and *Bacillus* sp. have been able to secrete a wide range of chemical substances which have shown an inhibitory effect on pathogenic bacteria (Oscaris et al. 1999, Gotteland et al., 2006; Vila et al. 2010). There is evidence that the probiotic strain of *L. salivarius* sub sp. *salivarius*UCC118 secrete a peptide, which has shown the inhibition capability against a broad range of pathogens, including *Staphylococcus*, *Enterococcus*, *Listeria*, *Bacillus*, and *Salmonella* species (Flynn et al. 2002).

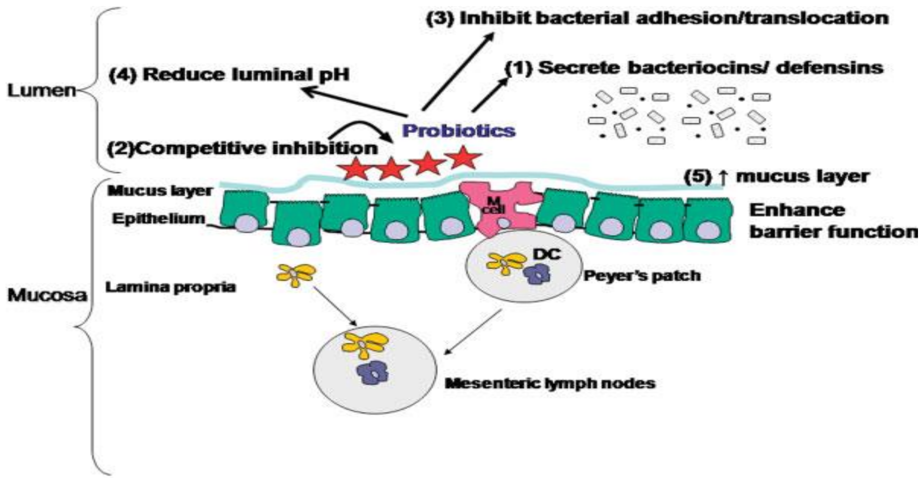

Plate 1. Inhibition of enteric bacteria and enhancement of barrier function by probiotic bacteria. Schematic representation of the crosstalk between probiotic bacteria and the intestinal mucosa. Antimicrobial activities of probiotics include the (1) production of bacteriocins/defensins, (2) competitive inhibition with pathogenic bacteria, (3) inhibition of bacterial adherence or translocation, and (4) reduction of luminal pH. Probiotic bacteria can also enhance the intestinal barrier function by (5) increasing mucus production. [Source: www.interscience.wiley.com].

Sources and competition for nutrients: Intestinal microorganisms play a crucial role in nutrition as well as the well-being of several animals (Floach et al. 1970) including fish (Sugita et al. 1989). The ability of intestinal microorganism to synthesize vitamins, essential growth factors (fatty acid, amino acid) and digestive enzymes are well documented (Teshima and Kashiwada 1967, Clements 1997). Microorganism's particularly anaerobic bacteria play a significant role in carp (*Cyprinus carpio*) nutrition by contributing fatty acids (Clements 1997) which are the end products of anaerobic fermentation (Smith et al. 1996). There is also evidence that the useful micro flora (aerobic, anaerobic) can serve as a source of food by providing vitamins, such as B_{12} and essential amino acids, which play a significant role in the host's nutrition (Sugita et al. 1991a, 1991b).

In fact, probiotics improve the health or well-being of the host by adhering to the mucus membrane, epithelial cells, gastrointestinal tract as well as other tissues (Gatesoupe 1999, Farzanfar 2006). Several researches have been conducted to evaluate the *in-vitro* and *in-vivo* attachment ability of many beneficial microorganisms and their results recommended that the some potential probiotic have ability to displace the pathogens by competing for necessary nutrients, space, etc. (Verschuere et al. 2000). They can cause the unavailability of nutrients and energy sources for essential growth of some pathogenic bacteria, by effectively utilizing those nutrients which would otherwise can be consumed by pathogenic microbes.

Prebiotics: The sustainable approaches practiced for disease control in aquaculture are the application of probiotics and prebiotics. However, the large scale use of probiotics in commercial aquaculture has been restricted due to problems associated with handling, pelleting and storage (Merrifield et al. 2010). Therefore, prebiotics have been proposed as an alternative in an attempt to overcome issues associated with probiotic applications. Instead of introducing probiotic bacteria, the aim of prebiotics is to stimulate selected indigenous microflora. Several studies have examined the health benefits of probiotics while knowledge about prebiotics is still limited.

The term prebioticcomes from the Greek words “pre” and “bios" meaning “before life”. Gibson and Roberfroid (1995) first introduced the concept of prebiotic about twenty years ago. They primarily defined prebiotic as “A non-digestible foods ingredient(s) comprising mainly carbohydrate being added to feed that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves the host health”. Several studies conducted in the last decade demonstrated that prebiotics play a significant role in maintaining a balanced intestinal microflora to enhance the wellbeing of the host. However, sometimes without due consideration to the criteria required, many food components exhibited the prebiotic effect. Though all dietary carbohydrates are not considered as prebiotics, but many oligosaccharides and polysaccharides in food (including dietary fiber) have shown to have prebiotic activity. Therefore, for a dietary substrate to be classified as prebiotic the following three characteristics are required:

1) Resistance against gastric acidity, hydrolysis by digestive enzymes and GI absorption.

2) Should be fermented by intestinal microflora.

3) Selectively stimulating the growth and/or activity of intestinal microflora related to health and wellbeing (Gibson et al. 2004).

The main positive aspect of using prebiotic over probiotic includes, prebiotics are natural feed ingredient, does not require particular precautions during pelleting and easy to handle (Gatesoup 2005). Numerous studies have been conducted on prebiotics in aquaculture. Most of these studies have emphasized the effect on growth performance, intestinal morphology, microflora, resistance against pathogenic bacteria and innate immune parameters (Ringø et al. 2010). Several well established prebiotic oligosaccharides used in aquaculture include inulin, fructo oligosaccharides (FOS), short-chain fructo oligosaccharides (scFOS), mannanoligosaccharides (MOS), galacto oligosaccharides (GOS), and GroBiotic®-A. Among the prebiotics studied, MOS is gaining prominence as a dietary supplement for fish and crustacean species (Sang and Fotedar 2010).

Mannan Oligosaccharide (MOS): Mannan oligosaccharide (MOS) is complex sugar extracted from the yeast cell wall of *Saccharomyces cerevisiae* (Gültepe et al. 2011). The cell wall of yeast mainly consists of four covalently linked components of which mannoproteins and β (1, 3) glucans are the most significant components which function as the backbone of the yeast cell wall (Lipke and Ovalle 1998). Another two components are chitin which is important for the maintenance of cell wall solubility, and β (1, 6) glucans which are responsible for making the link between halix shaped β (1, 3) glucans and some of mannoproteins (Lipke and Ovalle 1998). The external yeast cell wall mainly contain mannoprotein accounts of 50-90% carbohydrate as mannoproteins support N-linked glucans which in turn contained of 50-200 mannose molecule (Moran 2004). The initial interest in using mannan oligosaccharide (MOS) as a feed additive for animal nutrition was adapted from studies at the end of 1980s by Oyofo et al. (1989).

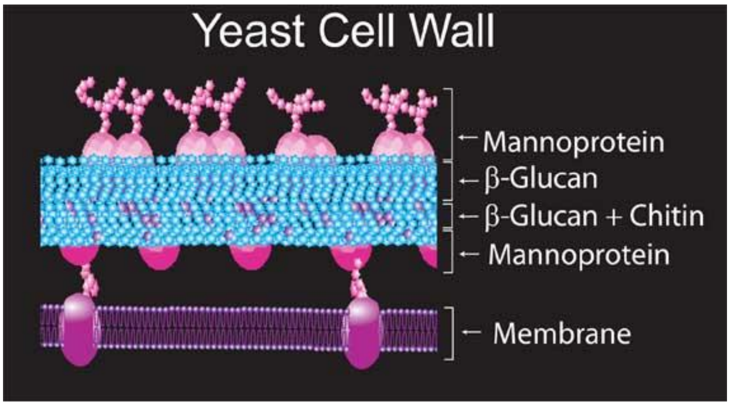

Plate 2. The cell wall structure of yeast (*Saccharomyces cerevisiae).*
(Source:http://www.sigmaaldrich.com/technical/documents/articles/biofiles/antifungals.html).

Mode of action of prebiotics: Nowadays, the use of prebiotics have been of great interest in fish culture (Li and Gatlin 2005, Genc et al. 2007, Yilmaz et al. 2007, Hai and Fotedar 2009) due to its ability to enhance growth performance of aquatic animal by favouring the predominance of helpful intestinal microflora which are able to improve nutrient component assimilation and feed utilization by improving the intestinal morphology, enhancement of growth performance and immune response (Ringo et al. 2010, Vazquez et al. 2006, Vos et al. 2007, Sado et al. 2015, Akter et al. 2016, Munir et al. 2018, Akter et al. 2019). Several researchers have proposed various mechanisms to provide details of ideas about their specific action, such as the stimulation of selective beneficial microflora, improvement of intestinal morphology, development of immune functions and disease resistance, improvement of growth performance and feed efficiency.

Stimulation of selective beneficial microflora: Prebiotics are preferred because most of the nutritional and health benefits attributable to prebiotics are directly and indirectly related with their selectively stimulating ability on growth and /or activity of the beneficial microflora in the GI tract (Gibson and Roberfroid, 1995, Merrifield et al. 2010, Ringo et al. 2010). However, there are some concerns associated with the use of prebiotics in aquaculture practices. This is because some opportunistic pathogenic bacteria can utilize a wide variety of carbohydrates and can ultimately cause health hazards by proliferating in the intestine utilizing the prebiotics (Gatesoupe 2005). Similarly, another major concern is the continuous supply of the prebiotic substrate in the intestine which may generate the risk that the pathogenic bacteria could acquire the ability to utilize the native compound or its degraded products. It has been suggested to utilize the prebiotic in the diet with discernment, and before the practical applications in fish farms and hatcheries many experiments with microbial survey should be conducted first.

Recently all dietary carbohydrates are not considered as prebiotic (Gibson et al. 2004) while certain prebiotics (FOS, MOS, and GOS) are those functional substrates which can only be utilized by a "healthy" or "beneficial" microflora such as lactobacilli and bifidobacteria (Cummings et al. 2004). This is because the genera under these two groups do not have any known pathogens and they are carbohydrate fermenting bacteria, which can produce host health beneficial products, namely short chain fatty acids (Macfarlane et al. 2006). These short chain fatty acids (SCFAs) turn the GI tract as acidic and this can be favourable for the development of probiotic bacteria as well as unfavorable for the growth of harmful species (Blaut 2002). Several studies have shown that prebiotics are able to selectively motivate one or a limited number of beneficial bacteria, thereby causing a selective improvement of the host's intestinal microflora (Mussatto and Mancilha 2007, Venter 2007).

Mannan oligosaccharide is one class of prebiotic oligosaccharides that benefits the enteric health. In fact, the beneficial bacteria can utilize prebiotic oligosaccharides as a nutrient source and may promote the maintenance of bifidobacteria and certain LAB in the intestine of humans and animals (Mussatto and Mancilha 2007, Moura et al. 2007). The nutritional characteristics of prebiotics are directly related to the physiological changes in the host. MOS have been observed to enhance resistance against enteric disease by various ways (Ferket 2004). One important way is to inhibit the colonization of enteric pathogens by blocking bacterial adhesion to intestinal epithelial lining. However, the first critical stage leading to infection is the adhesion of pathogenic bacteria to the epithelial surface of the intestinal (colonization) (Moran 2004). The proliferation of pathogenic bacteria such as *E. coli*, *Vibrio* spp. and *Salmonella,* in the GI tract are mainly dependent on the attachment ability of the pathogenic bacteria to the mucosal epithelium of the intestine. The cell surface of bacteria contains mannose bearing specific lectins that are required to identify specific sugars and to permit those cells to attach to that sugar (Panigrahi and Azad 2007). Previous research has shown that the utilization of purified mannose and MOS successfully prevent the attachment of bacteria to the host animal by providing the bacteria a mannose-rich receptor that serves to occupy the binding sites on the bacteria and to prevent colonization in the animal (Fairchild et al. 2001). Instead of attaching to intestinal epithelial cells, gram-negative pathogens bearing mannose-specific Type-1 fimbriae will attach to the MOS, therefore, inability of bacteria to colonize in the intestine causes them to move through the intestine (Sadeghi et al. 2013). Panigrahi and Azad (2007) demonstrate that MOS (Bio-Mos®) could significantly reduce the colonization of *E. coli* and *Salmonella* spp. It is evident that instead of binding to the mucosal surface, some pathogenic bacteria will accidentally be attached to MOS present in the intestinal lumen, and hence will be unsuccessful to propagate vary widely (Denev et al. 2009). This will ultimately increase intestinal integrity. The controlling of bacteria-mediated attachment has been suggested as a possible way of reducing enteric infection.

Effect on intestinal morphology: Recently, many studies have given emphasis on the effects of MOS on gastrointestinal morphology of various species in fresh and marine water fish (Yilmaz et al.2007, Salze et al. 2008, Dimitroglouet al. 2009, 2010, Sadoet al. 2015, Akter et al. 2016). In fact, MOS supplement have shown a significant positive effect on adult rainbow trout (Dimitroglou et al. 2008) and sole in terms of internal and external perimeter ratio both in the anterior and posterior intestinal regions. This ratio represents the modification on the absorptive surface area of the intestine, for better nutrient utilization and retention (Spring and Privulesku 1998). The transmission of electron microscopy (TEM) revealed that MOS can significantly raise microvilli length and/or density and make available an enormous absorptive surface area (Sang and Fotedar 2010, Danials et al. 2010, Akter et al., 2016), which plays a significant role in nutrient captivation. The good condition of intestinal morphology is likely to support an improved nutrient utilization. The incorporation of the light and TEM result in MOS being recommended not only to protect but also to increase the functionality of the intestinal tract.

Improvement of haematological parameters: As the health status of fish being reflected through its haematological parameters, therefore, an understanding of the haematological parameters used as an effective index in evaluating physiological and pathological abnormalities in fish to verify its health status (De Pedro et al. 2005). Most of the studies reported the haematological parameters as a result of feeding MOS in the pre-challenged fish group. Very few literature available on the effect of MOS on haematological parameters after challenged with pathogenic bacteria. The literature showed that dietary MOS had no effect on some hematological parameters of Channel catfish (*Ictalurus punctatus*) (Welker et al. 2007); Nile tilapia (*Oreochromis niloticus*) (Sado et al. 2008); Giant Sturgeon (*Husohuso*) (Monsour et al. 2012); Gilthead Seabream (*Sparus auratus*) (Gultepe et al. 2012); snakehead (*Channa striata*) (Talpur et al. 2014); rainbow trout (*Oncorhynchus mykiss*) (Denji et al. 2015) and gilthead sea bream (*Sparus aurata*) (Gelibolu et al. 2018)in the pre-challenged fish group. However, feeding the fish with MOS supplemented diet not only increase the WBC count in the pre-challenged in *Labeorohita* (Andrews et al. 2009) but also in yellowtail infected with *Nocardia kampachi* (Ikeda et al. 1976). It is well recommended that WBC play an important role in non-specific immunity or innate immunity (Roberts 1978), and is being considered as an indicator of good health status of fish. Similarly, significantly increased PCV was reported in carp juvenile (*Cyprinus carpio*) fed with a lower (0.1%) MOS level (Akrami et al. 2012b). The significantly improved PCV indicates that MOS is safe for consumption and their effectiveness in improving health status, as a lower PCV is an indicator of unhealthy fish as a result of not eating properly or are suffering infections (Blaxhall 1972). The various responses obtained on the haematological parameters as a result of feeding MOS diet might be due to different sources and purity of MOS, the concentration used, culture period and different species assessed (Pryor et al. 2003, Akrami et al. 2012a).

Improvement of immune functions and disease resistance: Recently, commercial aquaculture has shown a keen interest on the stimulation of the immune response of fish through dietary supplements (Staykov et al. 2007). As aquatic animals are continually vulnerable to numerous opportunistic pathogens, the innate immune system is therefore the most significant aspect of the development of aquaculture because this part of immune response provides the first line of defence for the host (Magnadóttir 2006). Prebiotics is one group of these dietary supplements with health promoting effects that have been proven in fish culture (Staykov et al. 2007, Zheng et al. 2011, Akrami et al. 2012a, b, Akter 2015, Muniret al.2018,Akteret al. 2019). Like other invertebrates, immunity acts as a significant task in protecting against pathogens in fish. This protection can be performed either by non-specific or an acquired specific immunity thereby defending against pathogen (Cerezuela et al. 2012). Unlike mammals, fish primarily depends on non-specific defense mechanisms (Swain et al. 2007). The prebiotics can enhance non-specific immune responses by modifying the microbial community (beneficial microflora) of the GI tract (Bailey et al. 1991), which is able to inhibit the growth of pathogenic bacteria (Mussatto and Mancilha 2007). Several humoral and cellular components are predominantly responsible for non-specific immune system of fish that can afford innate protection against infection, regardless of the pathogenic type (Whyte 2007). The prebiotic, especially MOS, has been able to increase humoral and cellular immunity by triggering the production of lysozyme and antibody in rainbow trout (Staykov et al. 2007), gibel carp (Akrami et al. 2012a), common carp (Momeni-Moghaddamet al. 2015), snakehead (Munir et al. 2018) and striped catfish (Akter et al. 2019); and leukocytes in European sea bass (Torrecillas et al. 2007), snakehead (Munir et al. 2018) and striped catfish (Akter et al. 2019). Though, the exact mechanisms have still not been completely described (Ferket 2004, Moran 2004) but it can be speculated that MOS could provide the beneficial effects to the host due to the secret mannose-binding lectin which can use as an opsonin (Nochta et al. 2009) and able to bind the mannose bearing structures of many pathogenic bacteria and triggering the phagocytosis (Ofek et al. 1995, Staykov et al. 2007).

Effect of prebiotics on histopathology of liver: Fish liver is being considered as a key organ that performsmany functions and plays a significant role in fish physiology (El-Bakary and El-Gammal 2010). Due to its position (Van der Oost et al. 2003) in the fish's body, this organ can easily be infected by bacteria during intraperitoneal injection of pathogenic bacteria. There is evidence of severe cellular damage of Atlantic salmon (Stoskopf 1993), hybrid striped bass (Bowser et al. 2004) and striped catfish (Akter 2015) liver when they were exposed to pathogenic bacteria. Among various pathological changes, necrosis is being considered as the biomarker of histopathological changes of a tissue level (Mohamed 2009). These pathological changes as a result of infection by pathogenic bacteria might be due to secret toxins by them. A lower lipid vacuolization and almost regular morphology of hepatocyte was reported in rainbow trout (Staykov et al. 2007) and European sea bass (Torrecilas et al. 2007) fed with MOS supplemented diet compared to control fed group which showed swelling of hepatocyte by cytoplasm vacuolization and nuclei shifted to cellular periphery. Therefore, histopathological alteration of liver can be used as an effective indicator of overall health status of fish (Mohamed 2009), which can be determined cost effectively by investigating the structural changes of liver using histology (El-Bakary and El-Gammal 2010).

Effect on fish growth and feed utilization: The positive effects of MOS with regard to growth performance were examined in some terrestrial vertebrate animals (Savage et al. 1997) as well as the Crustaceans (Genc et al. 2007, Hai and Fotedar 2009). Previous studies have shown variability in the response of fish and crustaceans to MOS supplementation: growth enhancement was observed in rainbow trout (*Oncorhynchus mykiss*) (Staykov et al. 2007, Yilmaz et al. 2007), green tiger prawn *(Penaeus semisulcatus)* (Genc et al. 2007), European sea bass (*Dicentrarchus labrax)* (Torrecillas et al. 2007), juvenile tropical spiny lobsters (*Panulirus ornatus)* (Sang and Fotedar 2010), freshwater crayfish (*Cherax destructor*) (Sang et al. 2011), gilthead sea bream (*Sparusaurata)* (Gültepe et al. 2011), striped catfish (*Pangasianodonhypophthalmus*) (Akter et al. 2016) and snakehead (*Channa striata*) (Munir et al. 2016). Andrews et al. (2009) reported that the enhancement of the growth of lactic acid bacteria in the intestines of fish might be a reason of this positive response of MOS in the growth performance of fish.

On the other hand, growth performance, feed efficiency and survival have remained unaffected when fish was fed with mannan oligosaccharide diet **(**Pryor et al. 2003, Welker et al. 2007, Sadoet al. 2008, Dimitroglou et al. 2010, Gültepe et al. 2012, Mansour et al. 2012, Talpur et al. 2014). There are no distinct reasons for the different results which might be due to the different basal diet, inclusion level, acclimation and culture period, source of MOS, animal characteristics (species, age, stage of production), and hygienic conditions of the experiment (Reza et al. 2009).

CONCLUSION

In recent years, probiotics and prebiotics have become an integral parts of the aquaculture practices for improving the growth performance, feed utilization and disease resistance. Many studies revealed the effects of probiotics and prebiotics to improve feed conversion, growth rates, weight gain, immune system and disease resistance of fish. Most of the studies have evaluated the effectiveness of probiotics and prebiotics under laboratory conditions; therefore, their application under culture conditions is necessary in order to exactly evaluate their use. Although various beneficial effects are manifested due to application of probiotics and prebiotics in aquaculture, however prebiotics have much potential to increase the efficiency and sustainability of aquacultural production. Therefore, comprehensive research need to conduct for characterize the dominant intestinal microbiotaoffish species and their responses to prebiotics is warranted.

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