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BENEFICIAL EFFECTS OF TWO *Lactobacillus plantarum* STRAINS ON EGG HATCHABILITY AND LARVAL SURVIVAL OF AFRICAN CATFISH (*Clarias gariepinus*)

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ABSTRACT

This study evaluated the effect of two strains of Lactobacillus plantarum on egg hatchability and larval survival during artificial propagation of Clarias gariepinus. Each of the test bacteria were added to the incubating water in a 6-litre circular bowl at a concentration of 2×10^3 and 2×10^6 cfu/L respectively, while incubating bowl without test bacterial inclusion served as control. Two grammes of fertilized eggs were incubated in triplicate bowls for each experimental group under room temperature. The percentage hatchability of the incubated eggs and the survival of the resulting larvae on third and tenth day of hatching, as well as the microbiological analysis of 10 day old larvae in all experimental groups were determined. All data were statistically analysed using one-way analysis of variance (ANOVA) while Duncan Multiple Range test was employed to separate means among experimental groups. The results of this study showed that percentage hatchability ranged from 69.85% to 71.12%, and was found to be insignificantly differed among the experimental groups. The larvae from test bacterial treatment groups had significantly ($p<0.05$) higher percentage survival after third day of hatching than the control groups, although no lactobacilli was detected in the guts of larvae after tenth day of hatching in all experimental groups. It can be concluded that incubation of C. gariepinus eggs in water inoculated with adequate quantity of the test bacteria did not contribute to egg hatchability but had profound effects on larval survival during their first few days of life.

Keywords: test bacteria, hatching, larval survival, probiotics.

INTRODUCTION

The concept of probiotics in aquaculture has attracted research attention in recent years following increased understanding of their roles in conferring the cultured organisms with enhanced growth performance, feed efficiency, disease resistance and improvement in the general wellbeing of the animals (Gomez and Balcazar, 2008). Different microorganisms, including Gram-negative and Gram-positive bacteria, yeasts, and unicellular algae, have been examined for use as probiotics to control disease, as supplements for improving growth and in some cases as a mean of replacing antimicrobial compounds in many aquaculture species at different stages of growth (Prol, 2010). In spite of these perceived benefits, the use of commercially available probiotic products has not been adopted by farmers in Nigeria and other developing countries, either because they are ineffective under practical conditions, or are too expensive to procure due to shipping costs and high foreign exchange rates (Nwanna, 2010). These facts necessitate the need for further research on identifying indigenous novel probiotics microorganisms for aquaculture use in the developing nations.

Lactobacillus plantarum is a facultative hetero-fermentative lactic acid bacterium that occupies a diverse range of environmental niches and has numerous characteristics that make it suitable as probiotics (Ogunshe and Olabode, 2009). *L. plantarum* produced bacteriocin which inhibits the growth of Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Listeria innocua* and *Pseudomonas aeruginosa* (Lash *et al*., 2005). Many published reports demonstrated positive effects of *L. plantarum* on larvae of various fish species. For instance, *L. plantarum* (906) administration significantly decreased sea bream (*Sparus aurata)* larvae and fry mortality (Carnevali *et al*., 2004). The survival of Atlantic halibut larvae was improved after incubation with *L. plantarum*, while increase in mucous cells in this fish suggested a stimulation of cellular defences (Gatesoupe *et al*., 2008) However, recent studies have shown high divergence in functionality among different strains of *L plantarum* isolated from different food materials and other sources (Siezen and van Hylckama Vlieg, 2011), thus the need to clearly demonstrate various effects

each strain have on the intended host before they are employed as probiotics. Available information on application of probiotics during hatchery propagation of African catfish (*Clarias gariepinus*) is scanty. The present study therefore examined beneficial effects of two strains of *L plantarum* at different levels of inclusion during hatchery propagation of *C. gariepinus* in order to determine if the addition of these probitocs bacteria to water at the onset of egg incubation could improve egg hatchability and larval survival.

MATERIALS AND METHODS

The two strains of *Lactubacillus plantarum* used in this study were initially recovered from sorghum and soyabeans, and their sequenced nucleotides were accorded accession numbers LC333559 and LC333560 by the DNA Data Bank of Japan (DDBJ) (Diyaolu *et al*., 2018). The probiotic bacteria were prepared following standard procedures, and kept in phosphate buffered solution (PBS 5.7) until use. Artificial propagation of two pairs of African catfish (*Clarias gariepinus*) brooders (1.35±0.08 kg) were carried under laboratory conditions. Each female brooder was injected at the rate of 0.5 ml of Ovaprim per kilogramme of fish, while the males were not induced. The injection, stripping and fertilization were done following the methods described before (Diyaolu, 2012).

Egg incubation was carried out in 6-litre circular plastic bowls under static water conditions at room temperature. Each bowl was prepared by adding cells of *L plantarum* strains into fresh water at varying bacterial concentrations as follows: no bacterial addition as control (TR1); 2×10^3 cfu/L of *L plantarum* strain LC333559 (TR2); 2×10^6 cfu/L of *L. plantarum* strain LC333559 (TR3); 2×10^3 of *L. plantarum* strain LC333560 (TR4), and 2×10^6 cfu/L of *L. plantarum* strain LC333560 (TR5). Briefly, 2 g of fertilized eggs (equivalent to 1000 eggs) were taken, mixed with 10 ml water, allowed to stay for 1 minute, and quickly spread for incubation in each designated bowl. The incubation lasted 30 hours after which dead eggs and egg shells were siphoned. Water in each bowl was replaced with fresh water, while the hatchlings were counted to determine percentage hatchability of the fertilized eggs. Thereafter, rearing water was renewed twice daily. The hatchlings were not fed for two days.

On the third day of hatching, percentage survival of the hatchlings from each experimental treatment was determined prior to commencement of exogenous feeding. Thereafter, 100 hatchlings from each bowl were siphoned gently into a separate experimental bowl containing fresh water with no test bacteria. The larvae were fed three times daily with commercial decapsulated *Artemia* nauplii at 0.5 g per litre of rearing water. The uneaten feed and dead larvae were siphoned and water in each bowl was renewed twice daily (in the morning and evening) prior to feeding. The larval survival on 10 days post hatching was determined. The microbiological evaluations of larvae were also examined 10 days post hatching, as this period was considered to be sufficient enough for probiotic to colonise gut of larvae. All procedures followed the method described earlier by Ringo *et al.* (1995). The data obtained in this study were statistically analysed using one-way analysis of variance (ANOVA) while Duncan Multiple Range test was employed to separate means among experimental groups.

RESULTS

The inflow water in all incubating bowls (experimental groups) had the following parameters throughout the experimental period: temperature 26.3±1.53 ^{0}C, dissolved oxygen 6.26±0.38 mg/L and pH 6.6±0.31. The result of the hatchability of *C. gariepinus* eggs incubated in different concentration of two strains of *L. plantarum* as well as the survival of the resulting larvae was presented in Figure 1. Percentage hatchability ranged from 69.85% to 71.12%, and was found to be insignificantly ($p>0.05$) differed among the experimental groups (Figure 1A). The larvae from probiotic treatment groups had higher percentage survival after third day of hatching than the control (Figure 1B). The larvae in the TR1 (control) groups had the significantly ($p<0.01$) lowest percentage of survival on the third day of hatching while highest larval survival of 90.75±1.91 % were recorded in TR3 and TR5 groups.. However, there was no significant ($p>0.05$) difference observed in the survival of larvae in all experimental groups on 10 day post-hatching (Figure 1C). Table 1 showed the bacteriological analyses of gut of larvae on 10 day post hatching. The total bacterial counts were not significant

(p>0.05) among experimental groups, while presumptive lactobacilli were not detected in all the treatment groups, indicating that test bacteria might not colonise the gut of *C. gariepinus* larvae

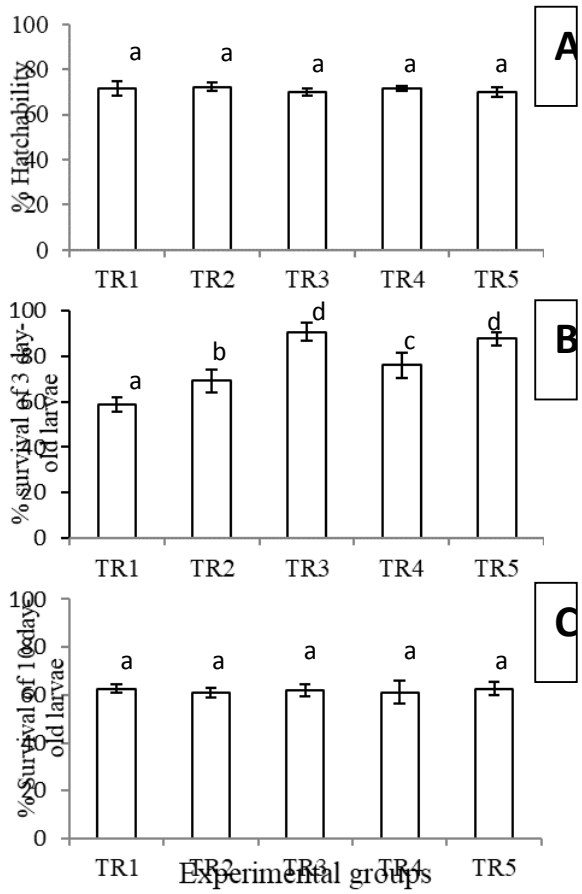


Figure 1: Egg hatchability and larval survival of *C gariepinus* incubated in water of different concentrations of two *L. plantarum* strains. A – Egg hatchability after 30 hours of incubation; B – Larval survival on day 3 post hatching; C – Larval survival on day 10 post hatching. Bars with different superscripts indicated significant difference ($p < 0.05$).

Table 1: Bacteriological analyses of gut of 10 day-old *C. gariepinus* larvae hatched in water of different concentration of two *L. plantarum* strains

Experimental groups	Total bacterial counts (cfu/g)	Presumptive lactobacilli counts (cfu/g)
TR1	**1.1. $2.17\pm0.12\times10^{4}$ a**	**1.2. ND**
TR2	**1.3. $1.60\pm0.15\times10^{4}$ a**	**1.4. ND**
TR3	**1.5. $2.06\pm0.29\times10^{4}$ a**	**1.6. ND**
TR4	**1.7. $1.73\pm0.42\times10^{4}$ a**	**1.8. ND**
TR5	**1.9. $1.63\pm0.61\times10^{4}$ a**	**1.10. ND**

1.11. The values were recorded as mean ± standard deviation from triplicate trials; ND – not detected; Values with the same superscripts in a column are not significantly (P>0.05) different; TR1 – Control; TR2 – 2×10^{3} cfu/L of *L plantarum* strain LC333559; TR3 – 2×10^{6} cfu/L of *L. plantarum* strain LC333559; TR4 – 2×10^{3} of *L. plantarum* strain LC333560 and TR5 – 2×10^{6} cfu/L of *L. plantarum* strain LC333560

DISCUSSION

The result obtained in this study showed no significant difference in hatchability among control groups and other probiotic-treated groups, implying that addition of probiotic bacteria during incubation of *C. gariepinus* eggs did not favour hatchability. However, according to Olaniyi and Omitogun (2013), it is essential to maintain adequate rearing environment for the successful rearing of the resulting hatchlings to avoid excessive mortalities. In practice, larval mortalities are known to be

more pronounced soon after hatching, and this may be due to larval developmental milestones, such as the depletion of the yolk sac, opening of the incipient gut tract, etc. (Prol, 2010). Poor water quality and microbial proliferations during this period must be avoided, as any stress or infection at this critical stage may result in massive larval mortalities (Olaniyi and Omitogun, 2013). In this study, *C. gariepinus* larvae from treatment groups incubated with *L. plantarum* LC333559 and LC333560 at concentration of 10^6 cfu/L had significantly highest survival, probably due to the presence of the probiotics on the surfaces of the larvae which might have protected them against harmful opportunistic microorganism at this critical period. In view of these results, incubation of fish eggs in water inoculated with adequate quantity of beneficial probiotics may not contribute to egg hatchability but might have profound effects on larval survival during their first few days of life. Presumptive lactobacilli were not detected in guts of larvae after 10 days post hatching. By implication, no strains of *L. plantarum* added to *C. gariepinus* fertilized eggs during incubation were able attach to or colonise guts of the resulting larvae. Similar observations on inability of lactic acid bacteria to attach to fish larval guts have been reported by other workers (Joborn *et al*., 1997)

CONCLUSION AND RECOMMENDATIONS

This study showed the ability of *L. plantarum* LC333559 and LC333560 strains to aid larval survival after three days post-hatching, when added during incubation of *C. gariepinus* eggs. Future trials may examine other modes of probiotic delivery (including bio-encapsulation in live food) that is the best in providing the larvae with the probiotics.

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