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USE OF PROBIOTICS FOR SUSTAINABLE AQUACULTURE PRODUCTION IN NIGERIA

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ABSTRACT
Aquaculture is fast developing in Nigeria but to ensure a sustainable development there is need to address problem of diseases which is an important issue affecting the aquaculture production. Though the use of antimicrobial drugs has helped in some ways, the notorious effects of antibiotics has necessitated seeking an alternative that is environmental friendly and safe for the organisms and consumers. Probiotics has been established to be a good alternative and its use is now gaining acceptance. This review aims to define the concept of probiotics, highlights the process of isolation and methods of application as well as its current status, challenges and prospects in Nigeria. Probiotics are entire or components of microorganisms that are beneficial to the health of a host. They are naturally present in the organism and or the culture medium and have different mechanisms of action. They are usually isolated from the gill, skin or culture medium and pass through isolation processes to obtain the desired strains and applied in-vitro or in-vivo. Probiotics is a natural ingredient in finfish, shellfish and culture environment and its appropriate application will save Nigeria aquaculture from losses due to diseases. It will make available, aquaculture products that are safe for consumption as well ensuring a healthy aquatic environment. However research should be conducted to make available, products that suit the local species and environment in commercial forms. Also, safety issues should be considered at all time.

Key words: sustainable production**,** fish culture**,** probiotics, antibiotics, diseases.

INTRODUCTION
Aquaculture is the fastest growing food-producing sector in the world, with an average annual growth rate of 8.9% since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (Subasinghe, 2005). World aquaculture has grown tremendously during the last fifty years from a production of less than a million tonne in the early 1950's to 63.6 million tonnes by 2011, this level of production had a value of US$ 126.775 billion (FAO, 2012). Although aquaculture activity in Nigeria started about 50 years ago (Olagunju *et al*., 2007), aquaculture production in Nigeria is currently about 200,535 metric tonnes contributing about 24% of domestic fish production (FAO, 2012), this is a large increment considering the status as at 2006 where aquaculture production in Nigeria was about 40,000 metric tonnes contributing only 6% of domestic fish production (Adeogun *et al*., 2007). Nigerians are high fish consumers and offer the largest market for fisheries production in Africa. According to Atanda (2012), Nigeria requires about 2.66 million metric tons of fish annually to satisfy the dietary requirement of its citizens (160 Million) whereas the total

aggregate domestic fish supply from all sources (capture and culture fisheries) is less than 0.7 million metric tons per annum therefore Nigeria has to import about 0.7 million metric tons of fish valued at about $500 million annually to augment the shortfall while in 2009 specifically, about N97Billion was spent importing fish into Nigeria.

Fish production from captured fisheries in Nigeria has been erratic and on the decline in recent years (FDF, 2007). Therefore to solve the high demand for fish, aquaculture production remains the best option to bridge the wide gap between fish demand and domestic production. According to Qi *et al*., (2009), fish disease is more prevalent under intensive management, the crowded condition of large population of fish would result in heavy parasitic infection, disease and loss of fish. A global estimate of losses in aquaculture to diseases as reported by the World Bank in 1997 was in the range of USD 3million per annum. Disease is now a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries (Bondad-Reantaso *et al*., 2005). For decades, antibiotics routinely used for treatment of human infections were also used for aquatic animals, for therapy, prophylactic reasons or growth promotion. However, the adverse effects associated with the use of antibiotics in aquaculture are notorious. The development and spread of antimicrobial resistant human pathogens (motile *Aeromonas spp., Edwarsiella. tarda, Escherichia coli, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae* etc.) were well documented (WHO, 1999); aquatic bacteria can also develop resistance genes as a consequence of exposure to antimicrobial agents (Smith *et al*., 1994; Kim *et al*., 2004; Sørum, 2006). Also, the occurrence of antimicrobial residues in aquaculture products pose threat to human health (WHO, 2006).Thus, the indiscriminate use of antibiotics for fish has increasingly become a matter of public concern in some countries of the world and a legal framework is being enforced (Kesarcodi-Watson *et al.* 2008). At present, there is an urgent need to discover new alternatives or approaches for the abuse of antibiotics. The use of probiotics, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment (Verschuere *et al*., 2000). This paper is aiming at discussing the concept of probiotics in aquaculture, highlights the process of isolation and methods of application as well as to enumerate some prospects and challenges faced in the use of probiotics in Nigeria.

The term, probiotic, simply means "for life", originating from the Greek words "pro" and "bios" (Gismondo *et al*., 1999). The most widely quoted definition was by Fuller (1989), who defined a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". This definition by Fuller (1989) is more accurate for terrestrial organism compared to aquatic organism, when looking at probiotics intended for an aquatic usage it is important to consider certain influencing factors that are fundamentally different from terrestrial based probiotics. Aquatic animals have a much closer relationship with their external environment. Potential pathogens are able to maintain themselves in the external environment of the animal (water) and proliferate independently of the host animal (Hansen and Olafsen, 1999; Verschuere *et al*., 2000). These potential pathogens are taken up constantly by the animal through the processes of osmoregulation and feeding. Based on the intricate relationship an aquatic organism has with the external environment when compared with that of terrestrial animals, the definition of a probiotic for aquatic environments needs to be modified. Verschuere *et al*. (2000) suggested the definition "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response

towards disease, or by improving the quality of its ambient environment". Apart from the requirement of the probiotic to be a live culture, this definition is a lengthy way of describing a probiotic as defined by Irianto and Austin (2002) thus "a probiotic is an entire or components(s) of a microorganism that is beneficial to the health of the host". The latter definition is in accordance with that given by Salminen *et al*., (1999). Some organisms used as probiotics in aquaculture include; *Bacillus cereus var. toyoi, Bacillus licheniformis, Bacillus subtilis, Enterococcus faecium, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnosus, Pediococcus acidilactici, Saccharomyces cerevisiae* and *Streptococcus infantarius* .

Mechanisms of actions of Probiotics in Aquaculture

Enhancement of colonization resistance and/or direct inhibitory effects against pathogens are important factors where probiotics have reduced the incidence and duration of diseases. Probiotics strains have been shown to inhibit pathogenic bacteria both in vitro and in vivo through several different mechanisms (Balcazer *et al*., 2006). The methodological and ethical limitations of animal studies make it difficult to understand the mechanisms of action of probiotics, and only partial explanations are available.

Nevertheless, some possible benefits linked to the administering of probiotics have already been suggested as:

i. Competitive exclusion of pathogenic bacteria.
ii. Source of nutrients and enzymatic contribution to digestion.
iii. Direct uptake of dissolved organic material mediated by the bacteria.
iv. Enhancement of the immune response against pathogenic microorganisms.
v. Antiviral effects

Competitive Exclusion

Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms. However, the composition of microbial communities can be altered by husbandry practices and environmental conditions that stimulate the proliferation of selected bacterial species. It is well known that the microbiota in the gastrointestinal tract of aquatic animals can be modified, for example by ingestion of other microorganisms; therefore, microbial manipulation constitutes a viable tool to reduce or eliminate the incidence of opportunist pathogens (Balca´zar, 2002).

Source of nutrients and enzymatic contribution to digestion

Some researchers have suggested that microorganisms have a beneficial effect in the digestive processes of aquatic animals. In fish, it has been reported that *Bacteroides sp* and *Clostridium sp.* have contributed to the host's nutrition, especially by supplying fatty acids and vitamins (Sakata, 1990). In addition, some bacteria may participate in the digestion processes of bivalves by producing extracellular enzymes, such as proteases, lipases, as well as providing necessary growth factors (Prieur *et al*., 1990). Similar observations have been reported for the microbial flora of adult penaeid shrimp (*Penaeus chinensis*), where a complement of enzymes for digestion and synthesized compounds are assimilated by the animal (Wang *et al*., 2000).

Positive Influence on water quality

Improved water quality has especially been associated with *Bacillus sp*. The rationale is that gram positive bacteria are better converters of organic matter back to CO_2 than gram-negative bacteria. During the production cycle, high levels of gram-positive bacteria can minimize the build-up of dissolved and particulate organic carbon. It has been reported that the use of *Bacillus sp*. improved water quality, survival and growth rates and increased the health status of juvenile *Penaeus monodon* and reduced the pathogenic vibrios (Dalmin *et al*., 2001).

Enhancement of the immune response

The non-specific immune system can be stimulated by probiotics. Rengpipat *et al*., (2000) reported the use of *Bacillus sp.* (strain S11) provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*P. monodon*). Balca´zar, (2003) demonstrated that the administration of a mixture of bacterial strains (*Bacillus sp* and *Vibrio spp*.) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *Vibrio harveyi* and white spot syndrome virus. This protection was due to a stimulation of the immune system, by increasing phagocytosis and antibacterial activity.

Antiviral effects

Some bacteria used as candidate probiotics have antiviral effects. Although the exact mechanism by which these bacteria do this is not known, laboratory tests indicate that the inactivation of viruses can occur by chemical and biological substances, such as extracts from marine algae and extracellular agents of bacteria. It has been reported that strains of *Pseudomonas sp*., *Vibrios spp*., *Aeromonas spp*., and groups of coryneforms isolated from salmonid hatcheries, showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) with more than 50% plaque reduction (Kamei *et al*., 1988). Girones *et al*. (1989) reported that a marine bacterium, tentatively classified in the genus Moraxella, showed antiviral capacity, with high specificity for poliovirus.

CRITERIA FOR SELECTION OF PROBIOTICS

It has been widely published that a probiotic must possess certain properties (Verschuere *et al*., 2000). These properties were proposed in order to aid in correct establishment of new, effective and safe products. According to Merrifield *et al*. (2010), the properties include:

- Must not be pathogenic to both the host species and general aquatic organisms.
- Must be resistant to bile salts.
- Should be able to adhere to and/or grow well in the Intestinal mucosa.
- Should display advantageous growth characteristics.
- Should exhibit antagonistic properties towards one or more key pathogens.
- Should remain viable under normal storage conditions and robust enough to survive feed production processes.

The selection of probiotics is illustrated in figure 1. It is commenced with screening of health animals during outbreak, whereby the more healthy animals are screened out. This is followed by isolation of microbial strains from the animal whether from the gill, skin and or culture medium. The isolated strains can be tested in the diseased organism either in-vitro or in-vivo. If the strain is able to colonize the system and overpower the pathogen, the strains can then be tested for its

economic viability and put through the regulating body for registration before making available to the public as a commercial product.

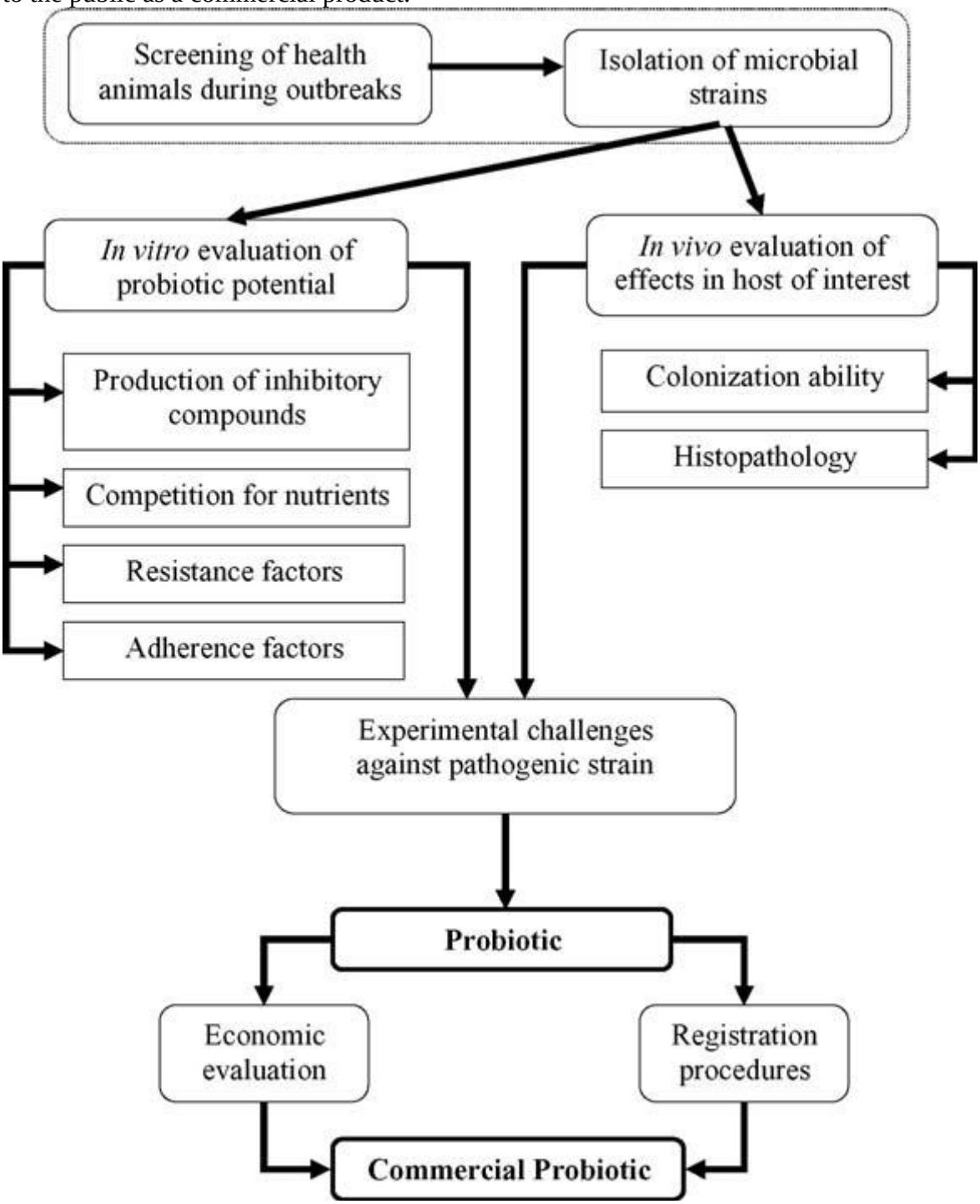

Figure 1: Diagram for selection of probiotics as biocontrol agents in aquaculture
Source: Balca'zar *et al*., (2006)

ISOLATION OF PROBIOTICS

Probiotics are generally organisms found on the aquatic animals and their ambient environment which is the culture medium. The probiotics can be isolated from the gill, the skin and the culture medium.

Process of Isolation

A clean cotton wool can be rubbed over the skin; the gill or little amount of water can be taken from the culture medium. The cotton wool is rinsed into petri dish and diluted before observed, characterized, Identified and counted under microscope. Little amount of the organisms is inoculated in a nutrient agar medium. This agar medium depend on the species of probiotics intended to be cultured because different organisms grows in different culture medium, for instance Man Ragosa and Sharp (MRS) medium is used for *Lactobacillus sp*, while Yeast Extract Agar (YEA) is used for *Saccharomyces cerevisiae*. The culture species is left under favourable condition and is left for a number of days or hours as required for the growth of the cultured organism after which a predetermined quantity of the cultured organisms can be applied to the fish in vivo or in vitro.

Applications of Probiotics in aquaculture

Probiotics can be provided to the host or added to its aquatic environment in several ways: Application directly to host is called in vivo while application to the culture environment is called in-vitro. Mayer (2012) listed the means of application as: addition via live food, bathing, addition to culture water or addition to any commercial diet. Addition via live food or to commercial diet can be categorized under the major heading in-vivo, while bathing and addition to culture water can be categorized under in vitro. All these methods had been used in various researches and positive results were achieved. Table 1, illustrates some probiotics that has been used in aquaculture and the method of application.

Table 1: Some tested Probiotics and the test method

Fish	Probiotics	Pathogen	Test method
Atlantic cod	*Carnobacterium divergens*	*Vibrio anguillarum*	In-vitro and In-vivo
Atlantic salmon	*Lactobacillus plantarum*	*Aeromonas salmonicida*	In-vitro and In-vivo
India carp	*Bacillus subtilis*	*Aeromonas hydrophila*	In-vivo
Nile Tilapia	*L.accidophilus* and *Saccaramyces cerevisiae*	Growth study	In-vivo
Gilthead sea bream	*Micrococcus sp*	*L. anguillarium*	In-vitro
Shrimp	*Pseudomonas aeroginasa*	*V.fluvialis, V.harveyi*	In-vitro
Rainbow trout	*L. rhamnosus*	Immune enhancement	In-vitro
Rohu rohu	*B. circulans, B. subtilis*	Digestive enzyme study	In-vivo
Silver perch	*A. media*	*Saprolegnia sp*	In-vivo
Tilapia	Commercial product	*Edwardsiella tarda*	In-vivo
F.W. prawns	*Lactobacillus spp*.	Gram negative bacteria	In-vivo
Turbot	Marine bacteria	Natural survival study	In-vivo

Source: Adapted from Kesarcodi-Watson *et al.* (2008).

STATUS OF PROBIOTICS USED IN AQUACULTURE IN NIGERIA

Generally the application of probiotics to aquaculture in Nigeria is still at its infancy, most information available on it are still experimental and yet to be used in commercial aquaculture. Ogunshe and Olabode, (2009) evaluated the ability of *Lactobacillus fermentum* LbFF4 isolated from Nigerian fermented food (fufu) and *L. plantarum* LbOGI from a beverage (Ogi) to induce immunity in *Clarias gariepinus* (Burchell) against some selected fish bacterial pathogens. While Folorunsho (2012) isolated four species of Lactobacillus from the skin and gill of African catfish (*Clarias gariepinus)* and Nile Tilapia (*Oreochromis niloticus*). The isolated species were *L. fermentum, L. acidophilus, L. xylosus*, and *L. brevis. L. fermentum* was found to be the dominating species and it was cultured using Man Ragosa and Sharp (MRS) as the agar media. The *L. fermentum* was administered as additive into the fish diet (0.25% inclusion) at 10^3 cfu/mL to 10^9 cfu/mL. The *L. fermentum* was able to promote growth and serves as antibacterial against *Pseudomonas aeruginosa* in the fish cultured.

Challenges in the Use of Probiotics in Nigeria

Challenges being faced in the use of probiotics are not in Nigeria alone except that it may be more intense in Nigeria where there is little information on the probiotics because in developed countries where a lot of research has been carried out, there are still issues on the use of probiotics. The information is limited and sometimes contrasting. Due to these uncertain and incomplete results, there is no standardized protocol to test the beneficial effects of these products and their impact on farmed fish welfare, growth and health status (Mayer (2012)). According to Moriarty *et al*. (2005), ineffective products that are sold as probiotics have caused farmers to question the probiotics concept, for example products for crustaceans containing *Lactobacillus sp* that were produced from human or land animals are not appropriate for shrimp where this bacteria are not occurring naturally. Some products in Asia have labels indicating *Clostridium sp*, *Pseudomona putida*, *P. aeruginosa*, *Enterococcus faecium* and *E. faecalis* which are human and or fish pathogens.

In Nigeria the challenges can be listed as:

- Inadequate specificity of which probiotics should be used and for what purpose
- Inadequate research
- Possible introduction of exotic microbes with future hazards
- Insufficient data on the use of Probiotics in Nigeria.

Prospects in the use of Probiotics in Nigeria

The use of probiotics in Nigeria will come with a lot of benefit for individuals, society, organization and Institutions, these among others includes:

- Creation of rooms for research, this is very essential as Nigeria scientist will have to be engaged to screen for novel strain of probiotics that will suit her own aquaculture.
- Job creation for a teeming population of unemployed youth, this can be directly or indirectly. Directly, government could establish a body or unit that will see to labeling and licensing of commercial products in order to ensure quality control in the use of probiotics as it gains more popularity in aquaculture. While indirectly, it creates jobs because of its potential boost of aquaculture activity and encouragement of more investors, thereby employing more people to manage farm and participate in the other value chain addition such as processing, marketing etc.

- Increase the aquaculture production of the country and hence gross domestic product.

Prospects for probiotics in Nigeria

Since aquaculture remains the best option to bridge the gap between fish demand and domestic production in Nigeria (FDF 2007), the incidence of fish disease and the corresponding health issue that can follow antibiotic treatment will give the use of probiotics a great prominence. Probiotics will be well accepted and promoted considering the fact that Nigeria needs to bridge the gap between fish demand and supply, the needs for production of fast growing, disease free fish that are safe for human consumption as well as the large expanse of land and water body which will continue to encourage more investor in fish farming business.

CONCLUSION AND RECOMMENDATIONS

Sustainable aquaculture production in Nigeria can be enhanced through the use of probiotics which is a natural ingredient in finfish, shellfish and the culture medium. The use of probiotics will not only save aquaculture production from losses due to diseases but will also promote fast growth of cultured species, ensuring healthy environment and save man from consumption of unhealthy antibiotics treated fish. No doubt that probiotics will find a good place in the developing aquaculture sector in Nigeria, it is therefore important to conduct more research to ensure efficient and appropriate use of probiotics, it is also necessary for the researchers to come up with products that suits our own local species and culture environment in other to save our aquaculture production from dependency on foreign products which tends to be more expensive and may not even be suitable for our local aquaculture system. However all efforts should be put in place to ascertain the quality of the probiotics produced for the safety of both the cultured organisms and final consumers.

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