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Isolation and identification of potential probiotic bacteria on surfaces of *Oreochromis niloticus* and *Clarias gariepinus* from around Kampala, Uganda

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Increased fish mortality due to infections has forced most farmers to resort the use of chemotherapeutic agents especially antibiotics. The continued use of these drugs in aquaculture is becoming limited as pathogens develop resistance and infer unpredicted long term public health effects. More research efforts are building to identify alternative disease prevention methods, among which the use of probiotics has been proposed. Therefore, the purpose of this study was to identify potential probiotics on surfaces of tilapia and catfish in areas around Kampala. Tilapia and catfish samples were aseptically collected from selected cages, ponds, tanks and hatcheries around Kampala, including Lake Victoria. The skin of fish was swabbed and then cultured on both general purpose and selective media. Probiotic screening was done using the agar spot method. Results revealed complete growth across all samples. The total microbial load was highest in fish from lakes ($1000\pm9.6\times10^5$ cfu) and cages ($1001\pm5.0\times10^5$ cfu). In all cases tilapia fish was significantly ($p<0.0001$) more contaminated than catfish. Out of the three strains of probiotics isolated, only *Lactobacillus spp* and *Lactococcus spp* showed antibacterial activity against pathogenic bacteria. The activity of *Lactobacillus spp* was significantly high ($p< 0.0001$) with *Streptococcus spp* (16.5±0.2 mm). *Lactobacillus spp* inhibited growth of only *Proteus spp* (5±0.2 mm). Our study shows that *Lactobacillus spp* and *Lactococcus spp* isolated from tilapia and catfish possess probiotic activity against a number of pathogenic bacteria. Our findings have significant implications for subsequent probiotic formulation and testing in aquaculture.

Key words: Probiotics, *Oreochromis niloticus*, *Clarias gariepinus*, aquaculture, Uganda.

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, presently 52% of the 600 wild fish species with economic value are threatened (Dudgeon et al., 2006), 17% over fished and 7% fully exploited (Naylor et al., 2000). In Uganda, the fish industry greatly contributes to the welfare of Ugandans in terms of employment, food security, government revenue and foreign exchange earnings (Nyombi and Bolwig, 2004). The

main fish species on the Ugandan market include; Nile perch (*Lates niloticus*), Nile tilapia (*Oreochromis niloticus*), and catfish (*Clarias gariepinus*) (Kabahenda and Hüsken, 2009). Aquaculture is currently the fastest growing food production sector in the world, expanding the total world production and diversity of cultured species (Naylor et al., 2000). The global production of fish from capture fisheries and aquaculture increased by 7.5 to 59.9% in 2010 from 55.7% in 2009 (Esteban et al., 2013).

Aquaculture is an important sector in Uganda providing alternative employment opportunities but fish diseases especially bacterial infections remain primary constraints to its continued expansion (Austin and Austin, 2007; Tellez-Bañuelos et al., 2010). In contrast to the intestines, little is known about the development or activity of bacterial flora on gills and surfaces of fish. Stress weakness is the fish's natural mechanism of defense, making it more susceptible to disease (Plumb and Hanson, 2011). With the growing concern of diseases, most farmers especially in shrimp farming have turned to antimicrobial drugs to cure the bacterial infections (Holmström et al., 2003). Although antibiotics improve survival, they also alter the microbial communities and induce resistant bacteria populations, with unpredictable long term effects on public health (Luis Balcázar et al., 2006). The use of antibiotics to cure bacterial infection and prevent fish mortality in aquaculture is becoming limited as pathogens develop resistance to the drugs and accumulation of antibiotic residues in fish tissues (De La Peña and Espinosa-Mansilla, 2009).

Furthermore, beneficial bacterial flora are killed by antibiotic administration, leading to more efforts to find alternative disease prevention methods such as use of nonpathogenic bacteria called probiotics (Kesarcodi-Watson et al., 2008). Probiotics are beneficial microorganisms with ability to reduce the use of antibiotics in aquaculture since their addition can assist in returning a disturbed microbiota to its normal beneficial composition (Defoirdt et al., 2011). According to Verschuere et al. (2000), the interaction between the probiotics and the host is, however not limited to the intestinal tract but also on the surfaces of skin and gills of the fish and its ambient environment. The majority of identified probiotics in fish belong to the lactic acid bacteria (*Lactobacillus*), *Vibrio*, *Bacillus* and *Pseudomonas* genera's (Gatesoupe, 1999). A number of commercially formulated probiotics are now being utilized in aquaculture but with mixed success results. Therefore, it is likely that for probiotics to be effective, they need to be isolated from the same environment where the fish is farmed. Therefore, the aim of the current study was to isolate and identify potential probiotic bacteria on the surface of Nile Tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*) from different production systems around Kampala. Results from this study will form a basis for the production of probiotic formulations and subsequent testing in aquaculture.

MATERIALS AND METHODS

Study design

A total of 45 Nile Tilapia (*Oreochromis niloticus*) and 45 Catfish (*Clarias gariepinus*) were purposively collected from Mulungu Island in Lake Victoria, hatcheries (fingerlings) in Kawempe, ponds and tanks at Kajjansi Research Institute and fish cages at Kitinda, all around Kampala district. Fish from the lake and ponds were captured using a cast net, with each fish put in a separate plastic bag and immediately transported to the Microbiology Laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity in iceboxes. Only freshly captured fish samples were included in the study, dead fish at capture were excluded. In the Laboratory, the skin of both catfish and tilapia were aseptically swabbed with subsequent culturing on sterilized media plates. Plates were incubated at 37°C for 24 h. Isolates were sub cultured to obtain pure cultures that were further identified using gram staining and biochemical tests.

Bacteria isolation and identification

Bacteriological media: Nutrient agar, MacConkey agar, Potato Dextrose Agar (PDA), de Man, Rogosa and Sharpe agar (MRS), Mannitol Salt Agar (MSA), Thiosulfate-citrate-bile salts-sucrose agar (TCBS) and Blood agar were prepared according to manufacturer's instructions (Sigma-Aldrich, USA). The media were sterilized at 121°C for 15 min in an autoclave and later poured into sterilized disposable plastic petri dishes. The petri dishes were then stored in the incubator after media drying. A sterile cotton swab was brushed all over the skin of the fish. Swabs were then swirled into sterilized peptone water that was serially diluted into five dilutions of 9ml. Bacterial cultures followed the method as described by Boone et al. (2001). Briefly, a quantity of 0.1ml of 10^{3} and 10^{-5} dilution was inoculated in Petri dishes of Nutrient Agar, MacConkey agar, TCB, PDA, MSA agar plates in duplicates and spread using a sterile glass rod, then incubated aerobically for 24 to 48 h at 37°C and anaerobically for MRS agar plates at the same temperature and hours.

Colony count was calculated by dividing the bottom of the Petri dish into four and the sum of bacterial count was multiplied by the dilution factor. Each distinct colony was further sub cultured on freshly prepared Nutrient agar for evaluation of purity and colonial morphology. The isolates were further subjected to Gram stain to determine their Gram reaction and biochemical test as described by (Cheesbrough, 2006) and (Mac Faddin, 1976) and also, to determine the identity of bacteria isolates.

Antimicrobial activity

The probiotic strains were screened for antimicrobial activity against selected pathogens using an agar spot method as described by (Schillinger and Lücke, 1989). Briefly, overnight cultures of

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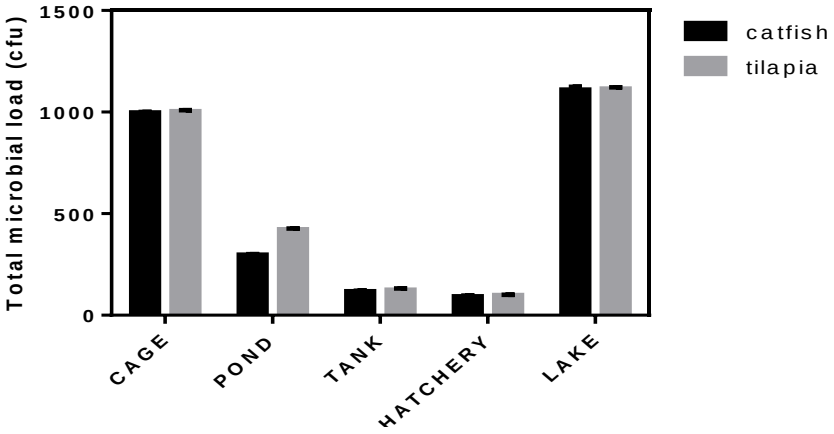

Figure 1. Total microbial load on surfaces of tilapia and catfish from selected sampling systems.

Lactobacillus spp, Bacillus sabtillis and *Lactococcus spp* were spotted onto the surface of MRS agar (1.2% w/v agar, 0.2% w/v glucose) plates, which were then incubated anaerobically for 24 h at 37°C.The indicator species (*Staphylococcus aureus, Streptococcus spp, Proteus spp and Pseudomonas spp*) were inoculated into 7ml of soft agar medium (nutrient broth containing 0.7% w/v) to a final concentration of approximately 10^5cfu. The soft media were later poured on the plates and incubated for 24 h at 37°C. Zones of clearance were later measured in mile meters.

Statistical analyses

Statistical analyses were done using Graph pad 6.0 statistical software. Total microbial load across sampling sites and fish species were done using a Two-way ANOVA. Significant differences in antibacterial activity across the different pathogenic bacteria were analyzed using a One-way ANOVA set at significance level of ($p< 0.05$). Multiple comparisons between groups (sampling sites and pathogenic bacteria strains) were done using Tukey`s multiple comparison test, differences were taken as significant at $p< 0.05$.

RESULTS

Total microbial load

The results revealed that the sampling site and fish type had a significant effect on total microbial load ($p< 0.0001$, $F_{(4,10)} =72.15$, $P> 0.0001$, $F_{(1,10)} =111.1$) respectively. On comparison between sampling sites, microbial load on fish surfaces was significantly higher ($p<0.05$) in lakes ($1000\pm9.6\times10^5$ cfu) and cages ($1001\pm5.0\times10^5$ cfu) as compared to tanks ($121.6\pm6.3\times10^5$ cfu), ponds ($360.5\pm72.2\times10^5$ cfu) and hatcheries ($90\pm4.3\times10^5$ cfu) (Figure 1). Hatcheries and tanks had the least microbial load. On comparison between tilapia and catfish, microbial load in tilapia from ponds was significantly higher ($p< 0.05$) as compared to catfish from the same source. No significant difference ($p>0.05$) between the two fish species were observed across the other sampling localities.

Prevalent bacteria isolated on surfaces of tilapia and catfish

The data showed that the most commonly isolated bacteria on the surface of tilapia across sampling systems were: *Escherichia coli* (82%), *Lactococcus spp* (80%), *Staphylococcus aureus* (73%), *Streptococcus spp* (69%), *Proteus spp* (60%), *Lactobacillus spp* (48%) and *Klebsiella spp* (47%). The least isolated being *Pseudomonas spp* (38%), *Bacillus subtillis* (27%), *Corynebacteria spp* (24%), *Bacillus cereus* (20%), and *Enterobacteria spp* (16%) (Table 1). For catfish, the most commonly isolated bacteria on the surface across the sampling systems were*: Lactococcus spp* (87%), *Escherichia coli* (80%), *Staphylococcus aureus* (64%), *Streptococcus spp* (60%), *Lactobacillus spp* (60%) *Proteus* spp (53%) *and Klebsiella* spp (47%) (Table 2).

Antibacterial activity of selected probiotic genera

When antibacterial activity as a measure of probiotic potential was determined, only two genera (*Lactobacillus and Lactococcus)* showed probiotic potential. The results revealed that antibacterial activity significantly varied ($p<0.05$) across the different pathogenic bacteria utilized. When antibacterial activity was compared across the different pathogenic isolates, *Lactobacillus spp* had a significantly ($p<0.0001$) higher activity. *Lactobacillus spp* showed the highest activity on *Streptococcus spp* (16 ± 0.2mm), compared to *Proteus spp* (9 ± 0.2 mm) and *Pseudomonas spp* (7 ± 0.2mm, Figure 2A). Antibacterial activity of *Lactococcus spp* was observed for only *Proteus spp* (5 ± 0.2mm, $p< 0.0002$) (Figure 2B). No probiotic potential was observed for *Bacillus subtillis.*

Table 1. Bacteria isolated on surfaces of tilapia.

Bacteria	Cage	Pond	Hatchery	Tank	Lake	Total
Eschericchia coli	8(80%)	7(70%)	6(60%)	7(70%)	9(90%)	37(82%)
Lactococcus spp	8(80%)	7(70%)	6(60%)	5(50%)	10(100%)	36(80%)
Staphylococcus. auerus	9(90%)	7(70%)	2(40%)	5(50%)	10(100%)	33(73%)
Streptococcus spp	10(100%)	8(80%)	0	4(40%)	9(90%)	31(69%)
Proteus spp	8(80%)	7(70%)	0	4(40%)	8(80%)	27(60%)
Lactobacillus spp	5(50%)	3(30%)	4(40%)	4(40%)	6(60%)	22(48%)
Klebsiella spp	7(70%)	5(50%)	1(20%)	2(20%)	6(60%)	21(47%)
Pseudomonas spp	7(70%)	0	0	3(30%)	7(70%)	17(38%)
Bacillus spp	2(20%)	2(20%)	5(50%)	0	3(30%)	12(27%)
Corynebacteria spp	5(50%)	0	0	0	6(60%)	11(24%)
Bacillus cereus	0	4(40%)	0	0	5(50%)	9(20%)
Enterobacteria spp	6(60%)	5(50%)	1(20%)	2(20%)	6(60%)	7(16%)

Table 2. Bacteria isolated on surface of catfish.

Bacteria	Cage	Pond	Hatchery	Tank	Lake	Total
Lactococcus spp	9(90%)	8(80%)	7(70%)	5(100%)	10(100%)	39(87%)
Escherichia coli	7(70%)	5(50%)	5(50%)	6(60%)	8(80%)	36(80%)
Staphylococcus aureus	8(80%)	6(60%)	1(20%)	4(40%)	10(100%)	29(64%)
Streptococcus spp	9(90%)	7(70%)	0	3(30%)	8(80%)	27(60%)
Lactobacillus spp	6(60%)	4(40%)	5(50%)	5(100%)	7(70%)	27(60%)
Proteus spp	7(70%)	6(60%)	0	3(30%)	8(80%)	24(53%)
Klebsiella spp	6(60%)	4(40%)	1(20%)	4(40%)	7(70%)	21(47%)
Bacillus subtillis	3(30%)	3(30%)	6(60%)	0	4(40%)	16(36%)
Pseudomonas spp	6(60%)	0	0	2(20%)	6(60%)	14(31%)
Enterobacteria spp	5(50%)	5(50%)	1(20%)	0	0	11(24%)
Corynebacteria spp	4(40%)	0	0	0	5(50%)	9(20%)
Bacillus cereus	0	3(30%)	0	0	4(40%)	7(16%)

DISCUSSION

There has been a growing concerns about the adverse effects of bacterial diseases in aquaculture of many economically important marine and fresh fish species including Nile tilapia and Catfish (Ashley, 2007). Bacterial infections cause considerable losses to the fish industry especially from mortality and reduced growth (Austin and Austin, 2007), forcing most farmers to resort to use of chemotherapeutic agents especially antibiotics. The continued use of these

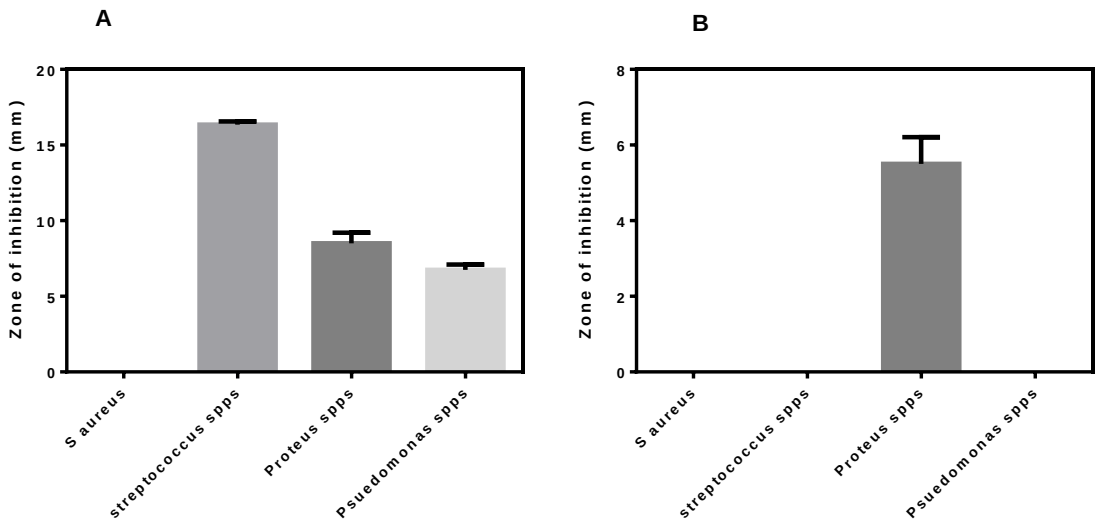

Figure 2. Probiotic activity of *Lactobacillus spp* (A) and *Lactococcus spp (B)* on selected pathogenic bacteria.

drugs in aquaculture has become limited as pathogens developed resistance to drugs (Alderman and Hastings, 1998). Probiotics have been proposed as possible alternatives to the use of antibiotics (Joerger, 2003). Therefore, the purpose of this study was to isolate and identify potential probiotic organisms on surfaces of tilapia and catfish from areas around Kampala district, Uganda.

In this study, the total microbial load was significantly high on both surfaces of tilapia and catfish from lakes ($1000\pm9.6\times10^{-5}$ cfu) and cages ($1001\pm5.0\times10^{-5}$ cfu). We further showed that microbial load in tilapia from ponds was significantly higher as compared to catfish from the same source. Total microbial load for both catfish and tilapia in this study was slightly higher compared to that reported by (Emikpe et al., 2011) in catfish ($117.33\times10^{-}$11cfu) and tilapia ($143.67\times10^{-}$11cfu). Generally, increase in total microbial load has been attributed to high aquatic temperatures resulting from organic matter recycling, self-cleaning potential, and re-mineralization (Fernandes et al., 1997; Hossain et al., 1999). Variations in bacterial counts between individual fish have been observed previously (Spanggaard et al., 2000) and were confirmed by our results.

The present study revealed 12 genera of bacteria on the surfaces of both catfish and tilapia from various aquatic environments. These results were in agreement with the findings of (Adebayo-Tayo et al., 2012). Some of the bacteria species recovered in this study were also identified from healthy *Clarias gariepinus* (Efuntoye et al., 2012). The presence of these isolated organisms was not surprising since fish live in water habitat full of micro-organism. Among these isolates, *Escherichia coli* were the most dominant in both catfish and tilapia. Increased presence of *E. coli* might demonstrate the level of habitat pollution because coliforms are not the normal flora of bacteria in fish (Mandal et al., 2009). Similarly, like in this study, other studies such as Ibrahim and Sheshi (2014) have demonstrated the presence of *Staphylococcus aureus*, which also less frequently occurs as natural microflora of fish.

From this study, three proposed probiotic genera were isolated; *Lactobacillus spp, Lactococcus spp* and *Bacillus* subtillis. These strains were similarly isolated from the gut of the Nile tilapia (Zapata and Lara-Flores, 2012). The findings are in agreement with (Ringø et al., 1997) who found that 10% microbiota population in Artic charr (*Salvelinus aplinus L.)* was lactic acid bacteria. The findings of our study also confirm a study by (Hamid et al., 2014) who isolated *Lactococcus spp and Lactobacillus spp* from catfish. However, reports on the presence of *Lactococcus spp* in freshwater fishes are scarce. In the present study, as reported previously by Einar Ringø and Gatesoupe (1998), *Lactobacillus spp* had the highest antimicrobial activity against all the selected pathogens tested except for *Staphylococcus spp*. Its activity was highest against *Streptococcus spp* (16±0.2mm), followed by *Proteus spp* (9 ± 0.2mm) and least for *Pseudomonas spp* (7 ± 0.2mm). The mechanism of antibacterial activity in *Lactobacillus* strains appears to be multifactorial (Servin, 2004). Ali et al. (2013) revealed that all lactobacilli tested (except *L. delbrueckii*) inhibited the growth of *S. aureus*. This probiotic activity of *Lactobacillus spp* on pathogenic bacteria has already been demonstrated in a number of studies in fish (Kim et al., 2007; Nayak, 2010; Nikoskelainen et al., 2001; Suzer et al., 2008). In our study, *Lactococcus spp* showed antimicrobial activity only against *Proteus spp* (5 ± 0.2mm). In another study, *Lactococcus spp* was reported to inhibit the fish pathogen, *Aeromonas hydrphila* in tilapia (Hamid et al., 2014).

In our study, surprisingly *Bacillus subtillis* isolated from

fish surfaces did not show any antimicrobial activity against the selected pathogenic bacteria. According to (Domrongpokkaphan and Wanchaitanawong, 2006) *Bacillus subtillis* isolated from hepato-pancreas of black tiger shrimp were found active against four shrimp pathogenic *Vibrio spp.* Indeed, (Sugita et al., 1998) observed *Bacillus subtillis* from fish gut to produce antibacterial substances. It is difficult to comment on the reason for this variability in antimicrobial activity amongst isolates from different fish anatomical compartments. However, as stated by Jacobsen et al. (1999) it is likely that the environment from which the bacteria are isolated might have a role in determining probiotic potential.

Conclusion

In conclusion, our study shows that total microbial load was highest in both tilapia and catfish sampled from cages and lakes compared to fish species from ponds, tanks and hatcheries.

The most commonly isolated potentially pathogenic organisms on both surfaces of catfish and tilapia included; *Escherichia coli, Staphylococcus aureus, Streptococcus spp and Proteus spp, Klebsiella spp* and *Pseudomonas spp.* Three probiotic species: *Lactococcus spp, Lactobacillus spp* and *Bacillus spp* were isolated. *Lactobacillus spp* showed the highest antimicrobial activity followed by *Lactococcus spp. Bacillus subtillis* showed no antimicrobial activity against selected pathogenic isolates.

However, future studies characterizing the observed probiotic species would be important to aid their use in aquaculture. Furthermore, studies evaluating probiotic potential using a combination of two or more organisms would be important for improved activity.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Adebayo-Tayo B, Odu N, Anyamele L, Igwiloh N, Okonko I (2012). Microbial quality of frozen fish sold in Uyo Metropolis. Nature Sci. 10(3):71-77.

Alderman D, Hastings T (1998). Antibiotic use in aquaculture: development of antibiotic resistance–potential for consumer health risks. Int. J. Food Sci. Technol. 33(2):139-155.

Ali F, Saad O, Hussein S (2013). Antimicrobial activity of probiotic bacteria. Egypt Acad. J. Biol. Sci. 5(2):21-34.

Ashley PJ (2007). Fish welfare: current issues in aquaculture. Appl. Anim. Behav. Sci. 104(3):199-235.

Austin B, Austin DA (2007). Bacterial fish pathogens: disease of farmed and wild fish (4th ed.). UK: Springer Science & Business Media.

Boone DR, Castenholz RW, Garrity G (2001). Bergey's manual of systematic bacteriology. Garrity GM (ed), 188(2):282.

Cheesbrough, M (2006). District laboratory practice in tropical countries: Cambridge university press.

De La Peña AM, Espinosa-Mansilla A. (2009). Analysis of antibiotics in fish samples. Anal. Bioanal. Chem. 395(4):987-1008.

Defoirdt T, Sorgeloos P, Bossier P (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr. Opin. Microbial. 14(3):251-258.

Domrongpokkaphan V, Wanchaitanawong P (2006). *In vitro* antimicrobial activity of Bacillus spp. against pathogenic Vibrio spp. in black tiger shrimp (*Penaeus monodon*). Kasetsart J. Nat. Sci. 40:949-957.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Stiassny ML (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81(2):163-182.

Efuntoye M, Olurin K, Jegede G (2012). Bacterial flora from healthy Clarias gariepinus and their antimicrobial resistance pattern. Adv. J. Food Sci. Technol. 4(3):121-125.

Emikpe B, Adebisi T, Adedeji O (2011). Bacteria load on the skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus* from Ibadan, South West Nigeria: Public health implications. J. Microbiol. Biotechnol. Res. 1(1):52-59.

Esteban M, Cuesta A, Chaves-Pozo E, Meseguer J (2013). Influence of melatonin on the immune system of fish: A review. Int. J. mol. sci. 14(4):7979-7999.

Fernandes CF, Flick GJ, Silva JL, McCaskey TA (1997). Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. J. Food Protect. 60(1):54-58.

Gatesoupe F (1999). The use of probiotics in aquaculture. Aquaculture 180(1):147-165.

Hamid THTA, Khan AJ, Jalil MF, Azhar S (2014). Isolation and screening of lactic acid bacteria, Lactococcus lactis from *Clarias gariepinus* (African catfish) with potential use as probiotic in aquaculture. Afr. J. Biotechnol. 11(29).

Holmström K, Gräslund S, Wahlström A, Poungshompoo S, Bengtsson, BE, Kautsky N (2003). Antibiotic use in shrimp farming and implications for environmental impacts and human health. Int. J. food Sci. Technol. 38(3):255-266.

Hossain M, Uddin M, Islam M, Chakraborty S, Kamal M. (1999). Study on the intestinal bacteria of Labeo rohita (Ham.). Bangladesh J. Fisheries Res. *3*(1):63-66.

Ibrahim B, Sheshi M (2014). Isolation and Identification of Bacteria Associated with Fresh and Smoked Fish (*Clarias gariepinus*) In Minna Metropolis, Niger State. Nigeria. J. Appl. Environ. Microbiol. 2(3):81-85.

Jacobsen CN, Nielsen VR, Hayford A, Møller P, Michaelsen K., Paerregaard A, Jakobsen M (1999). Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol. 65(11):4949-4956.

Joerger R (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poult. Sci. *82*(4):640-647.

Kabahenda M, Hüsken S (2009). A review of low-value fish products marketed in the Lake Victoria region. Regional programme fisheries and HIV/AIDS in Africa: investing in sustainable solutions. Penang, Malaysia: WorldFish.

Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008). Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274(1):1-14.

Kim PI, Jung MY, Chang YH, Kim S, Kim SJ, Park YH (2007). Probiotic properties of Lactobacillus and Bifidobacterium strains isolated from porcine gastrointestinal tract. Appl. Microbial. Biotechnol. 74(5):1103-1111.

Luis Balcázar J, Decamp O, Vendrell D, De Blas I, Ruiz-Zarzuela I (2006). Health and nutritional properties of probiotics in fish and shellfish. Microb. Ecol. Health Dis. 18(2):65-70.

Mac Faddin JF (1976). Biochemical tests for identification of medical bacteria: Williams & Wilkins Co.
Mandal SC, Hasan M, Rahman MS, Manik MH, Mahmud ZH, Islam M S (2009). Coliform bacteria in Nile Tilapia, Oreochromis niloticus of shrimp-Gher, pond and fish market. World J. Fish Marine Sci. 1(3):160-166.
Nayak S (2010). Probiotics and immunity: A fish perspective. Fish shellfish immunol. 29(1):2-14.
Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, Troell M (2000). Effect of aquaculture on world fish supplies. Nature 405(6790):1017-1024.
Nikoskelainen S, Ouwehand A, Salminen S, Bylund G (2001). Protection of rainbow trout (Oncorhynchus mykiss) from furunculosis by Lactobacillus rhamnosus. Aquaculture 198(3):229-236.
Nyombi K, Bolwig S (2004). A qualitative evaluation of alternative development strategies for Ugandan fisheries. The International Food Policy Research Institute. Washington. DC USA.
Plumb JA, Hanson LA (2011). Health maintenance and principal microbial diseases of cultured fishes: John Wiley & Sons.
Ringø E, Gatesoupe FJ (1998). Lactic acid bacteria in fish: A review. Aquaculture 160(3):177-203.
Ringø E, Olsen R, Øverli Ø, Løvik F (1997). Effect of dominance hierarchy formation on aerobic microbiota associated with epithelial mucosa of subordinate and dominant individuals of Arctic charr, *Salvelinus alpinus* (L.). Aquaculture Research, 28(11):901-904.
Schillinger U, Lücke FK (1989). Antibacterial activity of Lactobacillus sake isolated from meat. Appl. Environ. Microbiol. 55(8):1901-1906.
Servin AL (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS microbiology reviews 28(4):405-440.
Spanggaard B, Huber I, Nielsen J, Nielsen T, Appel K, Gram L (2000). The microflora of rainbow trout intestine: a comparison of traditional and molecular identification. Aquaculture 182(1):1-15.
Sugita H, Hirose Y, Matsuo N, Deguchi Y (1998). Production of the antibacterial substance by Bacillus sp. strain NM 12, an intestinal bacterium of Japanese coastal fish. Aquaculture 165(3):269-280.
Suzer C, Çoban D, Kamaci HO, Saka Ş, Firat K, Otgucuoğlu Ö, Küçüksari H (2008). Lactobacillus spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280(1):140-145.
Tellez-Bañuelos MC, Santerre A, Casas-Solis J, Zaitseva G (2010). Endosulfan increases seric interleukin-2 like (IL-2L) factor and immunoglobulin M (IgM) of Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromona hydrophila*. Fish shellfish immunol. *28*(2):401-405.
Verschuere L, Rombaut G, Sorgeloos P, Verstraete W (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiol. Molecular Biol. Rev. 64(4):655-671.
Zapata AA, Lara-Flores M (2012). Antimicrobial activities of lactic acid bacteria strains isolated from Nile Tilapia intestine (*Oreochromis niloticus*). J. Biol. Life Sci. 4(1).