International Scholars Journals

Advances in Aquaculture and Fisheries Management ISSN: 9424-2933 Vol. 3 (7), pp. 264-273, August, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

The use of probiotics in fish feed for intensive aquaculture to promote healthy guts

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Accepted 28 July, 2015

The use of probiotics in feed for fish and its inclusion in intensive aquaculture to promote healthy gut is growing. The need for alternative measures that will perform closely and effectively to the use of antibiotics after its annulment in European Union (EU) in 2006 is an issue which call for concern. Several definitions of probiotics mainly for aquaculture were also dig out from published papers. Among them is a definition considered appropriate for aquaculture which is described as" any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, in part at least, by improving the microbial balance of the fish". In this context, they regard direct benefits to the host as immuno-stimulants, improved disease resistance, reduced stress response, improved gastro intestinal morphology and benefits to the fish farmer or consumer. The benefits to the fish farmer and consumers includes improved fish appetite, growth performance, feed utilization, improvement of carcass quality, flesh quality and reduced malformations. The mode of action with benefits of probiotics in aquaculture from published journals was also looked into. The use of probiotics is growing especially as new strains were being discovered that can withstand processing stress in feed manufacturing. But process optimization knowledge to effectively work with these additives is insufficient. Combination of strain isolated from the gastro-intestinal (GI) tracts of host animal had also being proving to be effective. Need to find proper parameters to work with probiotics will be of great importance to the feed industry in the near future and the stability of the strains of probiotics as a synbiotic relation with prebiotics. Examples and effects of known strains were also stated and the future perspective of probiotics for fish.

Key words: Probiotic, fish farming, aquaculture, feed processing.

INTRODUCTION

Aquaculture is currently the fastest growing food production sector in the world, but diseases especially bac-terial infections remain primary constraints to its continued expansion (Abd El-rhman et al., 2009; Pieters et al., 2008; El-Haroun et al., 2006). Micro-organisms have been implicated in this problem and its control in aquaculture is a challenge (Ringo and Birkbeck, 1999). Thus heavy reliance on vaccines and antibiotics to combat these diseases is inevitable and their use for decades.Adverse effects on the use of antibiotics to control diseases has creates some problems. These effects includes among others, accumulation in the tissue and immuno-suppression (Tukmechi et al., 2007; Nayak et al., 2007; El-Haroun et al., 2006) and ecological threat to coastal areas heavily exploited for industrial cultivation of fish and shell fish (Gildberg et al., 1997). Because of these issues, growing concern about the use of chemical compounds, not only in human medicine and agriculture

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but also in aquaculture has led to interest in finding other methods of preventing losses in hatcheries (Rollo et al., 2006). Also, the emergent of antibiotic resistance of human commensal bacteria led to loss of confidence in the use of antibacterial leading to its total ban in European Union (EU) countries in 2006 (Angelis et al., 2006). Probiotics is recognised as an alternative therapy for health management due to restrictions on antibiotics and limitations of vaccinations and chemotherapy (Panigrahi et al., 2010). Its use as farm animal feed supplements such as in pig, cattle and poultry nutrition is from the 1970’s, but the concept in aquaculture is a bit new (Tukmechi et al., 2007). The research on its use for aquatic animals is increasing due to the demand for environment friendly aquaculture (Abdelhamid et al., 2009) in terms of use of eco-friendly alternatives to the therapeutic use of antimicrobials (Merrifield et al., 2010c). The aim of this paper was to review some published articles on the use of probiotics in aquaculture and to mention some of its challenges in feed processing.

Digestive system of fish

Fish are classified as either detritivores, herbivores, omnivores and carnivores. Within each category, they are thought of being euryphagous (eating a great variety of foods), stenophagous (eating a limited variety of foods) or monophagous (eating only one type of food). Fishes such as salmon, basses and halibut are known to be euryphagous carnivores. Fishes such as channel catfish and tilapia are refers to as euryphagous omnivores while carp and milkfish are known to be euryphagous herbivores. These classifications have made diversity in the anatomy of their digestive system (Halver and Hardy, 2002). The oral cavity in fish is the area where food is first consumed by the fish. It extends from the jaw to the esophageal sphincter. The posterior portion of the mouth, near the esophagus and gills is called the pharynx. The teeth are also part of the oral cavity with their position relating to the feeding habit of the fish. The structure and function of the gill rakers complement that of the teeth. While in some species, the gill rakers are fine and comb-like which are used to filter small particles from the water and in others, such as predatory fish the shape are sharp and pointed to hold and puncture prey. In some species the gill archers with molar like teeth are used for grinding. The food then moves after grinding from the oral cavity through the esophagus into the digestive system, where nutrients are absorbed from the food into the system. There is diversity in pattern of fish stomach and these are different in each different breed of fishes. The stomach can be straight with an enlarged lumen (for example halibut and catfish), U or J-shaped stomach with an enlarged lumen (salmon) and Y-shaped stomach with a caecum (tilapia). Some fish such as carp has no stomach (Halver and Hardy, 2002). The digestive tract is a long passage filled with mucus membrane with main purpose for prehension, ingestion, comminution, digestion and absorption of nutrients, and the elimination of solid waste material (McDonald et al., 2002). Apart from the function above, it is also a site for mineral exchange homeostasis, harbouring a complex microbiota and a highly evolved mucosal immune system (Lalles et al., 2007). This complex microbiota is concentrated in the intestinal region i.e. small and large intestine.

Function of gut microflora

The roles of microbes in digestion may be significant in fish. For example, microbes are responsible for significant cellulose activity in a wood-eating catfish and rohu carp. The marine herbivore *Sarpa salpa* also digests antibiotic treated green algae as well as untreated algae, indicating that endogenous fish enzymes were responsible (Halver and Hardy, 2002). Therefore, understanding and manipulating the gut microflora is an important area of nutrition as well as prevention from disease (Ringo, 2004). The intestinal microbiota does not exist as an entity by itself, but there is a constant interaction with the environment and the host functions (El-Haroun et al., 2006). The gastro intestinal tract (GIT) is a home to complex and dynamic microbial ecosystem, the composition of which differs between individual and gastrointestinal location and time. It provides pivotal stimuli for the development of the host immune system and physiology such as gastric development, differentiation and its integrity (Merrifield et al., 2010a). The main function of the microbiota (immense number of micro-organisms) includes degrading dietary compounds, influencing nutrient partitioning and lipid metabolism, providing essential nutrients generated as a result of microbial metabolism, protecting against invading pathogens and stimulating gut morphology (Mulder et al., 2009). Apart from these, the flora also acts as a barrier to gut pathogens by blocking their attachment to gut binding sites which is the first step of pathogenicity (Rollo et al., 2006). It can also play an important role in maintaining immune function. Thus, members of the natural aquatic microflora are effective at inhibiting fish pathogens (Pieters et al., 2008). In addition to limiting pathogen attachment, several members of the indigenous microflora, for example, *Lactobacilli* species produce bacteriocins known for its antibacterial actions. Therefore stability of the intestinal microflora is very important for the health of the organism (Rollo et al., 2006).

Gut microflora and causes of induced changes in gut flora

There are differences in micro-organism found in the gut microflora with respect to fish from both sea water and fresh water. Thus salinity and differences in species may play a role in the gastro intestinal microbiota (Ringo, 2004). The gastro-intestinal tracts of fish and shellfish are peculiarly dependent on the external environment, due to the water flow passing through the digestive tract (Gatesoupe, 1999). Establishment of a balanced gut flora as early as in the fish larvae is important but complex, and it seems to be influenced sometimes by the micro-flora of the egg (if any), the live feed and bacteria present in the rearing environment that is, water (Ringo and Birkbeck, 1999). Most bacteria cells are transient in the gut; with continuous intrusion of microbes coming from water and food (Gatesoupe, 1999) which itself readily supports and spreads bacteria pathogens (Merrifield et al., 2010b). Classification of intestinal microbiota of fish as "autochthonous" or indigenous (able to colonise the host's epithelial surface or associated with the micro-villi) or "allochthonous" (incidental visitors in the GI tract that are rejected after some time). Abundance and diversity of autochthonous bacterial populations are different from the allochthonous microbiota sampled from the same part of the intestine, indicating that some bacteria species poorly colonize gut mucosa. When evaluating the gut microbiota of fish, the bacterial population levels are influenced by day to day fluctuations and inter-individual differences (Ringo et al., 2008).

Changes due to the environment

Environmental exposure in early life has a significant impact on microbiota composition of the adult gut and the immune transcriptome during development. It is known that the larval gut of several marine fish species with a less developed digestive tract contains few, if any, bac-teria at the time of hatching. However it increases soon after hatching and the colonisation of the larval intestine (Ringo and Birkbeck, 1999). Even though this is impor-tant, care should be taken because inappropriate expo-sure of the gut in early stage of development to bacteria can increased incidence of infections, inflammatory and autoimmune diseases. Early life environment has a major impact on microbial diversity and these differences are sustainable through-out adult life (Mulder et al., 2009). As many pathogens naturally occur in aquatic environments, all forms of aquaculture are prone to disease outbreaks which are largely determined by host susceptibility (Pieters et al., 2008). Also physiological stress contributes to diseases and increase mortality in aquaculture. It leads to decreased disease resistance, impaired reproduction and reduced growth. It could also lead to intestinal microbiota disorders which decrease the level of beneficial micro-organism and thereby gives room to invasion from bac-teria disease; a significant cause of mortality in most fish hatcheries (Rollo et al., 2006; Ringo, 2004). Intensive aquaculture practises may create a negative impact on the pond environment, leading to outbreaks of infectious diseases (Tukmechi et al., 2007; Nayak et al., 2007) and high mortalities especially during transition from the yolk sac to the first feeding stage of development (Ringo and Birkbeck, 1999). Reason may be because the larvae stage and fish are highly exposed to gastro intestinal microbiota associated disorders from the start feeding even when the digestive tract is fully developed and the immune system is still incomplete (Gatesoupe, 1999).

Changes due to antibiotics

Aquaculture growth and its consequence in out-beak of high number of diseases have made the use of antibiotics and vaccines a compulsion. But its consequence has created antibiotic resistance. The use of antibiotic alters indigenous gut mirobiota in terms of total viable num-bers and diversity of population. Reduction of diversity or quantity of the indigenous microbiota is likely to reduce the effective barrier mechanism normally provided by the commensal microbiota; this could lead to a reduction of competition against secondary potential pathogens from the surrounding environment. Surviving bacteria species carrying genes for resistance may exchange genetic ma-terial conferring resistance to pathogens re-entering the gastro-intestinal tract, leading to the spread of antibiotic resistance and a reduction of antibiotic efficacy against future disease outbreaks. This is a more dangerous sce-nario in fish because the fish gut microbiota is largely transient and heavily influenced by the microbiota of the rearing environment, which itself readily supports and spread bacteria pathogens (Merrifield et al., 2010b) due to the breakdown of the natural barrier between the host and pathogens as a result of intensive fish farming (Ringo and Birkbeck, 1999). This prompted the search for alter-native additives with growth and health promoting effects thus; the use of probiotics especially in farm animals (Fuller, 1989; Lodemann et al., 2007; El-Haroun et al., 2006). Several other alternatives are also being used to improve animal health such as nutrition, water quality, lower stocking densities, non-specific immuno-stimulants to mention but a few (Tukmechi, et al 2007; Gildberg et al., 1997). Alternative control of pathogens instead of the use of antibiotics is the manipulation of the gut flora with probiotic which can be added to the diet to increase the proportion of health promoting bacterial in the gut (Ringo and Birkbeck, 1999) and its use in aquaculture is gaining acceptance (Rollo et al., 2006). Infact its use in feed has being on for decades as a growth promoters that lessen the diarrhea and commercial losses occurring around weaning period for animals such as piglets (Janczyk et al., 2009; Simon, 2010). The use of probiotics as a feed supplement in farms became popular due to the total ban in 2006 in European Union of the use of antibiotics (as growth promoting and therapeutic agent causing in-balance in the gut environment). Consumer demand for product without residues that may pose as a health risk also plays a part in the search for alternative to antibio-tics. It is noted that the use of antibiotics is a double-edge sword in for example recently born piglets as they could be used in eliminating the originating agents and likewise favors the approach of opportunistic micro–organism (such as *Salmonella species* and *Escherichia* *coli*) which may eventually prove resistant to antibiotics (Delforge, 2004). Prevention of GI tract colonization by a variety of microbial pathogens is the primary mechanism mediated by probiotics (Angelis et al., 2006). Probiotics ensure that the host maintains a beneficial microbial population in the gastro-intestinal tract, thus conferring a healthy effect (Abdelhamid et al., 2009). A good advantage is that it can be implemented during the early stage of fish develop-ment when vaccination by injection is impractical (Ringo and Birkbeck, 1999). Other advantages of probiotics in aquaculture are growth performance improvement, disease control through immunity enhancement and pathogens exclusion (Yun-Zhang Sun et al., 2010).

Probiotics

Probiotics is defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989). It can also be define as “a viable micro-organism which when ingested through the oral cavity in a sufficient quantity confer on the host a beneficial effect due to an improvement in the intestinal microbial balance” (Giorgio et al., 2010). A live microbial dietary additive confers health advantages and had has its long history of use in humans and animals (Tzortzis et al., 2005). A good definition for application for aquaculture is “a live, dead or component of a microbial cell that when administered via the feed or to the rearing water benefits the host by improving either disease resistance, health statues, growth performance, feed utilization, stress response or general vigour, which is achieved at least in part via improving the hosts microbial balance or the microbial balance of the ambient environment” (Merrifield et al., 2010c). Another proposed definition of probiotics used in aquaculture is “live microbial cultures added to feed or environment (water) to increase viability (survival) of the host” (Ringo et al., 2010). They are also refer to as bio-proteins containing living microbial cells that optimize the colonization and composition of the growth and gut microflora in animals and stimulate digestive processes and immunity (Dhanaraj et al., 2010). Probiotics may reduce the incidence of disease or lessen the severity of outbreaks in aquaculture. It can be used as alternative to microbial chemotherapeutics. They are primarily used as feed additives to prevent infectious intestinal diseases through the secretion of micro-toxins that inhibit the growth of other virulent micro-organism (such as *Escherichia coli* and *Salmonella*) in the intestinal lumen (Barths et al., 2009). A definition of probiotic to be considered appropriate for aquaculture is given in a review by Merrifield et al. (2010c) which is “any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, in part at least, by improving the microbial balance of the fish”. In this context, they regard direct benefits to the host as immuno-stimulants, improved disease resistance, reduced stress response, improved gastro intestinal morphology and benefits to the fish farmer or consumer. These benefits to the fish farmer or the consumer includes improved fish appetite, growth performance, feed utilization, improvement of carcass quality, flesh quality and reduced malformations. Probiotic species metabolize carbohydrates that are resistance to attack by indigenous cecal bacteria. They may also provide energy for indigenous bacteria to proliferate and thereby to utilize ammonia and branch chain fatty acids for the synthesis of protein (Fujieda and Sakata, 2001). The mucosal immune system has to retain the ability to respond actively to pathogens, while avoiding active potentially inflammatory responses to pathogens (Bailey, 2009).

Mode of action to stimulate a healthy gut

Probiotics modulate the growth of intestinal microbiota, suppress potentially harmful bacteria and reinforce the body’s natural defence mechanisms (Giorgio et al., 2010) thus improving resistance against infectious diseases (Gildberg et al., 1997). Bacteria probiotics do not have a mode of action but act on species-specific or even strain-specific and the immune response of the animal and its interaction with intestinal bacterial communities will play a key role (Simon, 2010). Probiotic produces inhibitory substances which may be antagonistic to the growth of pathogens in the intestine. The ability of some probiotics to adhere to intestinal mucus may block the intestinal infection route common to many pathogens (Ringo et al., 2010; Gatesoupe, 1999). They can also stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and by breakdown of indigestible components (Abdelhamid et al., 2009). They exert their effects in a number of ways which is presented in the following:

1. Competitive adhesion to the digestive tract wall to prevent colonisation by pathogenic micro-organisms. For example *E.coli* action on the gut wall may be prevented by *Lactobacilli* which compete successfully for these sites (Barth et al., 2009; McDonald et al., 2002; Lalles et al., 2007; Ringo et al., 2010; Gatesoupe, 1999). The ability to inhibit pathogens adhesion appears to depend on the specific probiotics and pathogens and on the mucosal site (Young-Hyo et al., 2001; Collado et al., 2007). Probiotics is known to have antagonistic properties against harmful bacteria entering the intestinal tract. Microbial aberrancies in the intestinal tract can produce diseases which lead to inefficient digestion and nutrient assimilation. The first step of intestinal infections is mediated by adhesion of pathogenic bacteria to mucosal surfaces and disruption of the microbiota. The protective role of probiotic bacteria might be mediated through adhesion and colonization of the mucosal simulation. Ability to adhere to intestinal mucosa is considered an important prerequisite for micro-organism intended for probiotic use;

this has relationship to many of the health benefits attributed to probiotics (Collado et al., 2007; Vendrell et al., 2008; El-Haroun et al., 2006). Gastric pathogen has to overcome or colonise the mucus layer in order to attach, interact with and infect the host epithelium (Merrifield et al., 2010a). Because the intestinal mucosal barrier including the epithelium cells, tight junctions controlling the paracellular pathways and a superficial mucous layer forms an effective physical barrier that separate the individual from the complex microbial populations which constitute the normal intestinal microflora (Ringo and Birkbeck, 1999). The ability of some of probiotics to adhere to intes-tinal mucus may block the intestinal infection route com-mon to many pathogens (Ringo et al., 2010; Gatesoupe, 1999). The inter-relationship between gut mucosal epi-thelial cells, mucus, anti-microbial products, commensal organisms resident in the gut and immune cells, in the mucosal/sub mucosal are vital for the health and well being of the fish. Microbiota stimulates intestinal epithelial proliferation and influenced enterocyte morphology (Merrifield et al., 2010c).

2. Probiotic produces inhibitory substances which may be antagonistic to the growth of pathogens in the intestine. Antagonism may arise because of competition for nutriaents that favour the growth of probiotics strain or the expression of their inhibitory effects in the gastro intestine (Gatesoupe, 1999). Mucus-associated populations of probiotics are likely to provide competition, release bacteriocins and other antimicrobial compounds in the mucus layer and exert a host immuno-stimulatory role (Merrifield et al., 2010a). An example is production of bacteriocins from lactic acid bacteria, which can inhibit the growth of fish pathogens such as *Vibrio anguillarum* and *Aeromonas salmonicida* (Gildberg, et al, 1997).

3. Bactericidal activity: Lactobacilli ferment lactose to lactic acid, thereby reducing the pH to a level that harmful bacteria cannot tolerate. Hydrogen peroxide is also produced, which inhibits the growth of gram negative bacteria. It has also been reported that lactic acid producing bacteria of the *Streptococcus* and *Lactobacillus species* produce antibiotics (McDonald et al., 2002; Klose et al., 2010). The antagonistic effects of gut microbiota against pathogens and other organisms are due to competition for nutrients and adhesion sites, formation of metabolites such as organic acids and hydrogen peroxide and production of bacteriocins (Ringo et al., 2010). Probiotics also modify the metabolism of the microbial ecosystem in the large intestine to increase short chain fatty acid production and thereby increase sodium and water absorption and decrease colonic motility (Sakata et al., 1999).

4. Prevention of amine synthesis: *Coliforms* bacteria decarboxylate amino acids to produce amines, which irritate the gut, are toxic and are concurrent with the incidence of diarrhoea. If desirable bacteria prevent the coliforms proliferating then amine production will also be prevented (McDonald et al., 2002). Probiotics may alter metabolism of the hind gut bacterial ecosystem to increase production of short chain fatty acid and other organic acids, decrease production of ammonia and isovaleric acid and is also very likely done through increasing the breakdown of hard to degrade carbohydrates thereby reducing the net breakdown of protein. Such changes in bacterial metabolism seem to be responsible for the anti-diarrheic actions of probiotic preparations (Sakata et al., 1999; Young-Hyo et al., 2001; Fujieda and Sakata, 2001; Modesto et al., 2009).

5. Enhance immune competence: Activities of probiotics aid the development of the immune system by stimulation of the production of antibodies and increased phagocytic activity. They may also inhibit virulence gene expression, enhanced immune response, improves gastric morphology and aid digestive function (Merrifield et al., 2010c). It needs to be mention that some bacterial act as an immuno-stimulant in fish and shrimp by improving the defence system of host against pathogens by enhancement of antibody production (Tukmechi et al., 2007).

6. They also compete for essential nutrients and enzymes resulting in enhanced nutrition in the host and the modulation of interactions with the environment and development of beneficial immune responses (Ringo et al., 2010). Probiotic bacteria ferment food derived from indigestible carbohydrate to produce short chain fatty acid in the gut. The short chain fatty acid cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver indicating a better health statues in fish (Tukmechi et al., 2007).

Beneficial effects of probiotics

Probiotics favourably influence both the development and the stability of the host's normal microbiota and inhibit colonisation by pathogens. Probiotics also influence the mucosal barrier by their trophic effect on intestinal epithelium and stimulate both specific and non-specific components of the immune system (Vendrell et al., 2008). They also contribute to higher growth and feed efficiency, prevent intestinal disorders and pre-digestion of anti-nutritional factors present in the ingredients thus improving nutrients utilization. Probiotics may also detoxify the potentially harmful compounds in feeds, by denaturing the potentially indigestible components in the diet by hydrolytic enzymes such as amylase and protease. They can also improve feed utilization that is, probiotics can decrease the amount of feed necessary for animal growth, and thereby reduce production cost. The fish (in this case, Nile tilapia) also utilize the nutrient more efficiently when supplemented with probiotics (El-Haroun et al., 2006). Other benefits includes competitive exclusion of pathogenic bacteria, it could also be a source of nutrient and enzymatic contribution to digestion, enhancement of the immune response against pathogenic micro-organisms and it could have antiviral effects (Ringo et al., 2010). The activity of probiotic may result in elevated health status, improved disease resistance, growth performance, body composition, reduced malformations, improved gut morphology, improvement of water quality, reduced malformations and microbial balance (Merrifield et al., 2010c). Probiotics may also stimulate appetite and improve nutrition by the production of vitamins, detoxification of injurious compounds in the diet and by the breakdown of indigestible components (Abd El-rhman et al., 2009). Maintenance of healthy gut microflora may provide protection against gastrointestinal disorders, including gastrointestinal infections and inflammatory bowel diseases. Probiotics can also be an appropriate alternative to the use of antibiotics in the treatment of enteric infection or to reduce the symptoms of antibiotic associated diarrhoea (Giorgio et al., 2010) and also promoting growth rate and increasing the efficiency of feed conversion (Young-Hyo et al., 2001). Probiotic administration is capable of reducing both necrotizing enterocolitis (NEC) and potential pathogens by altering the intestinal bacteria community (Siggers et al., 2008). Probiotic preparations increase the *in-vitro* breakdown of carbohydrates and decrease that of protein by mixed cecal bacteria in the absence of readily fermentable materials (Fujieda and Sakata, 2001). The inclusion of probiotics in feed is to encourage certain strains of bacterial in the gut at the expense of less desirable ones (McDonald et al., 2002). Not all microbes represent a pathogenic threat ; resident commensal microbes help maintain efficient functioning of the gut by supporting gut mucosal barrier function; mounting efficient immune responses to pathogens that break through barrier defences' or maintaining tolerance to luminal contents which allow for nutrient absorption (Merrifield et al., 2010c).

Different strains of probiotic used in aquaculture

Most probiotics belong to Lactic acid bacteria, genus *Vibrio, Bacillus, Pseudomonas* and *Roseobacter* (Ringo et al., 2010). The population level of lactic acid bacteria associated with the gastro intestinal tract of the fish is affected by physiological, nutritional and environmental factors related to age, food habits, season, salinity and stress. Though they are rarely present in juvenile fish reared on artificial feed but may dominate in the intestinal flora if they are supplemented in the feed (Bucio Galindo et al., 2009). To mention but few, strains of probiotic use in aquaculture are: *Lactococcus lactis, Lactobacillus plantarum*, *Lactobacillus rhamnosus, Lactobacillus sakei, Lactobacillus delbrueckii, Leuconostoc mesenteroides, Bacillus subtilis, Bacillus licheniformis, Aeromonas sobria, Saccharomyces cerevisiae, Carnobacterium divergens* (Merrifield et al., 2010c). The advantage of using *Bacillus* strains as a probiotic in feed is their ability to survive the the pelletization process (El-Haroun et al., 2006).

Selection criteria

Selection of probiotics is very critical because inappro-priate strains can lead to undesirable effects in the host (Yun-Zhang Sun et al., 2010). It is known that colonisa-tion of the gut is a criteria to be used for selecting po-tential probiotics in aquaculture (Ringo et al., 2004). The strain should also be able to establish and multiply in the host gut after colonisation (Yun-Zhang Sun et al., 2010). It must not be pathogenic both to the host and to the aquatic environment and the human consumers (Merrifield et al., 2010c). A good strain must survive passage through the upper gastrointestinal tracts and reach its site of action and it must be able to function in the gut envi-ronment (Bucio Galindo et al., 2009; Giorgio et al., 2010). It must be tolerant to gastric juice and bile (Merrifield et al., 2010c; Young-Hyo et al., 2001), adhere to epithelial surfaces, persistent in the Gastro-intestinal tracts and must have immune stimulation. It must also possess antagonistic activity toward intestinal pathogens (such as for example in salmonids; *Aeromonas salmonicida, Vibrio anguillarum, and Yersinia ruckeri*) and the capacity to stabilize and modulate the intestinal microbiota. It should also be free of plasmid encoded antibiotic resistance genes. It should be indigenous to the host or the rearing environment and be registered as feed additives. Tech-nological aspect to be considered for probiotic selection may include phage resistance, sensory properties, and variability during processing and stability in production and storage. It should not carry acquired genetically exchange antibiotic resistance (Merrifield et al., 2010c; Giorgio et al., 2010). Factors needed to be considered for appropriate bacteria to be included in probiotic mixtures should include: Animal diet, age, epithelial cell chemistry, adherence mechanisms and gut compartment (Denis et al., 1997). The main strategies in the use of probiotics are to isolate intestinal bacteria with favourable properties from mature animals and include large numbers of these bacteria in the feed for mature animals of the same species (Rollo et al., 2006; Gildberg et al., 1997). Probio-tics strains like *Lactobacilli* can be isolated from the skin, gills and the gut of fish. It is sometimes believe to be a normal part of the microbiota at larva, fry and fingerling stages (Ringo et al., 2004). Through the combination of multiple favourable probiotic strains, it may be possible to produce greater benefits than the application of individual strain (Merrifield et al., 2010c).

Administration

Administration of strains such as *Lactobacillus* and other lactic acid bacteria to fish is generally carried out by adding viable micro-organism to the feed (Bucio Galindo et al., 2009). Choosing a strain will depend on the fish species, rearing conditions and desired outcome of supplementation such as immuno-stimulation, disease prevention, improved growth performance to mention but few (Merrifield et al., 2010c). For successful application of probiotic strains as microbial ingredients in fish, other characteristics seem to be essential, such as high viability during processing, storage and after gastro-intestinal transit (Bucio Galindo et al., 2009). Another way of administering probiotic strains could be the use of rotifers as carriers or by inoculating the rearing water with live bacteria, as the sowing of the gut with harmless bacteria may prevent infection by pathogenic bacteria (Ringo and Birkbeck, 1999). This is done by either intro-ducing the specific bacteria into the digestive system via the live feed/inert diet or by adding the beneficial bacteria to the rearing water or by adding naturally occurring compounds to the inert diet which might selectively stimulate beneficial gut bacteria. In salmon's studies, live cultures are sprayed or top-dressed into basal diets and use of freeze-dried/lyophilised cells, dead cells, disrupted cells, cell free supernatants and spores have all showed effectiveness (Merrifield et al., 2010c). Probiotic treatments may be desirable during the larval stages because the larvae stage in fish are highly exposed to gastro intestinal microbiota associated disorders due to the start feeding even when the digestive tract is fully developed and the immune system is still incomplete (Gatesoupe, 1999). Appropriate dosage level should also be given consideration.

Though getting an appropriate depends on some factors. These factors varies and it depends on the probiotic species, host fish species, host physiological status, rearing conditions and the specific goal of feeding application that is, is it for maintaining good health statue, disease resistance or nutrition enhancement (Merrifield et al., 2010c).

Probiotics and production efficiency in fish

The use of probiotics may be useful to prevent diseases and reduce the use of antimicrobial compounds in aquaculture (Ringo, 2004). Ingestion of specific strains of lactobacilli by farmed fish may have an impact on their immunity which can be a way of reducing pathogenic infections in farmed salmon and to improve health in marine fish (Bucio Galindo et al., 2009). There may how-ever be specific strain effects. The application in salmo-nids does resulted in improved health status, improved disease resistance, growth performance, feed utilisation, carcass composition, gastric morphology, reduced mal-formations, gastro intestine colonisation and subsequent microbial modulation (Nayak et al., 2007; Merrifield et al., 2010c). Probiotics in aquaculture could prove effective at improving broodstock performance, growth performance, immunostimulation, increase resistance to disease (Merrifield et al., 2010b) and also increase survival of larvae for some days after hatching (Ringo, 2004). A commercial preparation of *Enterococcus faecium* improves growth and efficiency of Israel carp and sheat fish (Ringo, 2004). Administration of *Lactobacillus fructivorans*, isolated from Seam bream (*Sparus aurata*) gut and *L. plantarum*, isolated from human faeces influ-enced gut colonisation and significantly decreased larvae and fry mortality and gave improved tolerance to acute pH stress (from 8.6 to 6.3) in seam bream (Rollo, et al, 2006). It does this by inducing higher HSP70 gene expression which is an indication of greater potentiality to respond to the harmful conditions possibly present in fish farms. Carp fed combined strains of lactic acid bacteria preparation (*Streptococcus faecium* M74, *E. faecium* PDFM and *E. faecium* SF68) showed reduced *E. coli* in the microbiota after 14 days and complete elimination of *Clostridium* species in another experiment (Ringo, 2004). Lactic acid bacteria supplementation in the diet can also induce greater levels of immunoglobulin in rainbow trout. Immunoglobulin confers protection against diseases in animals and humans (Tukmechi et al., 2007). In a study carried out by Panigrahi et al. (2010), alteration in the blood profile was reported with an increase in plasma cholesterol, triglyceride and alkaline phosphatase activity level after feeding with probiotic strains. The increase in alkaline phosphate activity level is an indicator of good fish health condition (Tukmechi et al., 2007). There were alterations of metabolism and/or by the stimulation of host immunity. The strain used for this study enhanced fish growth, feed and nutrients utilization, fish chemical composition and muscular structure, besides fish resistance for pathogenic bacteria (Panigrahi et al., 2010). Another example where a probiotic strain was use to enhance the fish growth and health is in a research paper by Abd El-rhman et al. (2009). *Micrococcus lutens* was found to exert an inhibitory effect against *Vibrio vulnificus* and *Aeromonas hydrophila*. Higher growth performance, survival rate and feed utilization were also noticed with diet supplemented with the strain. They suggested that it may be due to improved intestinal microbial balance and reduced pathogenic flora which accelerates food absorption. The strain may also enhance the non-specific immune parameters such as the lysozyme activity, migration of neutrophilis and plasma bactericidal activity resulting in the improvement of fish resistance against infection. The use of combined strain of *B. subtilis* and *B. licheniformis* was also found to give some benefits. In their paper Merrifield et al. (2010b) showed that fish fed these com-bined strains had a significant improvement of feed con-version ratio (FCR), specific growth rate and protein efficiency ratio; this may be due to improvement in digestibility (El-Haroun et al., 2006; Dhanaraj et al., 2010). High levels of the strain survived transit through the gastro intestinal tract and are present in the posterior intestine where benefits such as aiding digestive function and enhancing the microbial defensive barrier mechanism may occur. The reduction of fish mortality was reported for rainbow trout by Vendrell et al. (2008). The probioticstrains*L.mesenteroides*CLFP196and*L.plantarum*

CLFP 238 was administered orally to rainbow trout for 30 days at 10^7CFU g^{-1} feed reduced mortality significantly from 78% in the control group to 46-54% in the probiotic group when challenged with *Lactococcus garvieae*, a zoonotic agent that causes serious economic losses in cultured marine and fresh water fish species especially in summer. This effect may be due to the manipulation of the intestinal microbiota which is a valuable mechanism to increase fish growth and survival rates. The same author (Vendrell et al., 2008), also showed that the use of *Lactobacillus rhamnosus* (strain ATCC 53103) supple-mented to rainbow trout for 51 days reduced the fish mortality caused by *A. salmonicida* from 52.6% in the control to 18.9 and 46.3% in the 10^9 CFU g^{-1} feed and 10^{12} CFU g^{-1} feed groups, respectively. It has been de-monstrated in the same fish species that supplementation of *Carnobacterium maltaromaticum* (strain B26) or *C. divergens* (strain B33) for 14 days at 10^7 CFU g^{-1} feed conferred protection after challenge with *A. salmonicida* and *Y. ruckeri*. In terms of cost benefit, improvement was recorded in the use of probiotic strain. El-Haroun et al. (2006), recorded an increase in production performance and subsequent cost benefit analyses in terms of net return and low total cost on Nile tilapia production experiment. The fish fed control diet and another fed with diet containing combination of probiotic strain of *B. licheniformis* and *B. subtilis* were worked with. They stated that according to market price in 2006, the use of pro-biotics gives higher total net return on fish production. An increase from 0.5 to 2% compare to the control. In ano-ther work with brewers yeast, *S. cerevisiae* (use as a supplement in animal feed to compensate for amino acid and vitamin deficiencies) in combination with *Lactobacillus acidophilus*, included in a diet fed to koi carp (*Cyprinus carpio*), change in growth was noted. The fish grew signi-ficantly faster than those fed on a control feed (indicating an improved growth performance) with an increase in microbial load in the gut (Dhanaraj et al., 2010). The inclusion of life yeast strain of *Debaryomyces hansenii* was found to stimulate the immune system of juvenile leopard grouper *Mycteroperca rosacea* (Yun-Zhang Sun et al., 2010). Also the use of combination of putative strains of *A. sobria* and *Brochothrix thermosphacta* is able to prevent fin rot cause by *Aeromonas bestiarum* and *Ichthyophthirius multifiliis,* a protozoan pathogen in rainbow trout (Pieters et al., 2008). The use of *Shewanella* species, a strain found in the microbiota in *Senegalese sole* and cultured in natural environment as a probiotic strain and added as a diet supplement up to 10^9 CFU g^{-1} showed a protection against *Photobacterium damselae* subspecies *piscida*. The fish also showed a better growth, higher total protein in muscle with signi-ficant differences in muscles and liver fatty acid compo-sition (De la Banda et al., 2010). The beneficial effects of probiotics were also found in fish reared in the tropics. The use of *Lactobacillus acidophilus* fed to *Clarias gariepinus* (African catfish) fingerling with a diet containing up to 35g/kg protein and 10g/kg Lipids for 12 weeks shows improved growth. The specific growth rate, relative growth rate, protein efficiency ratio, feed conversion ratio and survival were significantly ($P<0.05$) high. The haema-tology parameters (packed cell volume, haemoglobin, erythrocyte sedimentation rate, red blood cell and white blood cell, total serum protein, Ca^{2+} ,Mg^{2+}, Cl^-, glucose and cholesterol) and total immunoglobulin concentrates were also significantly better in fish fed the probiotic supplemented diet than in control. This may be due to the immune response in the probiotic group (Al-Dohail et al., 2009; Nayak et al., 2007), as it is suggested that probiotic bacteria could stimulate immunoglobulin production in fish thereby increasing disease resistance (Yun-Zhang Sun et al., 2010). Their is a general consensus that pro-biotics from autochthonous source have a greater chance of competing with resident microbes and of becoming predominant within a short period of intake, which can assist in returning a disturbed microbiota to its normal beneficial composition and therefore enhanced the disease resistance of host. An example is a significantly decreased larvae and fry mortality by using *Lactobacillus fructivorans* isolated from gut of adult Sea bream (*S. aurata*) (Yun-Zhang Sun et al., 2010).

Challenges

Total replacement of indigenous populations with probio-tics may not be desirable with regards to improving growth performance. As a probiotic presence within the digestive tract may modulate the complex microbial com-munities to a more functional population which is more effective than the complete replacement of the indige-nous microbiota by the probiont. Another challenge is variability of experimental results with probiotics supple-mentation test found to be highly complicated and not always reproducible (Merrifield et al., 2010b). This may be due to the composition of microflora associated with the gastro-intestinal tract which is highly variable de-pending on the species and its development stage.

Inhibition in feed processing

Heating is the major stress which may affect probiotic micro-organism during manufacturing of pelleted feed (Angelis et al., 2006). It is known that many processes in feed manufacturing uses heat and this can cause chan-ges in the chemical components of raw materials. Pelleting requires 70-80°C or more while extrusion pro-cess goes as high as from 95°C even sometimes to 130°C (Svihus and Zimonja, 2008). Most probiotic strains cannot survive at this temperature range. Research carried out by Simon et al. (2007) showed that *Bacillus species* which are spore formers, are much more stable during feed processing (including pelleting and in feed storage). They found out that the recovery of *Bacillus* *cereus var. toyoi* was 95% after pelleting (conditioner 80◦C, dye 87◦C), while the recovery of viable counts of an *E. faecium* strain decreased with increasing treatment temperature. Stability of vegetative cells can also be improved by various techniques (such as soaking on globuli and coating). They suggested that viability losses can be compensated by initial overdosing during feed production if the rate of inactivation is known. But a challenge pose in the use of spore forming bacilli might be that being an alien in the gut, the local immune system of the animal may treat it as a potentially pathogenic threat. In a published paper by Angelis et al. (2006), the survival of some particular strains during processing was also looked carefully into and a promising result was also obtained. Species such as *Lactobacillus reuteri, Lactobacillus mucosa, Lactobacillus plantarum, Lactobacillus kitasatonis, Lactobacillus rossiae, Lactobacillus ultunensis, Lactobacillus crispatus* and *Lactobacillus intestinalis* were selected based on their ability to resist heat treatments (approximately 70°C for 10 s), acid and bile salt resistance and antibacterial activity. They were freeze-dried and mixed (1% w/w) into pig feed before pelleting. After pelleting, pig feed contained 10^{10}-10^{9} CFU kg^{-1} of lactobacilli. The mixture was pelleted at 60°C for 40 s. No decrease in cell survival was observed within a 15 days period of pelleted feed stored at room temperature for any of the strains. New technologies have been developed in other to introduce strains into feed during processing. For example micro-encapsulation of probiotic bacteria (strain is protected against adverse conditions) which can even resist temperatures up to 90°C is available; however the technology may not be sufficient to protect live bacteria during extrusion (Merrifield et al., 2010c). Post pelleting spraying in oil or water is the best option for now. However, these can increase the cost of production as it may require considerable investment in coating technology and thus increase cost of feed. Many farmers may not be willing to pay the extra cost. Another challenge is survivability problems of the organism after ingestion. The use of prebiotics may help in this way. They induce a specific colonic fermentation by potentially health promoting indigenous bacteria such as Lactic acid producing flora (Tzortzis et al., 2005). Some of these can use natural feed additives such as inulin, sugar beet pulp and wheat starch, which can significantly affect microbiota composition and functionality. For example, a high dietary lactose favoured *Bifidobacteria* and *Lactobacilli* while decreasing *E. coli* (Lalles et al., 2007). Also it should also be mention that their is no guarantee that the use of oligosaccharide as a prebiotic will favour the growth of beneficial species in a complex microflora such as found in pigs intestine (Macdonald et al., 2002). Another major stumbling block these days are stringent rules which potential probiotic strain are being made to satisfy in the European Union (Merrifield, et al. 2010c). These may or already have started to hinder the discovery or the use of potential species as it takes several years before it can be approve.

EU regulation concerning administration

The EU regulations concerning probiotics goes back to 1970 with directives no. 70/524/EEC which was amended five times (Anadon et al., 2006). The directives were replaced with new regulation (EC) no. 1831/2003 of the European parliament and of the council of 22 September 2003 on additives for use in animal nutrition (OJ NO. L 268, 18.10.2003). The regulation set out the rule for its authorization, use, minority, labelling and packaging. In the regulation (EC) no. 1831/2003, the micro-organisms are included in the category "Zootechnical additives" and as functional group within the "gut flora stabilisers" defined as micro-organism or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora. Requirement for the assessment of microbial feed additives include:

1. Identity, characterisation and conditions of use, method of control; identity of the additive, characterisation of the active agents, characterisation of the additives; physico-chemical and technological properties, conditions of use of the additives and control methods.
2. Efficacy, studies on efficacy of probiotics strains must be performed in target species and animal categories. The demonstration for the microbial advantage claim should be based on a minimum of three trials demonstrating a statistically significance ($p<0.05$) on the specific animal categories.

Safety under condition for use

Regulation states that detailed safety assessment should be carried out on:

Studies on target species

For each animal category, a target specie tolerance testing shall be designed to determine a safety margin. This is done to evaluate for the animals the risk of an accidental overdosing originated during feed production. Studies on the effect of the microbial additive on the microflora of the digestive tract are also required when a claim is made concerning an effect on the intestinal microflora

Consumer safety assessment

Certain toxicological test (genotoxicity studies and oral toxicity test) are required to exclude the possibility that when probiotic product are accumulated in the animal it will not form a consumer risk. Safety of the workers formulating the product should also be addressed. Also due to the impact of the microbial additive on the environment, an environmental risk assessment is also required in some cases (Anadon et al., 2006).

Future perspectives

Attempts to discover more efficient strains are on course. Especially strains that will be able to attach to gut epithelial cells, heat stabile strains and growth rate in the intestine (Fuller, 1989). The use of *Bifidobacterium specie* and *Lactobacillus specie* as probiotics in fish might be a further future development if technological solutions such as non-thermal feed processing can be developed, which overcome their instability in common feed processing techniques (Simon et al., 2007). The use of combination of strain isolated (originating) from GIT of the host animal (for example, fish) should be further developed as some of them are thought to improve the colonization resistance of the host because they are well adapted to the GIT and should be more competitive than bacteria from other sources (Klose et al., 2010; Merrifield et al., 2010c). These can decrease incidence of aberra-tions in intestinal microbiota for example, diarrhoea (Lalles et al., 2007; Collado et al., 2007). The complexity of the intestine which may lead to variations between animals is also an issue, making probiotic strains to have different effects upon individual animals (Angelis et al., 2006). The provision of probiotic strain with technological traits suitable for direct inclusion in pelleted feeding may enhance microbial survival during GIT transit and may offer a series of industrial advantages. Combining probiotics and prebiotics in what has been called a synbiotic could beneficially affect the host by improving survival and implantation of live microbial dietary supplements in the gastrointestinal flora, by selectively stimulating the growth or activating the catabolism of one or a limited number of health-promoting bacteria in the intestinal tract, and by improving the gastrointestinal tract's micro-bial balance, but the creation of a synbiotic has to be investigated. Combining probiotics with prebiotics could improve the survival of the bacteria crossing the upper part of the gastrointestinal tract, thus enhancing their effects in the large bowel. Moreover, probiotic and prebiotic effects might be additive or even synergistic. This has been the case as stated in a by Roberfroid (2000). Optimization of process technology will also play a crucial role in the near future as we look forward on how best to apply both strains of probiotics and samples prebiotic into feed and they are still going to be effective even after rigorous heat processing. It has also being mention that the future studies should consider its effects on immune responses, gut microbiota and challenge studies as more molecular methods is being developed and should be included (Merrifield et al., 2010c). Further studies should include molecular approaches to analyse bacteria communities as for endothermic animals and due to conflict report, further tests should be done to clarify if lactic acid bacteria have a positive effect on fish welfare (Ringo, 2004). Investigation should also be done to see the interactions between probiotics and pathogens in the digestive tract of fish and also important endogenous reaction and the interactions by isolating gut cells of probiotic fed salmonids and assessing the expression of immune activatory or immuno-regulatory cytokines, pattern recognition receptors and anti-microbial proteins. Studies should also include experiments to truly assess the full potential of candidate probiont. It should also include feeding duration with the probiotic prior to the challenge; these should be considered to be prolong (Merrifield, et al, 2010c).

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