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Effects of Selected Probiotics on the Growth and Survival of Fry – Fingerlings of Clarias Gariepinus

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Abstract: *The effects of selected probiotic candidates - Lactobacillus plantarum, Pseudomonas flourescens and Saccharomyces cerevisiae - on the growth and survival of the fry and fingerlings of the catfish Clarias gariepinus was investigated. The specific growth rate and percentage survival of the fry and fingerlings exposed to the probiotic candidates were evaluated. The fingerlings showed significant improvement in specific growth rate (P<0.05) in all the treatments except in the treatment with Saccharomyces cerevisiae. Percentage survival was highest in the treatment with Pseudomonas and Lactobacillus (96.22%) and least in the treatment with Saccharomyces cerevisiae (42%). There were no significant improvements in the water quality parameters in the treatment groups when compared with the control. This study therefore shows that Lactobacillus plantarum and Pseudomonas fluorescens can serve as good probiotic candidates in the control of bacterial and fungal diseases of the fry and fingerlings of Clarias gariepinus.*
Key Words: *Clarias gariepinus, Fingerlings, Fry, Microbial control, Probiotics.*

I. Introduction

The African catfish, Clarias gariepinus is a scaleless slimy fish, darkly pigmented in the dorsal and lateral parts of the body. There are 24 species of Clarias in Nigeria among which are Clarias gariepinus, C. lazara, C. macromystes, C. agboyiensis, C. buthupogon (Idodo-Umeh, 2003). Clarias gariepinus has been singled out as the best for culture in Nigeria. Clarias lazara was favoured in Central Africa where it was first cultured intensively by Dutch Scientists working in Bangui, Central African Republic in the late 1970s. Clarias gariepinus is a preferred species due to several biological characteristics. A number of other Clarias species do not have the propensity for as much growth as Clarias gariepinus. Many fish farmers have purchased catfish fingerlings from the wild and found that they consumed large quantities of food and yet only grew to a size of 300-400 grams and 30cm in length (Potongkam and Miller, 2006).

Clarias species are commonly found in swampy waters or slow moving streams and reproduce during the rainy season. They are equipped with an accessory breathing organ that enables them to live out of water for some time. This makes them very hardy; proving, therefore, to be a fish adapted to African consumers (who lack refrigerators). In markets, the fish are held in shallow tubs, with water for days (Potongkam and Miller, 2006). During the culture of the fry and fingerling of the catfish, they are usually affected by various diseases which make the overall yield much less than expected. The production of catfish fry is a relatively simple process. However, success in many hatcheries is poor year after year. Problems usually include poor fry growth, low fry survival and high incidence of infectious diseases of eggs and fry. Environmental conditions prevailing in the aquatic environment such as temperature, dissolved oxygen, nitrate, ammonia, biochemical oxygen demand, pH, water hardness, water quality and quantity, whether high or low also have roles to play in the incidence of diseases (Tucker, 1991).

Conventional approaches, such as the use of disinfectants and antimicrobial drugs have had limited success in the prevention or cure of these diseases. Resistant strains of bacteria can transfer their resistance genes to other bacteria that have never been exposed to the antibiotic. The prophylactic use of antibiotics related to those used in human medicine or the use of any antimicrobial agent known to select for cross-resistance to antimicrobials used in human medicine could therefore, pose a particularly significant hazard to human health (Witte, et al., 1999). Furthermore, there is growing concern for the use of unapproved chemicals in aquaculture practices which could pose serious health hazards to the consumers of such products (Subasinghe, 1997).

Environmentally friendly alternative to antibiotics which are called probiotics have been developed. Probiotics are live microbial adjunct which have beneficial effect on the host by modifying the host- associated or ambient microbial community.This is achieved either by ensuring improved use of the feed, enhancing its nutritive value by enhancing the host response towards diseases or by improving the quality of its ambient environment (Laurent, 2000). They can be mono-or mixed cultures of live microorganisms. They beneficially improve the growth and development of the normal desirable microbial population in the host, allowing them to maintain domination over the undesirable organisms. The objective of this study was to evaluate the effects of selected probiotics on the growth and survival of fry and fingerlings of Clarias gariepinus.

II. Materials And Methods

Microbiological Analysis

Samples of water from each of the incubated fish tanks and the rearing water of the fry and fingerling were analyzed. The spread plate technique was used for the isolation of bacterial species from the water samples.

Selection of Probiotic Bacteria and Fungi

Pseudomonas fluorescens was obtained from the culture water, it was isolated using Centrimide Agar. Lactobacillus plantarum was obtained from a local beverage, (raw pap) using Man Rogosa Sharpe Agar. Saccharomyces cerevisiae was obtained from palm wine using Glucose Malt Agar. All the potential probiotics were subjected to biochemical tests. This was followed by the standardization of the probiotic for Susceptibility Testing using 1.0 McFarland's Standard. The probiotics were then finally subjected to preliminary screening for probiotic potential based on their antibacterial activity against the fish and egg pathogens. (Chythanya et al., 2002).

Broth cultures of the potential probiotics were prepared in nutrient broth and potato dextrose broth respectively and incubated for 24hours at 37^0c.

Experimental Set-Up

Plastic tanks of 42L capacity were used to culture the Clarias gariepinus fry and fingerlings. These were supplemented with artificial aeration. Three hundred fry were placed in each tank containing 20L water to which the potential probiotics have been applied. The fry were fed three times daily with Artemia for 4 weeks. Waste removal was carried out by partially siphoning water in and out of the tanks daily. Seven experimental groups with probiotics and a control (all in triplicates) were maintained. The control group was free of any probiotic inoculation.

Broth culture of One hundred mls(100mls) of each of the probiotic was directly added to the respective tanks in single and mixed inocula three times a week. A control tank was also set up without the inocula.

Evaluation of the Physicochemical Parameters of the culture water

Water samples were collected from each of the experimental tanks, three times every week, each was analyzed for changes in the water quality - pH, temperature, dissolved oxygen, ammonia and nitrite.

Growth Performance of the Fry and Fingerlings

The fry were weighed at the beginning and at the end of the experiment (as fingerlings) to assess the growth performance in terms of specific growth rate, percentage growth and weight gain.

Specific growth rate (SGR) is calculated using the following formula:

$$\text{SGR} = \frac{L_n\,(Final\,body\,weight) - L_n\,(Initial\,body\,weight)}{30days}\,x100$$

L_n = natural log

Weight gain = final body weight – initial body weight

Evaluation of the survival of the fingerlings at 4 weeks

$$\text{Percentage survival} = \frac{\text{Number of fishes alive at 4 weeks}}{\text{Number of fishes initially stocked}} \text{ x } \frac{100}{1}$$

III. Result

A number of bacterial and fungal species were isolated and identified from the incubated fry and fingerlings' culture water. The bacterial species were Staphylococcus, Escherichia, Streptococcus, Proteus, Enterobacter and Pseudomonas; the fungal species were: Candida, Aspergillus, and Penicillium.

The antibacterial activity of the potential probiotics using the well- in- agar- diffusion method showed that Pseudomonas fluorescens and Lactobacillus plantarum produced the largest zones of inhibition against Streptococcus, Staphylococcus, Enterobacter and Escherichia species. (Fig.1).

The effect of probiotic candidates on the survival of Clarias gariepinus fingerlings after four weeks is shown in

Fig. 2. The results show that Pseudomonas fluorescens played a major role in the growth and survival of the fishes. The percentage survival of the fingerlings at 4weeks was highest for the fishes treated with the combination of Pseudomonas and Lactobacillus plantarum; this was followed by Pseudomonas only and then,

the combination of the three probiotics. (Pseudomonas, Lactobacillus and Saccharomyces). The least survival rates were recorded for the fishes treated with only Saccharomyces cerevisiae.
The effect of probiotic candidates on the specific growth rate of Clarias garienpinus Fingerlings are shown in Figure 3. The specific growth rate of the

Fingerlings were observed to be highest in the tanks treated with the combination of Lactobacillus and Pseudomonas (Fig. 3), this was followed by the tanks Containing only Lactobacillus and then, the combination of Pseudomonas and Saccharomyces.

The Water quality parameters are shown in Figures 4a & 4b. There was no significant difference (P>0.05) in the water contained in all the treatment tanks, for all the parameters tested.

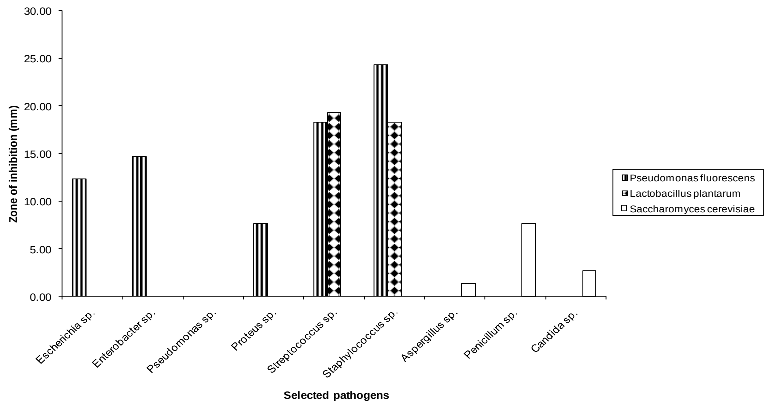
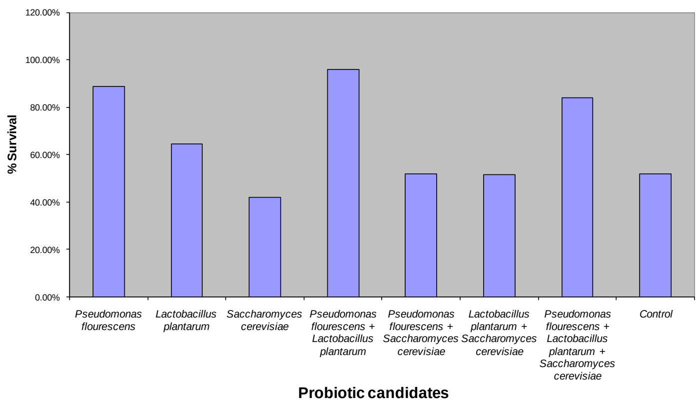


Figure 1: In vitro probiotic candidates - pathogens challenge test



2: Effect of probiotic candidates on survival of Clarias gariepinus fingerlings after four weeks

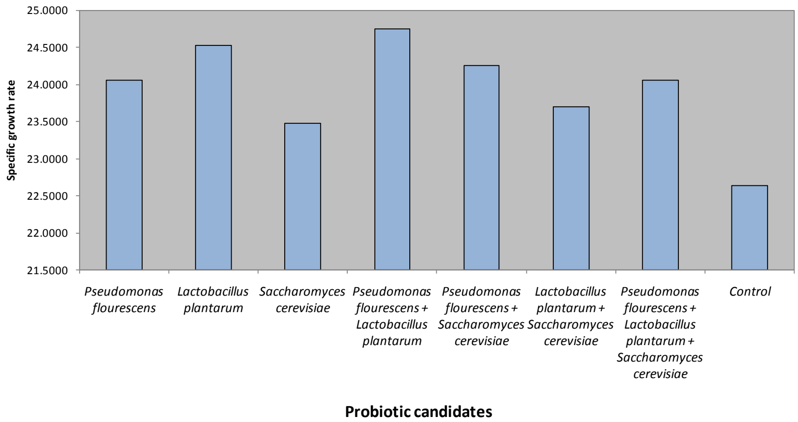


Figure 3: Effect of probiotic candidates on the specific growth rate of Clarias garienpinus fingerlings

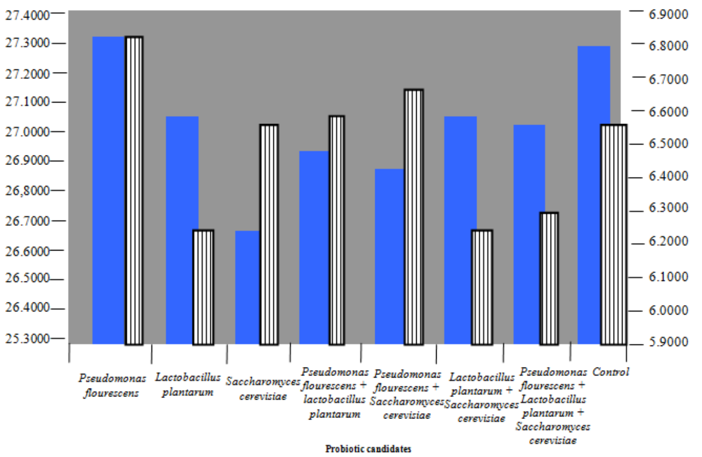


Figure 4a: Changes in temperature and pH values of *Clarias gariepinus* culture system following probiotic candidates application

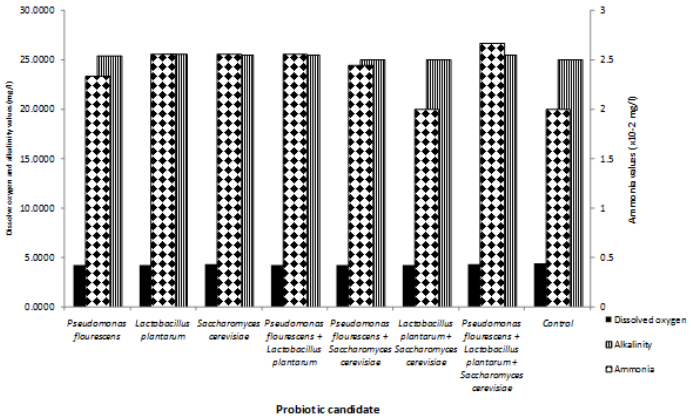


Figure 4b: Changes in dissolved oxygen, alkalinity and ammonia values in the Clarias gariepinus culture system following probiotic candidate application

IV. Discussion

Results of the antibacterial activity of the probiotics against the isolated pathogens revealed that Pseudomonas flourescens and Lactobacillus plantarum had better probiotic potential than Saccharomyces cerevisiae (Fig. 1) Pseudomonas flourescens showed antibacterial activity against all the isolated pathogens except other species of Pseudomonas while Lactobacillus showed antibacterial activity against Staphylococcus and Streptococcus species. Saccharomyces showed no inhibition on any of the bacterial pathogens but inhibited the isolated fungal pathogens. This observation of the antibacterial activity of Pseudomonas flourescens and Lactobacillus plantarum on the isolated pathogens were similar to those previously reported by Queiroz and Boyd (1998), O'Sullivan and O'Gara (1992), Gatesoupe (1994) and Gildberg et al. (1997) for fish and oyster cultures.

Lactobacillus and Pseudomonas have a synergistic effect on each other, this therefore accounts for their effects on the specific growth rate of the fingerlings. Highest growth rate was observed in the tanks treated with the combination of these two organisms (Fig. 3); this was followed by the tanks containing only Lactobacillus and then, the combination of the three probiotic candidates. The results were found to be significantly higher when compared with the control. However, the specific growth rate of the fingerlings in the tanks treated with the combination of Lactobacillus and Saccharomyces were low showing a kind of antagonistic relationship between the two microorganisms.

The highest percentage survival was observed in the treatment tanks with the combination of Pseudomonas and Lactobacillus (96.22%) and the least in the tanks with Saccharomyces only (42%). No significant difference was observed in the percentage survival of the fingerlings at 4 weeks between the treatment tanks with Pseudomonas only and the combination of the three probiotic candidates (Fig. 2). However, there was significant difference in percentage survival in these tanks (with probiotics) and the control (without probiotics).

These findings could be said to be in agreement with the results of Wang (2007), who observed a significantly higher weight gain in groups of Penaeus vannamei treated with probiotics than that of the control. Applications of the probiotics were thought to have induced enzyme activity in the digestive system of P. vannamei and therefore enhanced its survival. Nejad et al. (2006) and Venkat et al. (2004) also reported higher percentage specific growth rate of M. rosenbergii using Lactobacillus spp. Rahiman et al. (2010) also confirmed the effectiveness of probiotics in increasing the specific growth rate of cultured shrimp.The abiotic environment prevailing in the culture system (Figures 4a and 4b) was observed not to be significantly different (P>0.05), for all the treatment tanks when compared with the control. This may be because the treatment and control tanks were maintained at the same temperature and at controlled aeration. There was no significant variation in the average temperature (26.6^0C-27.3^0C) and dissolved oxygen (4.24MgL^{-1} – 4.30 MgL^{-1}) respectively in the different treatment tanks and the control. The lowest average pH was observed in the tank with Lactobacillus. The values of the physicochemical parameters tested (Figs. 4a and 4b) fell within the optimum for Clarias gariepinus culture. According to Tucker (1991), Clarias gariepinus tolerates a temperature of about 26^O– 28^OC, pH 6.5-7.5 and dissolved oxygen, 5ppm to saturation. The minor variation in the treatment groups did not seem to affect the survival of the fishes. Queiroz and Boyd (1998) reported that when probiotics are used to treat culture waste water, heterotrophic bacterial assimilation could be the major mechanism for organic matter removal and conversion of potential toxic inorganic nitrogen into relatively stable organic nitrogen. This may have accounted for the observed values of the ammonia in the water from the treatment tanks which ranged from 0.02 to 0.03ppm during the experimental period. Decamp et. al. (2008) concluded that improved water quality was a major factor for better performance in biomass increase and that probiotic bacteria have the potential to induce growth enhancement comparable to that obtained with antibiotics and it is cost effective.

V. Conclusion

Probiotics are environmentally friendly and therefore, more desirable when compared to the use of antibiotics and chemical disinfectants. It is clear from this study that the combination of Pseudomonas flourescens and Lactobacillus plantarum introduced directly through water into the culture system significantly enhanced the specific growth rate and increased the survival rate of the catfish fry and fingerlings. It is concluded, therefore, that they can serve as good probiotics in the control of bacterial and fungal diseases of Clarias gariepinus in the early growth stages.

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