Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.42, 2015

The Abundance of Lactic Acid Bacteria in the Gastrointestinal Tract of Lake Victoria Nile Perch

James. A. Obar[1], Alfred. A. Shitandi[2], Symon M. Mahungu[1], Agasa O. Lameck[2].
[1]Department of Dairy, Food Science and Technology, Egerton University
[2]Division of Research and Extension, Kisii University
Corresponding author:lameckagasa@gmail.com*

Abstract
This study was carried out to determine the presence and abundance of Lactic Acid from gastrointestinal tracts of Latesniloticus fish with the specific objective of determining fish size effect on lactic Acid Bacteria (LAB) population.
The study was conducted in Lake Victoria between April and September, 2012. Statistical analysis was performed by using Minitab 9.1.3 software version. Analysis of Variance (ANOVA) and Least Significant Difference (LSD) was used for statistical comparisons. Differences were considered significant at α=0.05 level.
The average weight of small fish sampled during the wet season was 667.9g that gave an average colony forming units/g of 9.2x 10^3; medium averaging 1485.1g had an average of 2.1x10^4cfu and big averaging 3210.8g had 4.9 x 10^4cfu. In the dry spells, the small fish averaging 614.6g had 6.7 x 10^3, medium averaging 1392.9g had an average of 1.7x10^4cfu and big averaging 2756.3g had 3.4 x 10^4cfu. The mean of counts of LABs are significantly different for the three sizes tested, with the big size giving the highest followed by medium and small respectively. This could be due to ability to access more variety of foods. The means of counts of LABs are insignificantly different for the eight sites tested. The mean of counts of LABs are significantly different for the wet and dry seasons tested.
In conclusion, this study showed availability of lactic acid bacteria in the gut of Lake Victoria Nile perch which can be used as a source of potential bio preservative.
Key words: Lactic Acid Bacteria (LAB), gastrointestinal tracts, Nile perch, L. Victoria, ANOVA

Introduction:
L. Victoria is the second largest fresh water lake by area and has the world's largest fresh water commercial fishery, largely based on the introduced Nile perch; supporting economically and socially important export fishery for Riparian countries. The high losses resulting from spoilage and cross contamination by pathogenic microbes requires processing techniques like bio preservation that retains the natural characteristics of the product. There is limited information on the isolated Lactic acid Bacteria in the guts of Nile perch that would be important in enhancing organic processing of its products. Bacteriocinogenic lactic acid bacteria and their isolated bacteriocins are considered safe additives, useful to control the frequent development of pathogens and spoilage microorganisms in foods. The spreading of bacterial antibiotic resistance and the demand for products with fewer chemicals create the necessity of exploring new alternatives, in order to reduce the abusive use of therapeutic antibiotics. Bacteriocins are indicated to prevent the growth of undesirable bacteria in a food-grade and more natural way, which is convenient for health and accepted by the consumers.

Fresh water fish is highly perishable and starts to deteriorate as soon as it is landed. FAO (2010) reported quality losses to range between 20–40 percent while George, et al. (2009), reported post-harvest losses of 25-50% in African fisheries. In the Lake Victoria basin, the spoilage of fresh fish is compounded by ambient temperatures which accelerate microbiological multiplication, and a lack of cooling facilities. There are considerable quality losses due to lack of modern fish processing and lack of adequate preservation technology to keep freshness of the harvested fish. Furthermore, human infections caused by pathogens transmitted from fish are quite common (Onyango et al., 2009). The industry has suffered the rejection of fish products by the European Union between 1996 and 1999 due to contamination by Salmonella spp. (Mwangi, 2004).

Onyango et al., (2009) reported that Nile tilapia within Winam Gulf of Lake Victoria are infected by human enteric pathogens of which Shigella spp.,Salmonella spp. and E. coli were the most frequently isolated, an indication that the beaches may be contaminated by untreated municipal sewage, runoff, and storm-water. S. typhimurium, S. typhi and S. enteritidis were the most common Salmonella isolates.The growing interest in processing products to retain characteristics similar to those of natural products has been the push for developing processing techniques like bio preservation (Leroi, 2010). The reasons for processing food products range from the removal of anti-nutritional components, increasing the shelf-life of the

final product and adding value. Fermentation and dehydration are some of the earliest and most important food processing technologies (Nieves et al., 2001).

Many lactic acid bacteria (LAB), including members of the genera Lactococcus, Lactobacillus, Carnobacterium, Enterococcus, and Pediococcus, are known to secrete antimicrobial peptides or bacteriocins (van Reenen,et al.,1998, Ross, et al.,2002). Attempts to harness these compounds to control Listeria monocytogenes in dairy and meat products have involved the addition of bacteriocins directly to the food in a purified or partially purified form (Ross, et al., 2002). A second approach is through the addition of the bacteriocin-producing bacteria to the food matrix so to facilitate the growth and production of bacteriocins in situ (Ross, et al., 2002). Since lactobacilli are known to produce many different bacteriocins; addition of these bacteriocin producers has been effective in reducing L. monocytogenes populations in many fermented meats.

Review by Leroi, (2010) indicate that Carnobacteria, commonly found in seafood, are able to limit the growth of L. monocytogenes in sea food products and reports that current work is focusing on the screening for other LAB species with wide antimicrobial spectrum. Fall et al., (2010) evidenced the in situ inhibition of Brochothrixthermosphacta, a major spoilage bacterium, L. monocytogenes and S. aureus by Lactococcuspiscium that could explain the protective effect observed in shrimp. Matamoros et al., (2009) identified seven strains from the genus Leuconostoc in various marine products that are active against many spoiling, pathogenic, Gram-positive and -negative marine bacteria. However, there is limited information on the extraction of LAB from Nile perch fish and their enumeration. Hence the study was carried out to determine the presence and abundance of lactic acid bacteria from gastrointestinal tracts of Latesniloticus fish with the specific objective of determining fish size effect on LAB population.

Materials and Methods

Study Area - The fish samples were purchased at Muhuru, Sori, Homa-Bay, Uhanya, Gaba, Got Kachola, Dunga and Mbita beaches of Lake Victoria.

Sampling of Fish - Three different sizes of fish (1 to 999, 1000 to 1999 and 2000 grams up) were randomly sampled from selected landing sites for each of the two seasons (April-June and July-Sept). The weights and lengths of the whole fish were taken, labelled and the fish were immediately layered with flaked ice and packed in insulated containers and transported to Laboratory at Dairy and Food Science and Technology Department, Egerton University.Sample preparation and LABs Isolation - In the Laboratory fish gut was obtained under aseptic conditions for LABs isolation. 25grams of fish guts were aseptically weighed, placed in 225ml of sterilized peptone water in a blender and blended for 2minutes.The homogenates were serially diluted to 10^{-6} in 9-ml volumes of sterile peptone water and pour plated on MRS agar plates. Triplicate plates were incubated anaerobically at 10^{o}C for 10days (Leila, et al., 2009) to select psychrotrophics and at 37^{o}C for 48h to select mesophylls, followed by catalyse reaction, and oxidase, gram staining, morphology, production of gas from glucose, tests to confirm LAB. Gas production from glucose were determined in MRS-broth supplemented with 1% glucose containing inverted Durham tubes at 30 ℃ for 48 h. Gram positive colonies that were oxidase and catalase negative were isolated, restreaked and cultured on prepared MRS Agar for 24h. Finally, the single colony of bacteria was isolated by observing their colonial morphology and some physiological tests (Gram staining, catalase reaction, oxidase reaction and arginine test).

Total Anaerobic Bacteria: Bacterial density as cfu/g for 3 replicates were initially averaged and used for final calculation. All equipment and chemicals used were sterilized properly prior to use.

Results and Discussion

Lactic acid bacteria counts in gastrointestinal tract of Latesniloticus

Total bacteria grown on MRS indicated bacterial population level of 3.5 x 10^3 to 1.5 x 10^5cfu/g wet weight of gastrointestinal tracts of all samples .Statistically there was no difference between LAB counts extracted from the samples drawn from most sites except Mbita that showed significance difference from Got Kachola and Uhanya (Table 1). There is a small river (R. Lambwe) that fills the Lake from Mbita point while these other points have bigger rivers for example R. Yala joins close to Uhanya and R. Migori and R. Kuja joins close to Got Kachola points. The river inflows have an effect on the LAB counts possibly due to the materials brought in that forms the food of the fish. The bacterial count was within the range reported from stomachs of marine fish (Buntin, et al, 2008; Austin and Al-Zahrani, 1988; Ringo, 1993).Similarly, Buntin, et al, 2008 findings showed LAB population level of 4.5x10^4 to 10^5cfu/g wet weights of GI tracts from all tropical marine

fish samples analyzed and Austin and Al-Zahrani, 1988; Ringo, 1993 reported bacterial population level in the range of $2x10^4$ to 10^5 in stomachs of salmon fish.

The mean of counts of LABs are significantly different for the two seasons tested. The wet season has higher mean count than dry possibly due to food materials that become available to fish during rainy seasons.

Lake Victoria supports the most productive freshwater fishery in the world, with annual fish yields exceeding 300,000 tons worth US $600 million annually, Kayambo and Sven, 2006. Nile perch is the basis of a lucrative export industry supporting about 30 fish-processing factories in the three countries (Tanzania, Uganda and Kenya). The percentage weight of guts is averagely1.87%. Since guts are considered a waste and their disposal is a menace around the lake and to the processing factories, it can be used to extract lactic acid for use as probiotic cultures. Thus, out of 43,650 metric tons Nile perch landed in Kenya in 2009 (Manyala, 2011), 816.255M.tons of guts were disposed as wastes around processing facilities thus ending in water bodies.

Though, it is well known that LAB are not under normal circumstances numerically dominant in the digestive tract of fish (Ringo and Gatesoupe, 1998), results in this study shows otherwise. Ringo (1993) indicated that LAB from the stomach of Arctic charr, Salvelinusalpinus (L) was only a minor part of the micro biota approximately 10%. On the contrary, the results from this study indicated high population of LAB in all fish samples (20 to 50%) similar with the findings of Buntin et al, 2008 from the same geographical region. The high LAB population in this study could be accounted on the predatory feeding habits and the warm geographical location of the fish under study (Buntin et al, 2008). LABS from the gastrointestinal tracts of cold-water fish are slow-growing with respect to incubation time hence low population recovery.

Most colonies were Gram-positive bacteria with catalase and oxidase negative, indicating majority population was LAB. This could be due to the fact that Nile perch fish is a predator heavy feeder and the warm geographical location (Buntin et al, 2008). This research was conducted with warm-water fish whereas most researches regarding LAB in fish have been conducted in cold-water fish, so scientists encountered certain obstacles in isolation of LAB. Ringo and Gatesoupe (1998) reported three prime important factors when isolating LAB from fish, which are nutrient medium, incubation temperature and incubation time. This could be one of the reasons which contributed to failure of growth of LAB at 10^oC for 10 days in this study as fish were from warm waters. Furthermore, lactic acid bacteria tend to grow slowly at refrigeration temperatures as reported by Huis (1996).

Butin et al (2008) suggested that very high incubation temperature may have been one of the limiting factors for initial isolation of LAB from fish intestines and noted that LAB from the gastrointestinal tracts of cold water fish are slow-growing with respect to incubation time. This study found the reverse; no growth of LAB when samples were incubated at low temperatures of10^oC. He further reports that in most investigations, trypticase soy agar (TSA), Marine-medium or brain-heart infusion agar (BHIA)have been used for recovery of LAB from intestines and internal organs such as kidney, liver, spleen, etc. However, Shotts and Teska (1989) reported that 5% (v/v) bovine or rabbit blood agar or tomato juice agar were also very useful for initial isolation.

The effect of fish size on Lactic acid bacteria counts

The mean of counts of LABs are significantly different for the three sizes tested, with the big size giving the highest followed by medium and small respectively. This could be due to ability to access more variety of foods including eating smaller fishes.Table 3 above shows clear effect of size on the LABs isolated during both seasons; the bigger the fish the higher the counts of colony forming units. This could be due to the bigger attachment surface area; the variety of foodstuffs fish is able to access, including smaller fishes. The bigger fishes' ability to move far into deeper waters hunting for food and eat bigger pieces of foods possibly contributes to these differences.The guts of fish is sterile until hatching when the fish comes into contact with the environment and live food that leads to successive colonization by a variety of microbes (Ringo et al., 1996).

The average weight of small fish sampled during the wet season was 667.9g that gave an average colony forming units/g of $9.2x\ 10^3$; medium averaging 1485.1g had an average of $2.1x10^4$cfu/gand big averaging 3210.8g had $4.9\ x\ 10^4$cfu/g. During the dry season, the small fish averaging 614.6g had $6.7\ x\ 10^3$cfu/g, medium averaging 1392.9g had an average of $1.7x10^4$cfu/g and big averaging 2756.3g had $3.4\ x\ 10^4$cfu/g. There is significant difference in the means of counts between dry and wet seasons (Table 2). The LAB counts are significantly higher during the wet season compared to dry season which could be due to the increased availability of foods that fish accesses from runoffs in to the lake.Being a predator, with the young stages feeding

on invertebrates and large on haplochromine cichlids, the zooplanktivorous cyprinid Rastrineobolaargentea, the prawn Caridinanilotica and juvenile Nile perch (cannibalism); this would be possibly a cause for LAB population variation levels. This is similar to what Welker and Lim, 2011, reported that gut microbial composition can also vary seasonally and with changes in diet of Tilapia. Al-Harbi and Uddin, 2004, also discovered that the bacterial composition of tilapia gut can fluctuate considerably depending on the time of year. Even with the most dominant bacterial species, numbers (total counts and as a percentage of the total population) change dramatically during the course of a year, an indication that modifications of diet can also affect the micro biota composition, Welker and Lim, 2011. In their reviews on the use of probiotics in diets of Tilapia, it was similarly found out that the gut bacterial population correlated well with the predominant species found in the rearing water and pond sediment, showing that the rearing environment plays a large role in the gut microbial composition. In rainbow trout, the gut micro biota make-up is altered when fish were switched from a fish meal to a plant meal based diet, Heikkinen et al, 2006. The findings of Hansen et al., 1992;Munro et al., 1994; Ringo et al., 1996 showed that gut is sterile until hatching, but soon after hatching, the fish comes in contact with the environment and live food that leads to successive colonization by a variety of microbes. This could further explain the progressive LAB population variation with size.The population level of LAB associated with the digestive tract is affected by nutritional and environmental factors like dietary polyunsaturated fatty acids, chromic oxide, stress and salinity, Ringoe and Gatesoupe, 1998. Therefore, the decrease in LAB counts during dry season could be due to stress associated with the decrease in food availability.

Phenotypic and Biochemical characteristics of the extracted LABs

The phenotypic and biochemical characteristics of the LABs extracted are given in Table 4. The isolation of one genus Lactobacillus in this study could be partly accounted to the use of one type of nutrient medium (MRS Agar) as previous literature have indicated nutrient medium as a factor besides incubation temperature and incubation time (Muigei, 2013; Ringo and Gatesoupe, 1998; Huis, 1996). Morphological and phenotypic analysis of the extracted LABs indicated that there were two facultative lactobacillus groups; homofermenters and heterofermenters. In their work on sea products, El Bassi et al., 2009, reported 97% Lactobacillus and 3% Lactococcus out of 100 LABs whereas this study gave 100% Lactobacillus.

Conclusion and Recommendations

The study established the availability of lactic acid bacteria in the gastrointestinal tract of fresh Lake Victoria Nile perch and can be used as a good source of potential bio preservative. The bigger sizes of fish have higher densities during wet seasons. The stomachs and intestines that are a menace to the processing industries can be used to extract the LABs for bio preservation. Further studies should be done to find out if the same can be used to treat waste water.

Acknowledgement

The authors are grateful to the National Commission for Science, Technology and Innovation (NACOSTI) for financial support through a research grant. We also thank Egerton University Department of Food Science for kindly availing their laboratory facilities.

REFFERENCES

Al-Harbi, H.A. and Uddin, M.N. 2004. Seasonal variation in the intestinal bacteria flora of hybrid tilapia (Oreochromisniloticus x Oreochromisaureus) cultured in earthen ponds in Saudi Arabia. Aquaculture 250: 566-572.

Austin, B., and Al-Zahrani, A. M. 1988.The effect of anti-microbial compounds on the gastrointestinal micro-flora of rainbow trout, Salmogairdneri Richardson. J.Fish Biol. **33**: 1–14.

Buntin, N., Chanthachum, S. and Hongpattarakere, T. 2008. Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. Songklanakarin Journal of Science Technology.**30**, 141-148.

El Bassi, L., Shinzato, N., and Matsui, T. and Hassuna, M. 2009.Biopreservation of Refrigerated and Vacuum-Packed Dicentrarchuslabrax by Lactic Acid Bacteria. Journal of Food Science.**74** (6) M335—M339.

Fall, P.A., Leroi, F., Cardinal, M., Chevalier, F., and Pilet, M.F., 2010. Inhibition of Brochothrixthermosphacta and sensory improvement of tropical peeled cooked shrimp by Lactococcuspiscium CNCM I-4031, Letters of Applied Microbiology. **50**, 357-361.

FAO.2010. Post-harvest losses in small-scale fisheries – Case studies in five sub-Saharan African countries. FAO Fisheries and Aquaculture Technical Paper. No. 550. Rome, FAO. 2010.

George F.O. A., Ephraim, R.N., Obasa, S.O. and Bankole, M.O. 2009. Antimicrobial Properties of some plant extracts on organisms associated with fish spoilage. 1st African organic conference, Sheraton Hotel, Kampala, 18-22nd May 2009.

Hansen, G.H., Strøm, E., Olafsen, J.A., 1992. Effect of different holding regimes on the intestinal micro flora of herring Clupeaharengus larvae.Appl. Environ. Microbiol.**58:** 461–470.

Heikkinen J, Vielma J, Kemilainen O, Tiirola M, Eskelinen P, et al. (2006) Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (Oncorhynchusmykiss). Aquaculture**261**: 259-268.
HuisIn't Veld JH.J., 1996. Microbial and Biochemical Spoilage of Foods: An Overview. Int. J. Food Microbiol.**33**:1–18
Kayambo S and Sven EJ (2006) Lake Victoria. Experience and lessons learned brief. Pp. 431- 446.
Leila, E.B., Mnasser, H., Naoya S. and Torumatsui, 2009.Biopreservation of refrigerated and vacuum-packed Dicentrarchuslabraxby Lactic Acid Bacteria. Journal of Food Science.**74** 335-339.
Leroi, F., 2010.Occurrence and role of lactic acid bacteria in seafood products. Food Microbiology.**27** (6): 698-709.
Nieves, S., Gabriela, C. and Jose, B. 2001. The effect of blanching on the quality of dehydrated broccoli florets. European Journal of Research and Technology.**213**: 474-479.
Manyala, J. O., 2011. Fishery Value Chain Analysis: Background Report – Kenya.
Matamoros, S., Pilet, M.F., Gigout, F., Prevost, H., and Leroi, F., 2009.Selectionand evaluation of seafood-borne psychrotrophic lactic acid bacteria as inhibitors ofpathogenic and spoilage bacteria.Food Microbiology.**26**: 638-644.
Muigei1, S.C.; Shitandi, A.; Muliro, P. and Ogata Rose Bitonga, O.R. 2013. Production of Exopolysaccharides in the Kenyan Fermented Milk, Mursik. International Journal of Science and Research **2** (12): 79-89.
Munro, P.D., Barbour, A., Birkbeck, T.H., 1994. Comparison of the gut bacterial flora of start- feeding larval turbot reared under different conditions. J. Appl. Bacteriol. **77:** 560–566.
Mwangi C. M. 2004. Quality of Lake Victoria Nile Perch (Latesniloticus) at the Landing Facility as a Potential Microbiological and Organ chlorine Pesticide Residue Hazards Critical Control Point.MSc(Food Science) Thesis-University of Nairobi, Kenya.
Onyango, M. D., Wandili S., Kakai, R. and Waindi, E. N. 2009. Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. J Infect Developing Countries.**3 (2):**99-104.
Ringo, E. and Gatesoupe, F. 1998. Lactic acid bacteria: A review. Aquaculture, **160**: 177–203.
Ringo, E., 1993. Does dietary linoleic acid affect intestinal microflora in Arctic charr, Salelinus alpinus. Aqua-cult.Fish. Manage.**24**: 133-135.
Ringo, E., Birkbeck, T.H., Munro, P.D., Vadstein, O., Hjelmeland, K., 1996. The effect of early exposure to Vibrio Pelagius on the aerobic bacterial flora of turbot, Scophthalmus maximus L. larvae.J. Appl. Bacteriol. **81**: 207–211.
Ross, R.P., Morgan, S. and Hill, C. 2002. Preservation and fermentation: past, present and future. International Journal of Food Microbiology **79**: 3 – 16
Van Reenen, C.A., Dicks, L.M.T. and Chikindas, M.L. 1998.Isolation, purification and characterization of plantaricin 423, a bacteriocin produced by Lactobacillus plantarum. Journal of Applied Microbiology.**84**: 1131–1137.
Welker, T.L and Lim, C., 2011.Use of Probiotics in Diets of Tilapia. Journal of Aquaculture Research Development. S1:014

Table 1 The LAB count (cfu/g) from guts of Nile perch fish from 8 different sites of L. Victoria

site	mean counts of LABs (cfu/g)
Mbita	38528[a]
Sori	28139[ab]
Muhuru	27161[ab]
Ggaba	26894[ab]
Dunga	25878[ab]
Homabay	24456[ab]
Got Kachola	19806[b]
Uhanya	16983[b]

Means in same column followed by the same letter are not significantly different (P< 0.05)

Table 2 The Means of LAB count (cfu/g) from guts of Nile perch fish during two different seasons of L. Victoria

season	mean counts of LABs (cfu/g)
Wet(April-June 2011)	30213[a]
Dry(July-Sept 2011)	21749[b]

Means in same column followed by the same letter are not significantly different (P< 0.05)

Table 3 The LAB count(cfu/g) from guts of 3 different sizes Nile perch fish

sizes	mean counts of LABs (cfu/g)
Big(1-999g)	48083[a]
Medium(1000-1999g)	20942[b]
Small(2000-4500g)	8917[c]

Means in same column followed by the same letter are not significantly different (P< 0.05)

Table 4: Phenotypic and Biochemical characteristics of the extracted LABs

Genus		**Rods/Coccobacilli**				
		Lactobacillus (homofermentaters)	Lactobacillus (heterofermentaters)	Weissella		Leuconostoc
				Arginine negative	Arginine positive	
No of isolates		8	1	0	0	0
Cell morphology		Rods	Rods	Rods	Coccobacilli	Coccobacilli
Cell arrangement		Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains
Phenotypic Characteristics						
1. Gas production in MRS broth		-	+	+	+	+
2. Arginine hydrolysis		±	+	-	+	-
3.Isolation Temperature	10°C	-	-	±	±	+
	30°C	+	+	+	+	+
	40°C	+	+	+	+	±
4.Tolerance to Sodium Chloride (NaCl)	2%	+	+	+	+	+
	4%	+	+	±	±	±
	6.5%	+	+	±	±	±
5. Vancomycin resistance (30 mcg disc): S- susceptible R- resistant		R/S	R/S	R/S	S	S