THE *IN VITRO* EFFECTS OF LACTIC ACID BACTERIA SCREENED FROM GASTROINTESTINAL TRACTS OF *LATES NILOTICUS* ON *E. COLI* AND *SALMONELLA* SPP.

OBAR JAMES ADERO

A Thesis Submitted to the Graduate School in Partial fulfilment for the requirement of the award of Master of Science Degree in Food Science, Department of Dairy and Food Science and Technology of Egerton University

Egerton University

October 2015

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been presented in any other institution/university for the award of any degree.

Signature……………………………………. **Date……………………**

Mr. Obar James Adero
KM16/2185/08

APPROVAL BY THE SUPERVISORS

We confirm that this thesis has been submitted with our approval as the University Supervisors

Signature………………………………….. **Date…………………..**

Prof. Alfred A. Shitandi, Ph.D.
Professor of Food Microbiology
Department of Dairy and Food Science and Technology
Egerton University

Signature…………………………………. **Date……………………**

Prof. Symon M. Mahungu, Ph.D.
Professor of Food Chemistry
Department of Dairy and Food Science and Technology
Egerton University

COPYRIGHT

© 2015, **Obar James Adero**

All rights reserved. No part of this thesis may be used or reproduced in any manner whatsoever or translated to any other language or otherwise without prior written permission of the author, except in the case of brief quotation embodied in critical articles or reviews for academic purposes only.

DEDICATION

This thesis is dedicated to my mother Roslidah Auma Obar, my wife Joyce Akoth Olal, and my children Geoffrey Griffins Adero, Remmy Quincy Adero and Brina Ashley Adero

ACKNOWLEDGEMENT

First and foremost I wish to thank the Almighty God for His providence, guidance and protection throughout this study. Secondly, I wish to acknowledge all parties who took part in making the whole undertaking a success. I wish to acknowledge the contribution made by the Department of Dairy and Food Science and Technology for accepting my studentship and offering facilities for the research including provision of the technical knowhow. My sincere thanks and appreciation goes to the Kenya National Council of Science, Technology and Innovation (NACOSTI) for funding this research. My appreciation goes to my supervisors Professor Alfred A. Shitandi and Professor Symon M. Mahungu for their guidance and supervision of this work up to the very end. My acknowledgement goes to my colleagues Maxwel Juty, Oloo Ben, Jared Omollo, Salome and Misiko for their mutual and social support. Special thanks go to Professor Abdul Faraj (Chairman, Department of Dairy and Food Science and Technology) and Professor J.W. Matofari for their continuous support during and after laboratory work. I wish also to extend my thanks to the Board of Post Graduate Studies and Egerton University as a whole for giving me the opportunity to study in the institution. Lastly, I wish to thank my dear wife Joyce Akoth and our children; Griffins Adero, Remy Adero and Brina Adero for their social, financial and material support during the whole period of study.

ABSTRACT

Biopreservation systems in foods are of increasing interest in the food industry. It is the use of naturally occurring microorganisms and/or their inherent antibacterial compounds of defined quality and quantity to extend shelf life and to enhance the safety of foods. Bacteriocinogenic LAB and/or 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 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 community. This study was carried out to screen lactic acid bacteria from gastrointestinal tracts of *L. niloticus* fish and to assess their *in vitro* effects on *S. enteritica* and *E. coli*. *S. enteritica* was chosen considering its being the causal agent of the largest number of enteric infections in the world, while *E. coli* as an indicator microorganism. A randomized complete block design was used. Random sampling was carried out at the landing sites on L. Victoria. A 25g portion of each GIT sample was aseptically obtained from fish followed by blending in 225ml sterile peptone water for two minutes in a blender. For bacterial enumeration and isolation, threefold serial dilutions of gut homogenates was pour plated in and spread plated on the surface of dry RMS agar and incubated at 37°C for 48 hours. The cultures of LAB were purified and identified (phenotypically and biochemically).
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. A total of 96 fish samples were analyzed and 9 Lactobacillus isolates were identified, out of these four (B4, M4, G4 and E4) were identified to have anti-bacterial properties. The Lactobacillus isolates showed an inhibitory spectrum against the indicator organisms tested. Lactobacillus isolate B4 exhibited the highest degree of inhibition against *S. enteritica* and *E .coli* followed by M4, G4 and E4 respectively. In conclusion, this study showed availability of lactic acid bacteria in the gut of L. Victoria Nile perch and can be used as a good source of potential bio preservative. Further research required to identify these LABs to species level and the metabolites responsible for inhibition.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION ... ii

COPYRIGHT ... iii

DEDICATION ... iv

ACKNOWLEDGEMENT ... v

ABSTRACT ... vi

TABLE OF CONTENTS ... vii

LIST OF TABLES ... ix

LIST OF FIGURES ... x

LIST OF APPENDICES ... xii

LIST OF ABBREVIATIONS ... xii

CHAPTER ONE ... 1

1.0 INTRODUCTION ... 1

1.1 Background information ... 1

1.2 Statement of the Problem ... 3

1.3 Objectives ... 3

1.4 Null hypotheses. ... 3

1.5 Justification ... 4

CHAPTER TWO ... 5

2.0 LITERATURE REVIEW ... 5

2.1 Nile perch ... 5

2.2 Chemical Intervention Technologies ... 6

2.3 Lactic acid bacteria ... 6

2.4 Practical Applications of LABs as Probiotics and biopreservatives ... 9

2.5 Nisin as a model food preservative ... 10

CHAPTER THREE ... 12

3.0 MATERIALS AND METHODS ... 12

3.1 Study Site ... 12

3.2 Sampling ... 12

3.2.2 Sample preparation ... 12

3.3 Microbial determination ... 14

3.4 Grouping of lactic acid bacteria 14
3.5 Test strains 15
3.6 *In vitro* antibacterial activity. 15
3.7 Data analysis 15
CHAPTER FOUR.. 16
4.0 RESULTS AND DISCUSSION 16
4.1 Lactic acid bacteria counts in gastrointestinal tract of *Lates niloticus*.................. 16
4.2 The effect of fish size on LAB counts 216
4.3 In vitro antibacterial activity 22
5.0 CHAPTER FIVE 28
5.1 CONCLUSIONS 28
5.2 RECOMMENDATIONS 28
6.0 REFERENCES.. 29
7.0 APPENDICES 41

LIST OF TABLES

Table 3. 1: Sampling 13
Table 4.1.1: The LAB count (cfu/g) from guts of Nile perch fish from 8 different sites of L. Victoria……………………………………………………………………………………..17
Table 4.1.2: The weights of gastrointestinal tracts, percentage vis-à-vis gross fish weights. 19
Table 4.1.3: Phenotypic and biochemical characteristic of the extracted LABs. 20
Table 4.2.1: The LAB count (cfu/g) from guts of 3 different sizes of Nile perch fish. 21
Table 4.2.2: ANOVA table showing level of difference for the 3 different sizes. 21
Table 4.3.1: Diameter of zones of inhibition (cm) produced by Lactobacillus isolates on the two test strains as assessed by the well diffusion method. 23
Table 4.3.2: Mean comparison of inhibition zones (cm) produced 24

LIST OF FIGURES

Figure: 4.1.1a and 4.1.1b: Inhibition zones from antimicrobial activity of LAB B on *Salmonella enteritica* and *E. coli* respectively 25

Figure: 4.1.2a and 4.1.2b: Inhibition zones from antimicrobial activity of LAB G on *Salmonella enteritica* and *E. coli* respectively 25

Figure: 4.1.3a and 4.1.3b: Inhibition zones from antimicrobial activity of LAB Mi on *Salmonella enteritica* and *E. coli* respectively 26

Figure: 4.1.4a and 4.1.4b: Inhibition zones from antimicrobial activity of LAB E on *Salmonella enteritica* and *E. coli* respectively 26

LIST OF APPENDICES

Appendix 1: Map of Lake Victoria 41

Appendix 2**:** Concentrated LAB culture on MRS agar……………………………………….42

Appendix 3: Streaked purified colonies of LAB G 42

Appendix 4: Photos of LAB B under microscope 42

Appendix 5: Photos of LAB E under microscope 43

Appendix 6**:** Photos of LAB G under microscope……………………………………………43

Appendix 7: Photos of LAB Mi under microscope 43

Appendix 8a and 8b: Photos of James and Maxwel during laboratory work ………………..…44

Appendix 9: Photos of LAB B under microscope 42

Appendix 10: Photos of LAB E under microscope 43

Appendix11**:** Photos of LAB G under icroscope………………………………………………...43

Appendix 7: Photos of LAB Mi under microscope 44

Appendix 8a and 8b: Photos of James and Maxwel during laboratory work ………………..…44

Appendix 9: Antagonistic activity of isolated LAB against two indicator strains; S.enteritica and E. coli at 1.4cm diameter well 45

Appendix10**:** Antagonistic activity of isolated LAB against two indicator strains; S.enteritica and E. coli at 0.2cm diameter well……………………………………………………………...45

Appendix 11: LAB counts and means for wet and dry seasons 46-48

Appendix 12: Authors own publications……………………………….. ………………..…49

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BHIB	Brain Heart Infusion Broth
Cfu/ml	Colony forming units per millilitre
FAO	Food and Agriculture Organisation
RCBD	Randomized Complete Block Design
LAB	Lactic acid bacteria
LSD	Latin Square Design Test
MHA	Muller Hinton Agar
MRS	deMan Rogosa Sharpe
SAS	Statistical Analysis for Sciences
GRAS	Generally Recognised as Safe
NACOSTI	National Commission of Science, Technology and Innovation
SD	Standard deviation
CV	Coefficient of variation

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background information

The Kenyan fisheries sub-sector supports about 55,000 fishermen and women and 800,000 individuals indirectly, through local income generation and export earnings (FAO, 2010). The sub-sector also plays an important role in meeting the food security and nutritional requirements of the riparian populations (Fisheries Department, 2006). In 2009, the fisheries sub-sector earned the country over KShs. 4.17 billion in foreign exchange from the export of 18,506 metric tons of fish and fisheries products (Fisheries Department, 2009). 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 percent in African fisheries. In the Lake Victoria basin, the spoilage of fresh fish is compounded by ambient temperatures which accelerate microbiological multiplication due to lack of cooling facilities. There are considerable quality losses due to lack of modern fish processing and adequate preservation technologies 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 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. enteritica* were the most common *Salmonella* isolates.

The growing interest in processing products to retain intrinsic characteristics has been the push for developing processing techniques like biopreservation (Leroi, 2010, Leila *et al*, 2009, Buntin, *et al,* 2008). 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 small, ribosomally synthesized antimicrobial peptides called bacteriocins (van Reenen *et al.* 1998). Bacteriocins are proteinaceous, antimicrobial compounds produced by many kinds of bacteria (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 as 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.

Reviews by Leroe (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 *Brochothrix thermosphacta*, a major spoilage bacterium, *L. monocytogenes* and *S. aureus* by *Lactococcus piscium* 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. Ndaw *et al* (2008) succeeded in inhibiting *Staphylococcus aureus*, *Salmonella* spp. and sulfite-reducing *Clostridia* using *Lactobacillus delbrueckii subsp. Delbrueckii* in sardine fillets. Studies by Altieri *et al*. (2005) found inhibition of *Pseudomonas* spp. and *P. phosphoreum* in Vacuum Packaging fresh plaice fillets at low temperatures by using a *Bifidobacterium bifidum* starter, and extending the shelf-life, especially under MAP. A French company commercializes a strain of patented *Lactococcus lactis* (patent no. PCT/FR02/03180, 2001) for an application in shrimp but most of the companies producing microbial starters do not sell LAB for a specific seafood application.

However, there is limited information on the extraction of LAB from Nile perch fish and their subsequent *in vitro* tests on pathogenic bacteria. Thus, the objective of this study was to evaluate the *in vitro* antimicrobial activities of LABs isolated from the guts of Lake Victoria Nile Perch fish on *Salmonella enteritica* and *E .coli. S. enteritica* was chosen considering its being the

causal agent of the largest number of enteric infections in the world (Onyango *et al.*, 2009), while *E. coli* as an indicator microorganism

1.2 Statement of the Problem

The purpose of this study was to find a more natural and acceptable way of preserving fish using lactic acid bactera isolated from gastrointestinal tracts of *Lates niloticus.*The losses incured by the stakeholders in the Lake Victoria Nile perch industry due to rejection of *Salmonella* contaminated fish products by the consumers requires attention and sustainable solution. Lack of acceptable organic additives that are antagonistic to *E. coli* and pathogenic *Salmonella* spp. calls for search of natural organic materials.

1.3 Objectives

1.3.1 General Objective

To find a more acceptable way of preserving fish using lactic acid bacteria isolated from gastrointestinal tracts of *Lates niloticus* hence contribute to food security.

1.3.2 Specific Objectives

1. To extract and identify Lactic acid bacteria strains in the gastrointestine tract of *L.niloticus* harvested from Lake Victoria.
2. To determine the effect of fish size on Lactic acid bacteria counts.
3. To test these lactic acid bacteria *in vitro* for their bacteriostatic effect on *Salmonella enteritica* and *E. coli.*

1.4 Null hypotheses.

1. There are no Lactic acid bacteria in the intestines and stomach of Nile perch (*Lates niloticus*) fish.
2. The size of fish has limited effect on Lactic acid bacteria counts in the gut.
3. These Lactic acid bacteria extracts have no bacteriacidal effects on *Salmonella enteritica* and *E. coli*.

1.5 Justification

Bacteriocinogenic lactic acid bacteria and/or their isolated bacteriocins are considered safe additives, able to control the frequently developing pathogens and spoilage microorganisms in foods and feed. Bacteriocins are indicated to prevent the growth of undesirable bacteria in a food and are considered a natural process, convenient for health and accepted by the consumers. The losses of 25-50% incurred by the stakeholders in L. Victoria Nile perch industry justify the need to find an acceptable method for fish preservation. The LAB extracts from the intestines of fish, which is currently a waste around L. Victoria and its potential use as a biopreservative, could be of benefit in mitigating the challenges presently faced by fishermen and processors.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 Nile perch

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 Nile perch (*Lates niloticus*) is a large freshwater fish found mostly in the rivers and lakes of Africa (Megapesca1997). Locally known as Mbuta (Luo) or Sangara (Kiswahili), can grow up to 200kg in weight and measure two meters in length. It was introduced to Lake Victoria in 1954 where it has contributed to the extinction of more than 200 endemic fish species through predation and competition for food. It is a large predator; feeding on *haplochromine cichlids*, the zoo planktivorous cyprinid *Rastrineobola argentea*, the prawn *Caridina nilotica* and juvenile Nile perch (cannibalism).Young stages feed on invertebrates. In Lake Victoria, male size at first maturity is 50-55cm TL (2 years), females 67.5-85cm TL (2-4 years). Fifty percent maturity at 60-74cm TL for males and 102-110cm TL for females. At the processing factories, the fish are offloaded, washed, sorted and weighed; Snoeks, 2005. The fish are then filleted by hand without gutting and the fillets then skinned and trimmed by hand as well.

The Lake Victoria Nile perch fish flesh have been shown to have the following nutritional profile; ash (%) 0.55-0.63, lipid (%) 0.59-6.3, Protein (%) 19.8- 17.7, Moisture (%) 78.5-79.6 (Okeyo *et al.*, 2009**)**. According to Okeyo, *et al.,*2009*,* the ungutted iced Nile perch handled under normal conditions in Lake Victoria fishery with a delay of 3-4 hours before icing, has shelf life of 22 days, while that which is iced on board has a shelf life of 28 days. Spoilage is caused by high temperatures, poor transportation, inadequate preservation methods and poor handling methods among others, and these results into losses of 25-50% in African fisheries (George, *et al.*, 2009).

2.2 Chemical Intervention Technologies

To date, the application of biopreservation is an additional hurdle to prevent transmission of foodborne pathogens and reduce the use of chemical additives especially the use of chlorine and chlorine-based sanitizers in fish processing industries, allowing minimal food processing treatments without compromising food safety. Furthermore, the application of chlorine and chlorine-based sanitizers at high concentrations has been linked to the generation of toxic and potentially carcinogenic by-products, including trihalomethanes, bromate, haloacetonitriles, and haloacetic acid (LeChevallier and Au 2004; Gopal *et al.,* 2007; Wang *et al.,* 2007). The most popular and frequently used chemical sanitizers in fish processing facilities and products are chlorine and chlorine-based sanitizers, hydrogen peroxide, neutral electrolyzed water, organic acids, ozone, polyphosphates, and potassium sorbate (Yuk *et al*., 2014). The use of chlorine and chlorine-based sanitizers has limitations such as having low efficacies when high amounts of organic matter are present and are also less stable at elevated temperatures and may adversely affect the organoleptic qualities of fish by inducing lipid oxidation (Ray 2003).

2.3 Lactic acid bacteria

Lactic acid bacteria (LAB) are widely distributed in various animal intestines (Mitsuoka, 1980; Sakata *et al*., 1980; Devriese *et al*., 1987; Perdigon *et al* ., 1995; Salminen and Wrightm, 1998) and some LABs due to their probiotic nature have played an important role in beneficial functions for industrial animals (Perdigon *et al* 1995). Studies have shown that some LAB improve the intestinal microflora and promote the growth and health of animals (Perdigon *et al* 1995). Most probiotics contain single or multiple strains of LAB and are part of the natural microflora of many animals; they are generally regarded as safe and may display antagonistic activities against pathogenic bacteria (Byun *et al*., 1997; Garriga *et al.,*1998).

The intestinal microflora, especially LAB, may influence the growth and health of fish. Although not common, it is generally accepted that LAB occur among the normal intestinal microbiota of fish from the first few days after hatchery (Yang *et al*., 2007; Ringo, 2008). These LAB are largely Gram positive, nonmotile, non-sporulating bacteria that produce lactic acid as a single major product of fermentative metabolism (Ringo *et al.,* 2001).

Lactic acid bacteria are used as natural or selected starters in food fermentations in which they perform acidification due to production of lactic and acetic acids flavour. Protection of food from spoilage and pathogenic microoganisms by LAB is through producing organic acids, hydrogen peroxide, diacetyl (Messens and De Vugst, 2002), antifugal compounds such as fatty acids or phenullactic acid and /or bacteriocins (De Vugst and Vandamme, 1994). LAB play an important role in food fermentation as the products obtains with their aid are characterised by hygienic safety, storage stability and attractive sensory properties. The LAB of importance for foods belong to the genera of *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Paralactobacillus, Pediococcus*, *Streptococcus* and *Weissella* (Sharpe, 1979; Kandler and Weiss, 1986; Axelsson, 1993; Vandamme *et al*., 1996; Stiles and Holzapfel, 1997;Ringo *et al*, 1998; Salminen and Von Wright, 1998 ; Leisner *et al*.,2000; Huber *et al*,, 2004).

Lactobacillus plantarum, have been found in pollock (Schroder *et al*., 1980),cod (Strom and Olafsen, 1990), Atlantic salmon (Ringo *et al*., 1997) and Arctic char (Ringo *et al*., 1998). Carnobacteria, including *Carnobacterium maltaromaticum*, *divergens, gallinarum* and *inhibens*, have been isolated from the above mentioned species, as well as from rainbow trout (Jöborn *et al*., 1999; Ringo *et al*., 2001; Huber *et al*., 2004). Carnobacteria have been quoted as being the dominant genus in the gastro-intestinal tract of juvenile Atlantic salmon (Ringo *et al*., 1997) and cod (Seppola *et al*., 2006). Others have reported the presence of *Leuconostoc mesenteroides, Lactococcuspiscium, Vagococcus salmoninarum, Lactobacillus fuchuensis, Streptococcus* spp., and *Weissella* spp. (Wallbanks *et al*., 1990; Williams *et al*., 1990; Ringo and Strom, 1994; Ringo and Gatesoupe., 1998; Liu *et al*., 2009; Matamoros *et al*., 2009). Factors, like salinity of the water or stress, can affect the presence of LAB (Ringo and Strom, 1994).

Although most LABS are generally recognized as safe by the US Food and Drug administration, their implication in fish disease has been reported. In marine farmed fish, numerous epizootics linked to streptococci have been notified, beginning in Japan and North America then spreading worldwide (Eldar *et al*., 1996). These bacteria, reclassified as *Lactococcus garvieae,* are responsible for septicemias, opthalmias and hemorrhages. *C. maltaromaticum* has been isolated from different diseased fish and its virulence has been clearly established in rainbow trout and

striped bass experimentally infected (Baya *et al*., 1991; Toranzo *et al*., 1993). A novel *Weissella* species has been described as an opportunistic pathogen for rainbow trout (Liu *et al*., 2009).

Lactic acid bacteria (LAB) have been used in industrial manufacture of fermented food products and are known to inhibit the growth of other bacteria by synthesizing a wide variety of low molecular weight antibiotics, metabolic (end) products, enzymes, defective bacteriophages, lytic agents and bacteriocins(Pal and Ramana 2010). Bacteriocins produced by LAB are ribosomally synthesized antimicrobial proteins/peptides, and some of these undergo posttranslational modifications (van Reenen *et al.* 1998). Most LABS have the GRAS (generally recognized as safe) status, and the use of LAB or their metabolites for food preservation is generally accepted by consumers as “natural” and “health promoting.”

The use of antimicrobial peptides (bacteriocins) produced by LAB as a natural food preservative has gained worldwide attention in recent years because of their inhibitory activity against food spoilage and food borne pathogenic bacteria (Pal and Ramana 2010). Bacteriocins associated with food preservation belong to heat stable class I lantibiotics (nisin) and class II small peptides (pediocin AcH/PA1). The classic example of a commercially successful, naturally produced bacteriocin is nisin. Known since 1928 to be produced by some *Lactococcus lactis* isolates and structurally characterized in 1971 as a lanthionine-containing peptide, nisin and nisin-producing strains have had a long history of application in food preservation, especially of dairy products (Jack *et al.* 1995). However, the use of nisin is limited because of its low solubility in water, narrow pH range (inactive at and above pH 7.0) and low antilisterial activity. Pediocin can be an ideal substitute for nisin as it is an antilisterial protein active over a wide pH range, heat stable, class IIa bacteriocin (Rodriguez *et al.* 2002). Several bacteriocins from *Pediococcus* species have been characterized biochemically and genetically, and their mode of action has also been investigated (Bhunia *et al.* 1988; Cintas *et al.* 1995; Kim *et al.* 2000; Jamuna and Jeevaratnam 2004; Albano *et al.* 2007). Pediocin has been used successfully as an antimicrobial treatment to control the growth of food borne pathogens (Bari *et al.* 2005; Nieto-Lozano *et al.* 2006). On the assumption that a specific bacteriocin will have its own unique properties and usefulness in targeting microbial pathogens, studies on characterization of new bacteriocins will always prove beneficial (Pal and Ramana, 2010). The present study will focus on the isolation, identification

and application of abundant LAB from *Lates niloticus* intestines to inhibit spoilage in fish/fillet and/or pathogenic bacteria multiplication in media.

Many studies have been conducted on the selection of bacteria with antimicrobial properties that could be inoculated at high level in seafood in order to inhibit the growth of undesirable micro-organisms. LABS are good candidates for biopreservation technology as they produce a wide range of inhibitory compounds (organic acids, hydrogen peroxide, diacetyl and bacteriocins). In addition, they often have the GRAS status granted by the US-FDA and benefit from the healthy image associated with dairy products (Rodgers, 2001). Despite the promising results obtained on the selection of bacteria exhibiting antimicrobial properties in liquid medium and the number of bacteriocins characterized is increasing every day, very few commercial applications have appeared in seafood products (Leroi, 2010) as compared to dairy and meat products, thus necessitating research.

A major hurdle is that these products are not fermented and the selected LAB strain should not change their organoleptic and nutritional qualities. Many bacteria that have showed promising results in liquid medium have proved to be ineffective in products, either because they were poorly established in the environmental conditions (Wessels and Huss, 1996), or because they produced unpleasant odours (Nilsson *et al*., 1999). Nevertheless, the importance of LAB in semi-preserved fish has been reported (Truelstrup Hansen *et al*., 1996; Basby *et al*., 1998; Leroi *et al*., 2001; Cardinal *et al*., 2004; Lyhs *et al*., 2007; Jaffrès *et al*., 2008; Andrighetto *et al*., 2009), and research into this subject has intensified to prevent growth of pathogenic and spoiling bacteria.

2.4 Practical Applications of LABs as Probiotics and biopreservatives

There is interest in novel approaches for minimal processing and the exploitation of microbial metabolites such as bacteriocins for biopreservation (Ross, *et al* 2002). The bacteriocin nisin is the lantibiotic which has found application as a shelf-life extender in a broad range of dairy and nondairy products worldwide, ranging from processed and cottage cheese to dairy desserts and liquid egg (De Vuyst and Vandamme, 1994). Bacteriocins of lactic acid bacteria have been found to inhibit the growth of food borne pathogens and spoilage micro flora, and hence, can be used as natural preservatives in the production of foods with enhanced shelf life and/or safety (Pal *et al*., 2009). With the emergence of antibiotic-resistant Gram-positive pathogenic bacteria, the

possibility of bacteriocins as supplements or replacements for antibiotics for therapeutic use is also being considered. Very few antimicrobial peptides have been used as preservatives in the food industry and/or as antibiotic substances in medicine. However, an increasing number of bacteriocins are being tested in model systems, some of them show promising results and may reach commercial acceptance in the near future (Pal *et al*., 2009). Altieri *et al*. (2005) succeeded in inhibiting *Pseudomonas* spp. and *P. phosphoreum* in Vacuum Packaged fresh plaice fillets at low temperatures by using *Bifidobacterium bifidum* starter and extending the shelf-life, especially under Modified Atmospheric Packaging.

Dhaw, *et al.* (2008), indicated that controlled LAB fermentation could be used as a successful process for biopreservation of sardines produced in huge quantities. A small French company commercializes a strain of patented *Lactococcus lactis* (patent n° PCT/FR02/03180, 2001) for an application in shrimp, but most of the biggest companies producing microbial starters do not sell LAB for a specific seafood application (Leroi, 2010).Pal *et al*., 2009,purified two bacteriocins from *Pediococcus pentosaceous* strains, which inhibited the growth of *Staphylococcus aureus* in liquid medium and *Salmonella typhimurium* in orange juice, justifying their potential as food biopreservatives. The bacteriocin was also found to be effective in such applications as inhibiting spoilage bacteria during beer and wine fermentations, while the exploitation of nisin-producing strains has been shown to improve certain vegetable fermentations. The success of nisin has prompted many research groups to search for novel bacteriocin-producing strains over the last 20 years. This has resulted in a growing arsenal of potential biopreservatives which may be used either singularly or in combination to protect food from spoilage and safety problems. These biopreservatives can be used in a number of ways in food systems from the use of the bacteriocin-producing strains directly in food as starter or protection cultures to the use of concentrated bacteriocin preparations as food additives in food systems. Fermented foods for which Lactic acid bacteria play a major role have been cited by Wong, *et al.,* 2007. Microorganisms commonly used as probiotics have been cited by O'May, *et al.,* 2005.

2.5 Nisin as a model food preservative

Nisin is a ribosomally synthesized peptide that has broad-spectrum antibacterial activity, including activity against many bacteria that are food-spoilage pathogens (Delves-Broughton, 1990). Nisin is produced as a fermentation product of a food-grade bacterium, and the safety and

efficacy of nisin as a food preservative have resulted in its widespread use throughout the world (Vandamme, 1984; Qiao *et al*., 1997). Nisin is a member of the class of antimicrobial substances known as lantibiotics, so called because they contain the unusual amino acid lanthionine (Qiao *et al*., 1997). Lantibiotics, in general, have considerable promise as food preservatives, although only nisin has been sufficiently well characterized to be used for this purpose.

Nisin, a GRAS (generally recognized as safe) compound, is the only approved bacteriocin for use in foods. Nisin is primarily effective against Gram-positive microorganisms, such as Micrococcus, Streptococcus, Lactobacillus, and Staphylococcus species, and inhibits the germination of bacterial endospores, such as Bacillus and Clostridium species (Delves-Broughton, 1990). Nisin is commercially made by the fermentation of Lactococcus lactis subsp. lactis in pH controlled batch fermentations using a milk-based fermentation medium (Qiao *et al*., 1997; Vandamme, 1984).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study site was the fish landing sites of Lake Victoria (Appendix 1: map of Lake Victoria). The fish samples were purchased at Muhuru (1° 01´S; 34° 06´E), Sori (0°50´S; 34° 09´E), Homa-Bay (0°31´S; 34° 27´E), Uhanya (0°50´S; 34° 09´E), Gaba(0°15´N; 32° 37´E), Got Kachola, Dunga(0°05´S; 34° 44´E) and Mbita(0°25´S; 34° 12´E) beaches.

3.2 Sampling

3.2.1 Sampling of Fish

Samples were randomly obtained from six different landing sites on the Lake Vitoria. Three different sizes of fish i.e. 1 to 999, 1000 to 1999 and 2000grams up were randomly sampled from each of the six randomly selected landing sites for each of the two seasons (April-June and July-Sept, 2012; Table 3.1). 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 the Food Laboratory of Dairy and Food Science and Technology Department of Egerton University.

3.2.2 Sample preparation

The fish were washed with running drinking tap water to remove any dirt or slime. The hands, knife and table were sterilized using 70% alcohol. The fish were placed on clean stainless steel surface and inverted with the belly facing upwards. The anal pores of the fish were located and cut in a V or notch shape. The knife was pointed into the cut and drawn towards the head splitting the fish to the base of the gills ensuring no damage to the gut. The fingers of the hand holding the knife were used to remove the gut from the cavity. The guts of thirty pieces of fish were weighed against each and recorded to determine the percentage.

Table 3.1: Sampling

Landing site	Code	April to June 2012			July to Sept 2012		
		small	medium	Big	small	medium	big
Muhuru	A	2 (A1,A2, A3)	2 (A4,A5,A 6)	2 (A7,A8,A 9)	2 (A10,A1 1,A12)	2 (A13,A1 4,A15)	2 (A16,A17,A 18)
Sori	B	2 (B1,B2,B 3)	2 (B4,B5,B6)	2 (B7,B8,B9)	2 (B10,B11 ,B12)	2 (B13,B14 ,B15)	2 (B16,B17,B 18)
Homa Bay	C	2 (C1,C2,C 3)	2 (C4,C5,C6)	2 (C7,C8,C9)	2 (C10,C11 ,C12)	2 (C13,C14 ,C15)	2 (C16,C17,C 18)
Uhanya	D	2 (D1,D2, D3)	2 (D4,D5,D 6)	2 (D7,D8,D 9)	2 (D10,D1 1,D12)	2 (D13,D1 4,D15)	2 (D16,D17,D 18)
Gaba-Uganda	E	2 (E1,E2,E 3)	2 (E4,E5,E6)	2 (E7,E8,E9)	2 (E10,E11 ,E12)	2 (E13,E14 ,E15)	2 (E16,E17,E 18)
Got Kachola	F	2 (F1,F2,F 3)	2 (F4,F5,F6)	2 (F7,F8,F9)	2 (F10,F11, F12)	2 (F13,F14, F15)	2 (F16,F17,F1 8)
Dunga	G	2 (G1,G2, G3)	2 (G4,G5,G 6)	2 (G7,G8,G 9)	2 (G10,G1 1,G12)	2 (G13,G1 4,G15)	2 (G16,G17,G 18)
Mbita	H	2 (H1,H2, H3)	2 (H4,H5,H 6)	2 (H7,H8,H 9)	2 (H10,H1 1,H12)	2 (H13,H1 4,H15)	2 (H16,H17,H 18)

Sample sizes

Small: 1g to 999g (ranged from 33 to 45cm)

Medium: 1000g to 1999g (ranged from 43 to 52cm)

Big: 2000g up (ranged from 51 cm up)

3.3 Microbial determination

About 25g 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 (in anaerobic jar) at 10ºC for 10 days (Leila, *et al.*, 2009) to select psychrotrophics and at 37ºC for 48h to select mesophylls, followed by catalase reaction (with 10% H_2O_2), and oxidase (N-dimethylparaphenylene diamine tablet), gram staining, morphology, production of gas from glucose, tests to confirm LAB. Total counts were taken and recorded. Gas production from glucose were determined in MRS-broth supplemented with 1% glucose containing inverted Durham tubes at 30°C for 48 h. Gram positive colonies that were oxidase and catalase negative were isolated, restreaked and cultured on prepared MRS Agar for 24h. These colonies were harvested from each plate and concentrated in MRS broth for 24 h before being used to test for their potentiality to inhibit *Salmonella enteritica* and *E. coli.* The media was sterilized at 121^0C for 15 minutes in an autoclave (Fishers scientific, USA) and was poured into sterile disposable petri dishes (Fishers scientific).

The selection of suitable strains was based on inhibition against *Salmonella enteritica* and *E. coli*. After 2 days (mesophylls); 10 days (psychrotrophics) of incubation, LAB isolates were selectively picked based on colour, size and shape and re-streaked on fresh plates similar to those from which they had been originally isolated from to ensure purity.

3.4 Grouping of lactic acid bacteria

Five to fifteen colonies were randomly picked from countable colonies in MRS agar plates, purified by repeated plating and studied for their Gram reaction, cell and colony morphology, and catalase reaction. Gram-positive, catalase-negative, oxidase-negative, cocci or rod-shaped isolates with characteristic cell arrangements were considered as possible lactic acid bacteria. Further grouping in to different genera was made by testing for gas production in MRS broth containing 1% glucose. Gas production was detected in inverted Durham tubes after incubation at 30^0C for 24-36 hours.

3.5 Test strains

Human pathogens that have been frequently isolated from fish within winum gulf of L. Victoria and also led to rejection of fish were identified and the two selected were *Salmonella enteritica* subsp. *enteritica serovar enteritidis* (ATCC 13076) and *Escherichia coli* (ATCC 25922) American Type Culture Collection.

3.6 *In vitro* antibacterial activity.

LAB isolates were separately grown in test tubes without agitation in 10 ml MRS broth (HIMEDIA) at 37°C for 48 hours. The antimicrobial activity of the LAB isolates was determined by the modified well diffusion method of Piddock, 1990. Sterile tubes (1.4 and 0.2cm) were used to make holes in solidified Muller Hinton Agar. Holes of 0.2cm were not giving consistent measurements possibly due to smaller units involved and hence the necessitation to use a bigger hole of 1.4cm. Sterilized pipettes were used to draw 2ml and 0.3 ml of the culture broth of lactic acid bacteria incubated for 48 hours and pipetted into the wells of solidified Muller-Hinton agar (HIMEDIA) priorly inoculated with 24 to 48h old cultures of test microorganisms. The plates were kept at 10°C for 3 to 4 h to permit diffusion on the assay material, and incubated at 37°C for 24 to 36 h. Wells filled with un-inoculated MRS broth and Erythromycin served as negative and positive controls respectively. Zones of inhibition were then measured. The antibacterial activity tests were done in duplicates and the mean values of the inhibition zones recorded.

3.7 Data analysis

The data was subjected to analysis of variance (ANOVA) by Randomised Complete Bolck Design (RCBD) using SAS and mean separation done by Latin Square Design test (LSD).

CHAPTER FOUR
4.0 RESULTS AND DISCUSSION

4.1 Lactic acid bacteria counts in gastrointestinal tract of *Lates niloticus*

Nine LABS were isolated from 144 fresh Lake Victoria Nile perch fish samples. The total bacteria grown on MRS indicated bacterial population levels of 3.5 x 10^3 to 1.5 x 10^5 cfu/g wet weight of gastrointestinal tracts of all samples. The bacterial count was lower than the range reported from stomachs of marine fish (Buntin, *et al*, 2008; Austin and Al-Zahrani, 1988; Ringo, 1993). In their studies, the reported levels ranged from 2 x 10^4 to 1 x 10^5 cfu/g. The difference could be attributed to the fact that salt favor the growth of LAB. Apendix 11 shows the LAB counts of fish of three sizes during wet and dry seasons.

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 and big averaging 3210.8g had 4.9 x 10^4. During the dry season, the small fish averaging 614.6g had 6.7 x 10^3, medium averaging 1392.9g had an average of 1.7x10^4 and big averaging 2756.3g had 3.4 x 10^4. The mean LAB counts for the wet and dry seasons' samples are shown in Appendix 11. The samples taken during wet season had higher mean counts of LAB as compared to those sampled during dry season.

There was no bacterial growth for plates incubated at 10°C for 10 days while duplicate plates incubated at 37°C for 48h produced growths. This is possibly due to the reason that the fish originated from tropical waters of high temperatures that favor the thermophillic nature of LABs. There was significant difference in the means of counts between dry and wet seasons (Apendix 11). 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 *Rastrineobola argentea*, the prawn *Caridina nilotica* 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), reported 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 microbiota 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 significant role in the gut microbial composition. In rainbow trout, the gut microbiota make-up was altered when fish were switched from a fish meal to a plant meal based diet, Heikkinen *et al*, 2006. Therefore, the decrease in LAB counts during dry season could be due to stress associated with the decrease in food availability.

The mean LAB counts for the eight different sites where samples were drawn from are shown in Table 4.1.2 below.

Table 4.1.1 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 ($P < 0.05$)

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 4.1.1). 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 weights of 30 pieces of fish, their guts' weights and individual and overall percentages are given in Table 4.1.2. The average percentage weight of guts is 1.87% (Table 4.1.2). Thus, out of 43,650 metric tons Nile perch landed in Kenya in 2009 (Manyala, 2011), 816.255M.tons were disposed as wastes around processing facilities thus ending in water bodies. 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 bacteria for further propagation and possible use as probiotic cultures.

The results from this study indicated averagely high population of LAB in all fish samples ranging from 20 to 50%. Butin *et al*, (2008) reported that there was high LAB population (4-5x10^4 to 10^5 cfu/g) wet weight of GI tracts from all fish samples from the same geographical region. However, our results differ significantly from those reported by Ringo in their work on Arctic charr, *Salvelinus alpinus* which were only a minor part of the microbiota approximately 10%. 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 (Butin *et al*, 2008). LABS from the gastrointestinal tracts of cold-water fish are slow-growing with respect to incubation time hence low population recovery. The phenotypic and biochemical characteristics of the LABs extracted are given in Table 4.1.3. The isolation of one genus (Lactobacillus as indicated in table 4.1.3) 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. Appendix 2 shows the concentrated LAB on MRS agar after purification and appendix 3 indicates single purified colonies of LABs. Appendix 4-7 shows the photos of the various antagonistic LAB strains identified.

Table 4.1.2: The weights of gastrointestinal tracts, percentages vis-à-vis gross fish weights

Fish wt.(g)	Stomach wt.(g)	GIT wt.(g)	Stomach (%)	GIT %
1580	25	35	1.58%	2.22%
1410	20	30	1.42%	2.13%
1380	20	30	1.45%	2.17%
7500	80	100	1.07%	1.33%
1460	25	30	1.71%	2.05%
1520	25	35	1.64%	2.30%
2100	30	40	1.43%	1.90%
3120	45	60	1.44%	1.92%
980	15	25	1.53%	2.55%
8560	130	160	1.52%	1.87%
640	10	15	1.56%	2.34%
750	12	16	1.60%	2.13%
870	12	15	1.38%	1.72%
3860	60	75	1.55%	1.94%
4150	65	80	1.57%	1.93%
1680	25	35	1.49%	2.08%
1860	32	44	1.72%	2.37%
520	8	12	1.54%	2.31%
680	10	14	1.47%	2.06%
960	14	20	1.46%	2.08%
720	12	16	1.67%	2.22%
840	13	20	1.55%	2.38%
560	8	13	1.43%	2.32%
1180	18	26	1.53%	2.20%
1260	18	27	1.43%	2.14%
1750	28	40	1.60%	2.29%
5580	70	90	1.25%	1.61%
6560	90	115	1.37%	1.75%
7400	95	120	1.28%	1.62%
3200	40	55	1.25%	1.72%
Total 74630	**1055**	**1393**	**1.41%**	**1.87%**

Table 4.1.3: Phenotypic and Biochemical characteristics of the extracted LABs

Genus		**Rods/Coccobacilli**				
				Weissella		
		Lactobacillus (homofermentaters)	Lactobacillus (heterofermentaters)	Arginine negative	Arginine positive	Leuconostoc
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	10ºC	-	-	±	±	+
	30 ºC	+	+	+	+	+
	40 ºC	+	+	+	+	±
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

4.2 The effect of fish size on Lactic acid bacteria counts

The mean LAB counts for the three different sample sizes (big, medium and small) are shown in Table 4.2.1. The bigger the fish the higher the mean counts of LAB in the gut.

Table 4.2.1 The LAB count (cfu/g) from guts of 3 different sizes of Nile perch fish

sizes	mean counts of LABs (cfu/g
Big(2000-4500g)	48083[a]
Medium(1000-1999g)	20942[b]
Small(350-999g)	8917[c]

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

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 4.2.1 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 also be due to the bigger attachment surface area of the guts; 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 (Hansen *et al*., 1992; Munro *et al*., 1994; Ringo *et al*., 1996).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). Table 4.2.2 below is the anova table showing strong level of significance difference (0.0001) at 5% levels of significance.

Table 4.2.2 ANOVA table showing level of difference for the 3 different sizes

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	10.55939476	5.27969738	54.82	<.0001
Error	141	13.57860523	0.09630216		
Corrected Total	143	24.13799999			

Dependent Variable: log cfu

Being a predator, with the young stages feeding on invertebrates and large on haplochromine cichlids, the zooplanktivorous cyprinid *Rastrineobola argentea*, the prawn *Caridina nilotica* and juvenile Nile perch (cannibalism); this would be possibly a cause for LAB population variation levels as already reported in section 4.2 above. 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. The increase in LAB counts with size could be associated with the increasing ability to hunt and access a variety of food types and sizes.

4.3 *In vitro* antibacterial activity

The results of inhibition by the *Lactobacillus* isolates on the enteric pathogens *Salmonella enteritica* and *E. coli* are shown in Table 4.1.Out of the nine lactic acid bacteria isolates, four of them showed antibacterial activity against the enteric pathogens tested with varying inhibition zones. The four isolates B4, G4, E4 and Mi4 were all identified to belong to genera lactobacilli which were all homofermenters. Out of the four LAB isolates evaluated, B4 had the best antimicrobial activity with a mean inhibition zones of 3.05cm and 0.19 cm at 1.4cm and at 02cm diameter well respectively against both microorganisms tested. This was followed by Mi4 (3.13 and 0.15cm), G4 (2.98cm and 0.12cm) and E4 (2.52cm and 0.1cm) respectively. Microbial inhibition was in the order *E. coli* (3.06 ± 0.33cm and 0.17 ± 0.07) and *S. enteritica* (2.81 ± 0.19 and 0.14 ± 0.06) for 1.4 and 0.2 cm respectively.

Table 4.3.1: Diameter of zones of inhibition (cm) produced by *Lactobacillus* isolates on the test strains as assessed by the Well diffusion method

	Salmonella enteritica		*E.coli*	
well diameter Lab Isolate	1.4	0.2	1.4	0.2
B4	2.78±0.08[A]	0.17	3.32±0.13[a]	0.22
E4	2.55 ±0.1[B]	0.11	2.48±0.13[c]	0.11
G4	2.87±0.12[A]	0.11	3.08±0.12[b]	0.13
Mi	2.85±0.1[A]	0.17	3.15±0.14[b]	0.17
Mean ± SD*	2.81 ± 0.19[C]	0.14 ± 0.06	3.06 ± 0.33	0.17 ± 0.07
%CV**	4.02	39.34	4.7	34.05
Positive Control	3.02±0.15	0.2	3.25±0.19	0.23

**SD, Standard deviation; **CV, Coefficient of Variation. Means in same column followed by the same letter are not significantly different (P< 0.05)*

This study confirms the efficacy of some Nile perch fish microorganism's gut's extracts as natural antimicrobials and suggests the possibility of employing them in fish preservation where spoilage is caused mainly by microbial activity.

The *Escherichia coli* pathogen was the most inhibited microorganism with a mean inhibition zone of 3.06+ 0.33cm while the *S. enteritica* was the least inhibited with a zone of 2.81 ± 0.19cm, at 1.4cm diameter well as shown in Table 4.3.1 The means of diameter of inhibition zone produced by the four isolates on *S. enteritica* are insignificantly different except one (E4). Only two LAB strains; G4 and M4 are not significantly different in inhibiting *E. coli*. In general,

both the test microorganisms were inhibited by all the four out of nine extracted LABs used in this study.

The overall means of comparison between the four extracted LAB strains on both pathogens are shown in Table 4.3.2. Further analysis of diameter zones produced on both *E. coli* and *S. enteritica* showed that the means of diameter of inhibition zones produced by the isolates B4, Mi4, G4 and E4 are significantly different (Table 4.3.2). This indicates that lactic acid bacteria isolates from Nile perch fish have different inhibition ability against the growth of *S. enteritica* and *E. coli*. There was no significant difference in inhibition between isolate B4 and G4 and M4. M4 and G4 are statistically the same in their inhibition strength against *E. coli* and *S. enteritica* Strain E4 is significantly different from all the others (Table 4.3.2). Strain B4 is statistically insignificant from the positive control (erythromycin). The differences could be due to the type and amount of metabolites produced by the lactic acid bacteria strain. In their report on the nature of antagonistic molecules resulting from Lactobacillus strains, El Bassi *et al.*, 2009 reported that the differences in strength could be due to pH decrease by organic acid, such as lactic acid production and or production of protein, such as bacteriocin.

Table 4.3.2: Mean comparison of inhibition zones (cm) produced by Lactobacillus isolates on the test strains as assessed by the Well diffusion method

	Diameter means	
isolate	At 1.4cm	at 0.2cm
B4	3.05^{ab}	0.19^{de}
E4	2.52c	0.1^{g}
G4	2.98^{b}	0.12^{fg}
M4	3^{b}	0.15^{ef}
+ve control by erythromycin	3.13^{a}	0.22^{d}

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

The inhibitory activity against test strains of *S. enteritica* and *E. coli* by the isolated strains cultured in MRS broth is shown in figures 4.1.1 to 4.1.4. The Lactobacillus isolate B4 exhibited the highest degree of inhibition followed by Mi4, G4 and E4 respectively. With the exception of

E4, the rest exhibited higher inhibition against *E. coli* than *S. enteritica.* The highest antimicrobial activity was against *E. coli*, which is similar with the findings of Butin *et al.,* 2008. The negative control set with only MRS broth in the well exhibited no inhibition.

The photos of inhibition diameter zones for the four identified antagonistic LABs are shown in figures 4.1.1 to 4.1.4.

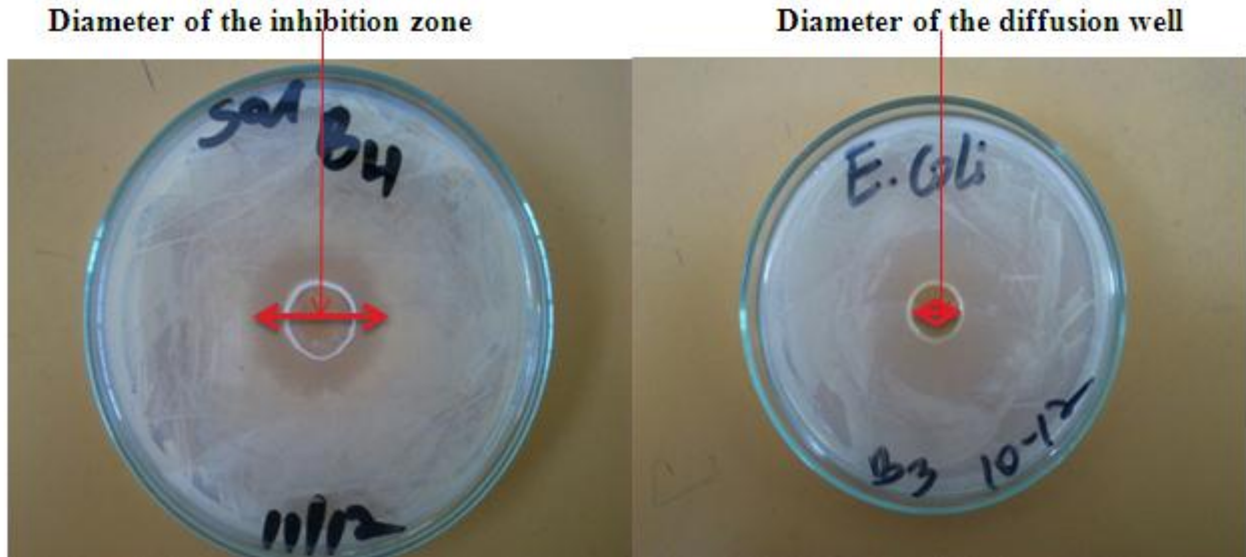
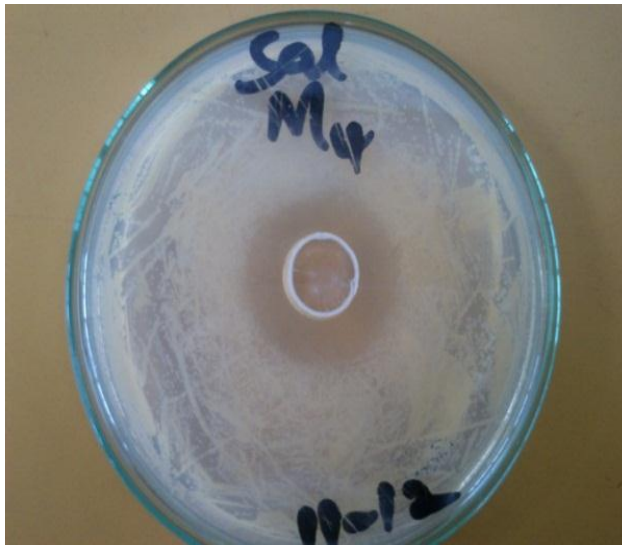


Figure: 4.1.1a Inhibition of *S. enteritica* by LAB B **Figure: 4.1.1b** Inhibition of *E.coli* by LAB B

Figure 4.1.1a and 4.1.1b illustrates the inhibition diameter zone of lactic acid bacteria against test bacteria and well diameter. *E. coli* is inhibited more than *S. enteritica* by LAB B.

Figure: 4.1.2a Inhibition of *S. enteritica* by LAB G **Figure: 4.1.2b** Inhibition of *E.coli* by LAB G *E. coli* is inhibited more than *Salmonella enteritica* by LAB G.



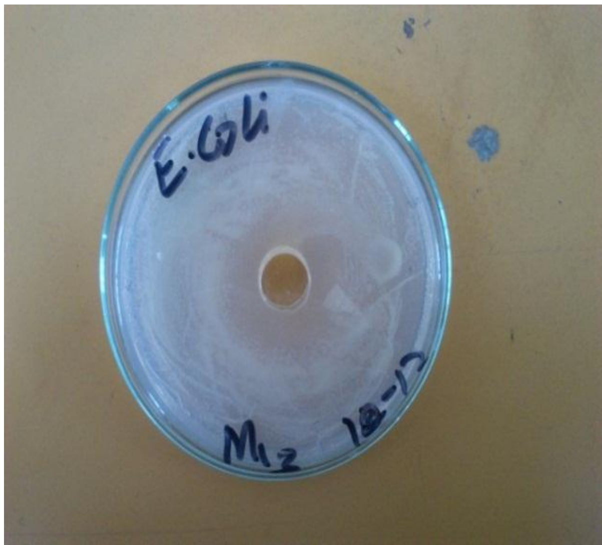
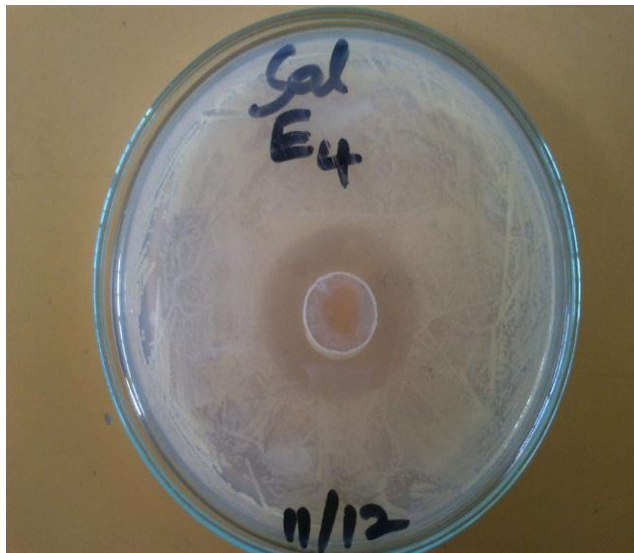


Figure: 4.1.3a Inhibition of *S. enteritica* by LAB M **Figure: 4.1.3b** Inhibition of *E.coli* by LAB M

E. coli is inhibited more than *Salmonella enteritica* by LAB M.



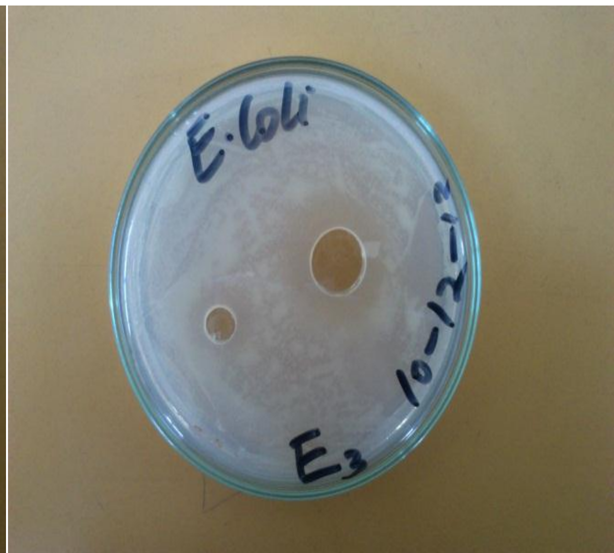


Figure: 4.1.4a Inhibition of *S. enteritica* by LAB E **Figure: 4.1.4b** Inhibition of *E.coli* by LAB E

Salmonella enteritica is inhibited more than *E.coli* by LAB E.

The culture filtrate from LAB, that was identified to be solely genera of Lactobacillus sp., isolated from gastro-intestinal tracts of Nile perch were tested by well diffusion method to know if the antimicrobial metabolites produced by LAB were extracellular and released into the growth medium. In this test, both the test strains were inhibited indicating that the inhibitory metabolites produced by the isolates were extracellular and diffusible because inhibition took place by diffusing through a layer of agar. Out of the nine LAB isolates four inhibited the growth

of the test strains to varying degrees. Similar to our findings, Kivanc (1990) observed varying degrees of inhibition of various food borne pathogens by cell-free filtrates of LAB. The *in vitro* study of Butin *et al* (2008) showed the inhibitory activity of *P. pentosaceus* APa4, *P. pentosaceus* AIa1 and *Ent. faecium* ARa1 on the growth of *S. aureus, Salmonella sp*., pathogenic *E. coli* and *L. monocytogenes*, with the highest antimicrobial activity being against *L. monocytogenes* and *E. coli*, respectively.

The *in vitro* study of Apella *et al* (1992) also showed the inhibitory activity of *Lactobacillus casei* and *Lactobacillus acidophilus* on the growth of *Shigella sonnei*. *In-vitro* inhibition of *E. coli* O157:H7 by LAB was observed in Trypticase Soy broth (Brashears, Reilly & Gilliland, 1998). In agar spot tests (Brashears, Jaroni & Trimble, 2003), and on agar plates (Caridi, 2002). *In-vitro* inhibition of *E. coli* (ATCC 25921) by *Lactobacillus delbrueckii subsp. delbrueckii* was observed in MRS broth on Mueller Hinton agar (Dhaw *et al*, 2008). Between the two test bacteria used in this study, *Salmonella enteritica* subsp. *enteritica serovar Enteritidis* (ATCC 13076) was relatively resistant to the antimicrobial activity of LAB than the *Escherichia coli* (ATCC 25922). The differences could be due to metabolic pathways controlled by gene expressions (Raghunathan *et al*, 2009), making them more tolerant to some organic acids than *E. coli*. There are also reports that indicate some microorganisms produce an acid-tolerance response system that protects them against severe acid stress for longer periods (Foster & Hall, 1991). This may be the reason for resistance of *Salmonella* test strain when compared to the *E. coli* test strain.

Similar to Tadesse *et al*, 2005, the observed inhibition might arise from the acid produced as our LABs were grown in broth media containing glucose. They further indicated that the media composition affects the production of antimicrobial metabolites produced by Lactobacillus isolates. Varadaraj *et al* (1993) observed moderate inhibition of some food borne pathogens and other bacterial species by neutralized culture filtrates of LAB using a well diffusion assay. McLean & cGroarty (1996) also showed that about 60% of the antimicrobial activity of culture filtrates of LAB was removed when the filtrates were neutralized to pH 6.5 with NaOH. The findings of Butin *et al,* 2008 also shows that the LAB extracts from gastrointestinal tracts of marine fish are inhibitory against human pathogens including *S. aureus*, *Salmonella sp*., pathogenic *E. coli* and *L.monocytogenes.*

CHAPTER FIVE

5.1 CONCLUSIONS

There are LABs in the gastrointestinal tracts of Nile perch from L. Victoria. The population of LABs in the gastrointestinal tracts of Nile perch from L. Victoria is affected by the size of fish; i.e the bigger the fish the higher the LAB population. There are LABs in the gastrointestinal tracts of Nile perch from L. Victoria with the capability of inhibiting enteric pathogens (*S. enteritica* and *E.coli*). The study indicated that there are strains of lactobacillus with potential functional probiotics for future in vivo studies for commercialization in the food/fish industry.

5.2 RECOMMENDATIONS

The taxonomic determination of the lactic acid bacteria isolates were done by use of morphological observations and biochemical tests only, so molecular method of identification need to be performed. In addition to evaluation of the promising isolates for their probiotic usage further research can also focus on the characterization of amino acid and nucleotide sequences of these antimicrobial compounds.

6.0 REFERENCES

Albano, H., Todorov, S.D., Van Reenen, C.A., Hogg, T., Dicks,L.M.T. and Teixeira, P. 2007. Characterization of two bacteriocins produced by *Pediococcus acidilactici* isolated from “Alheira”, a fermentedsausage traditionally produced in Portugal. International Journal of Food Microbiology.**116**: 239–247.

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

Altieri, C., Speranza, B., Del Nobile, M.A., and Sinigaglia, M., 2005.Suitability of bifidobacteria and thymol as biopreservatives in extending the shelf life of freshpacked plaice fillets.Journal of Applied Microbiology. **99**: 1294-1302.

Andrighetto, C., Lombardi, A., Ferrati, M., Guidi, A., Corrain, C., and Arcangeli, G., 2009. Lactic acid bacteria biodiversity in Italian marinated seafood salad and their interactions on the growth of *Listeria monocytogenes*. Food Control **20**: 462-468.

Apella, M.C., Gonzalez, S.N., Nader de Macias, M.E., Romero, N. & Oliver, G. (1992). In vitro studies on the inhibition of the growth of Shigella sonnei by Lactobacillus casei and Lactobacillus acidophilus. Journal of Applied Bacteriology **73:** 480-483.

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

Axelsson, L.T., 1993. Lactic acid bacteria: Classification and physiology. In: Salminen, S. and von Wright, A. (Eds.), Lactic acid bacteria. Marcel Dekkers Inc., New York, pp.1-65.

Bari, M.L., Ukuku, D.O., Kawasaki, T., Inatsu, Y., Isshiki, K. and Kawamoto, S. 2005. Combined efficacy of nisin and pediocin withsodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA in reducing the *Listeria monocytogenes* population of inoculated fresh cut produce. Journal of Food Proteins.**68**:1381–1387.

Basby, M., Jeppesen, V.F., and Huss, H.H., 1998. Characterisation of the microfloraof lightly salted lumfish *(Cyclopterus lumpus)* roe stored at 5°C. J. Aquatic Food Production Technology. **7**:35-51.

Baya, A.M., Toranzo, A.E., Lupiani, B., Li, T., Roberson, B.S., and Hetrick, F.M., 1991. Biochemical and serological characterization of *Carnobacterium* spp. Isolated from

farmed and natural populations of striped bass and catfish. Applied Environmental Microbiology. **57**: 3114-3120.

Bell C and Kyriakides A (2002) Salmonella: a practical approach to the organism and its control in foods. Publisher: Oxford; Malden, Mass. Blackwell Science Ltd. **59**: 269. In Onyango M. D., Wandili S., Kakai R. andWaindi E. N. 2009. Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. J Infect Developing ountries.**3(2):** 99-104.

Bhunia, A.K., Johnson, M.C. and Ray, B. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. Journal of Applied Bacteriology. **65**:261–268.

Brashears, M.M., Jaroni, D. & Trimble, J. (2003). Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of Escherichia coli O157:H7 in cattle. Journal of Food Protection **66**: 355-363.

Brashears, M.M., Reilly, S.S. & Gilliland, S.E. (1998). Antagonistic action of cells of Lactobacillus lactis towards Escherichia coli O157:H7 on refrigerated raw chicken meat. Journal of Food Protection **61**: 166-170.

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.

Byun, J.W., Park, S.C., Benno, Y. and Oh, T.K. 1997. Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). Journal of General Applied Microbiology. **43**: 305-308.

Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L., and Leroi, F., 2004. Sensory characteristics of cold-smoked Atlantic salmon *(Salmo salar)* from European market and relationships with chemical, physical and microbiological measurements. Food Research International. **37**: 181-193.

Caridi, A. (2002) Selection of Escherichia coli-inhibiting strains of Lactobacillus paracasei subsp. paracasei. Journal of Industrial Microbiology and Biotechnology **29**: 303-308.

Cintas, L.M., Rodriguez, J.M., Fernandez, M.F., Sletten, K.,Nes, I.F., Hernandez, P.E. and Holo, H. 1995. Isolation and characterizationof pediocin L50, a new bacteriocin from

Pediococcus acidilactici with a broad inhibitory spectrum. Applied Environmental Microbiology. **61**: 2643–2648.

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food Technology. **44**: 100-117

De Man, J.C., Rogosa, M. and Sharpe,E. 1960. A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology. **23**: 130-135.

Devriese, L.A., Kerckhove, A., Kilpper-Balz, R. and Schleifer,K.H. 1987. Characterization and identification of *Enterococcus species* isolated from the intestines of animals. International Journal of Systematic Bacreriology. **37**: 257-259.

De Vuyst, L. and Vandamme, E.J. 1994. Bacteriocins of lactic acid bacteria: Microbiology,genetics and applications. In Aguilar, C. and Klotz, B. 2010. Effect of the temperature on the antagonistic activity of lactic acid bacteria against *Escherichia coli* and *Listeria monocytogenes.*Journal of Food Safety. Available at http://www.google.co.ke. As on 5th Oct. 2010.

De Vuyst L., Vandamme, E. J. 1992. Influence of the carbon source on nisin production in *Lactococcus lactis subsp. lactis* batch fermentations. Journal of General Microbiology. **138**:571 578.

De Vuyst, L., Vandamme, E. J. 1994. Nisin, a lantibiotic produced by *Lactococcus lactis subsp. lactis*: properties, biosynthesis, fermentation and applications. In Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications, eds. L. De Vuyst and E. J. Vandamme, 151-221. London: Blackie Academic & Professional London.

Ndaw, A.D., Faid, M., Bouseta, A. and Zinedine, A. Effect of controlled lactic acid bacteria fermentation on the microbiological and Chemical Quality of Moroccan Sardines (*Sardinapilchardus*).International Journal of Agriculture & Biology. 2008; **10** (1) 21-27.

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

Eldar, A., Ghittino, C., Asanta, L., Bozzetta, E., Goria, M., Prearo, M., and Bercovier, H., 1996.*Enterococcus seriolicida* Is a Junior Synonym of *Lactococcus garvieae* , a causative agent of septicemia and meningoencephalitis in fish. Curr.Microbiol.**32**: 85-88.

Fall, P.A., Leroi, F., Cardinal, M., Chevalier, F., and Pilet, M.F., 2010. Inhibition of *Brochothrix thermosphacta* and sensory improvement of tropical peeled cooked shrimp by *Lactococcus piscium* CNCM I-4031, Lett. Appl. Microbiol. **50**, 357-361.

Fall, P.A., Leroi, F., Chevalier, F., Guérin C., and Pilet M.F., 2010. Protective effect of a non-bacteriocinogenic *Lactococcus piscium* CNCM I-4031 strain against *Listeria monocytogenes* in sterilised tropical cooked peeled shrimp. J. Aquat. Food Prod. Technol. **19**, 84-92.

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.

Fisheries Department. 2006. Fisheries annual statistical bulletin. Nairobi, Fisheries

Fisheries Department. 2009. Fisheries annual statistical bulletin. Nairobi, Fisheries. In Manyala, J. O., 2011. Fishery Value Chain Analysis: Background Report – Kenya.Retrieved 24th April 2014 at www.fao.org/.../Background_report_-_Kenya_-_April_2011.

Foster, J.W., & Hall, H.K. (1991). Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium*. Journal of Bacteriology **173**: 6896-6902.

Garriga, M., Pascual, M., Monfort, J.M. and Hugas, M. 1998.Selection of lactobacilli for chicken probiotic adjuncts. Journal of Applied Microbiology. **84**: 125-132.

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.

Gopal K, Tripathy SS, Bersillon JL, Dubey SP. 2007. Chlorination byproducts, their toxicodynamics and removal from drinking water. J Hazard Mater **140**:1–6.

Hamnes, W.P., Weiss, N., Holzapfel, W., 1992. The genera *Lactobacillus* and *Carnobacterium*. In: Balows,A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K.H._Eds.., The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, Vol. II. In E. Ringo, F.J. 1998. Gatesouper Aquaculture **160**:177–203.

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

Heikkinen J, Vielma J, Kemilainen O, Tiirola M, Eskelinen P, (2006) Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (Oncorhynchus mykiss). Aquaculture **261**: 259-268.

Huber, I., B. Spanggaard, K.F. Appel, L. Rossen, T. Neilson and L. Gram, 2004. Phylogenetic analysis and *in situ* identification of intestinal microbial community of rainbow trout (*Oncorhynchus mykiss* Walbaum). Journal of Applied Microbiology. **96**: 117-132.

Huis In't Veld JH.J., 1996. Microbial and Biochemical Spoilage of Foods: An Overview. Int. J. Food Microbiol. **33**:1–18.

Jack, R.W., Tagg, J.R. and Ray, B. 1995. Bacteriocins of gram-positive bacteria. InVijai P., Ajay P., Mahantesh P., RamanaK.V.and Jeevaratnam K. 2010. Isolation, biochemical properties and application of bacteriocins from *pediococcus Pentosaceous* isolates. Journal of Food Processing and Preservation. **6:** 1064-1079.

Jaffrès, E., Sohier, D., Leroi, F., Pilet, M.F., Prévost, H., Joffraud, J.J., and Dousset,X., 2008. Study of the bacterial ecosystem in tropical cooked and peeled shrimpsusing a polyphasic approach. International Journal of Food Microbiology**131**: 20-29.

Jamuna, M. And Jeevaratnam, K. 2004. Isolation and partial characterizationof bacteriocins from *Pediococcus* species. Applied Microbiology Biotechnology. **65**:433–439.

Joborn, A., Dorsch, M., Olsson, J.C., Westerdahl, A., and Kjelleberg, S., 1999.*Carnobacterium inhibens* sp. nov., isolated from the intestine of Atlantic salmon *(Salmo salar)*. International Journal of Systematic Bacteriology.**49**: 1891-1898.

Kandler, O. and Weiss, O., 1986. Regular, non-sporeforming Gram-positive rods. In:Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holz, J.G. (Eds.), Bergeys Manual of Systematic Bacteriology. Vol. 2, Williams and Wilkins, Baltimore, MD, pp. 1208-1260.

Kayambo S and Sven EJ (2006) Lake Victoria. Experience and lessons learned brief. Pp. 431-446.

Kim, C.H., Ji, G.E. and Ahn, C. 2000. Purification and molecular characterization of a bacteriocin from *Pediococcus* sp. KCA1303-10 isolated from fermented flat fish. Food Science Biotechnology.**19**: 270–276.

Kimoto, H., Kurisaki, J., Tsuji, N. M. Ohmomo, S. and Okamoto, T. 1999.*Lactococci* as probiotic strains: Adhesion to human enterocyte-like Caco-2 cells and tolerance to low pH and bile. Letters in Applied Microbiology. **29**: 313–316.

Kimoto, H., Ohmomo, S. and Okamoto, T. 2002. Cholesterol removal from media by Lactococci. Journal of Dairy Science. **85**: 3182–3188.

Kivanc, M. (1990). Antagonistic action of lactic cultures toward spoilage and pathogenic microorganisms in food. Nahrung **34:** 273-277

LeChevallier MW, Au K-K. 2004. Water treatment and pathogen control. London: IWA Publishers.

Leila, E.B., Mnasser, H., Naoya S. and Torumatsui, 2009. Biopreservation of refrigerated and vacuum-packed *Dicentrarchus labrax* by Lactic Acid Bacteria. Journal of Food Science.**74** 335-339.

Leisner, J.J., Vancanneyt, M., Goris, J., Christensen, H. and Rusul, G., 2000. Description of *Paralactobacillus selangorensis* gen. Nov., sp. nov., a new lactic acid bacterium isolated from chili bo, a Malaysian food ingredient. Int. J. Syst. Evol.Microbiol. **50:** 19-24.

Leroi, F., 2010. Occurrence and role of lactic acid bacteria in seafood products. Food Microbiology. **27** (6): 698-709.

Leroi, F., Joffraud, J.J., Chevalier, F., and Cardinal, M., 2001.Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. J. Appl. Microbiol., 578-587.

Liu, J.Y., Li, A.H., Ji, C., and Yang, W.M., 2009. First description of a novel *Weissella* species as an opportunistic pathogen for rainbow trout *Oncorhynchus mykiss* (Walbaum) in China. Vetenary Microbiology. **136**: 314-320.

Lyhs, U., Lahtinen, J., and Schelvis-Smit, R., 2007. Microbiological quality of maatjes herring stored in air and under modified atmosphere at 4 and 10 degrees C. Food Microbiology. **24:** 508-516.

Manyala, J. O., 2011. Fishery Value Chain Analysis: Background Report – Kenya.Retrieved 24th April 2014 at www.fao.org/.../Background_report_-_Kenya_-_April_2011.doc

Matamoros, S., Pilet, M.F., Gigout, F., Prevost, H., and Leroi, F., 2009.Selection and evaluation of seafood-borne psychrotrophic lactic acid bacteria as inhibitors of pathogenic and spoilage bacteria.Food Microbiology. **26**: 638-644.

McLean, N.W. & McGroarty, J.A. (1996). growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of Gardnerella vaginalis by lactobacilli in vitro. Applied and Environmental Microbiology **62**: 1089-1092

Megapesca. 1997.Nile perch - marketing success or ecological disaster? Retrieved on 5th October 2010 at http://www.gisl.co.uk/Megapesca/nilesupplement.htm

Messens, W., Neysens, P., Vansieleghem, W., Vanderhoeven, J. and De Vuyst, L. 2002. Modeling the growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471 in response to temperature and pH values used for sourdough fermentations. Applied Environmental Microbiology.1431–1435.

Mitsuoka, T. 1980. A Color Atlas of Anaerobic Bacteria.1st Edn. Tokyo University Press, Tokyo, pp: 20-29.

Muigei,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 (Lates niloticus) at the Landing Facility as a Potential Microbiological and Organochlorine Pesticide Residue Hazards Critical Control Point. MSc(Food Science) Thesis- University of Nairobi, Kenya

Ndaw, A.D., Faid, M., Bouseta, A. and Zinedine, A. 2008. Effect of controlled lactic acid bacteria fermentation on the microbiological and Chemical Quality of Moroccan Sardines (*Sardina pilchardus*). International Journal of Agriculture & Biology, **10 (1)** 21-27

Nieto-Lozano, J.C., Reguera-Useros, J.I., Pelaez-Martinez,M.C. and Deh Jorre, A.H. 2006.Effect of bacteriocin producedby *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* on Spanish raw meat. Meat Sci. **72:** 57– 61

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.

Nilsson, L., 1999. Control of *Listeria monocytogenes* in cold-smoked salmon by biopreservation.In: Leroi, F., 2010. Occurrence and role of lactic acid bacteria in seafood products. Food Microbiology.**27 (6)**: 698-709.

O'May, G. A. and MacFarlane, G. T. 2005. Probiotic efficacy: Are the claims justified? In Probiotic Dairy Products. Blackwell Publishing, London. Pp 138–166. of the evidence. Journal of Nutrition Research. **21**: 343–353.

Okeyo, G.O., Lokuruka, M.N. and Matofari, J.W. 2009. Nutritional composition and shelflife of the Lake Victoria Nile perch (L*ates niloticus*) stored in ice. African Journal of Food, Agriculture, Nutrition and Development **9(3)** 901-919

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.

Pal, A. and Ramana, K.V. 2010.Purification and characterization of bacteriocin from *Weissella paramesenteroides* DFR-8, an isolate from cucumber (*Cucumis sativus*). Journal of Food Biochemistry. **34(5)**: 932- 948.

Pal, V., Pal, A., Patil, M., Ramana, K.V. and Jeevaratnam, K. 2009. Isolation, biochemical properties and application of bacteriocins from *pediococcus Pentosaceous* isolates. Journal of Food Processing and Preservation. Available at http://www.google.co.ke. As on 5th Oct. 2010.

Perdigon, G., Alvarez, S., Rachid, M. Aguero, G. and Gibbato, N. 1995. Immune system stimulation by probiotics. Journal of Dairy Science.**78**: 1597-1602.

Piddock, L.J.V. 1990. A review. Techniques used for the determination of antibacterial resistance and sensitive in bacteria. Journal of Applied Bacteriology. **68**: 307–18

Qiao, M., Omaetxebarria, R., Oruetxebarria, I., Saris, P. E. J. 1997. Isolation of a *Lactococcus lactis* strain with high resistance to nisin and increased nisin production. Biotechnology Letters.**19**:199-202.

Raghunathan,A., Reed, R., Shin, S., Palsson, B. and Simon Daefler, S. (2009). Constraint-based analysis of metabolic capacity of *Salmonella typhimurium* during host-pathogen interaction. Journal of BMC Systems Biology, **3**:38

Ray B., 2003. Control of microorganisms in foods. Fundamental food microbiology. 3rd ed. London: In Yuk, H.G., Tocmo, R., Krizman,K., Khoo, W.J., Phua, K.L. and Kim, M. 2014. *Listeria monocytogenes* in Vacuum-Packed Smoked Fish Products: Occurrence, Routes of Contamination, and Potential Intervention Measures. Comprehensive Reviews in Food Science and Food Safety. **13**.

Robinson, R.K., Batt, C.A. and Patel, P.D. eds. 2000. Encyclopedia of Food Microbiology.**3**: 1864-1870 and: **1**: 633-660.

Ringo , E. and Gatesoupe, F. 1998. Lactic acid bacteria: A review. Aquaculture, **160**: 177–203.

Ringo, E. and Strom, E., 1994. Microflora of Arctic char, *Salvelinus alpinus* (L.); gastrointestinal microflora of free-living fish, and effect of diet and salinity on the intestinal microflora. Aquaculture Fish Management. **25**: 623-629.

Ringo, E., 1993. Does dietary linoleic acid affect intestinal microflora in Arctic charr, Salelinus alpinus. Aqua-cult.Fish. Manage. **24**: 133-135.

Ringo, E., 2008. The ability of *carnobacteria* isolated from fish intestine to inhibit growth of fish pathogenic bacteria: a screening study. Aquaculture Research. **39:** 171-180.

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.

Ringo, E., Olsen, R.E., Overli, O., and Lovik, F., 1997. Effect of dominance hierarchy formation on aerobic microbiota associated with the epithelial mucosa of subordinateand dominant individuals of Arctic char, *Salvelinus alpinus* (L.). Aquaculture Reserch **28**: 901-904.

Ringo,E. Serinewesmajervi, M., Heidi Rogn Bendiksen,H.R., Berg,A., Olsen,R.E., Johnsen,T., Helenemikkelsen,Seppola,M., Strom, E. and Holzapfel,W. 2001. Identification and characterization of carnobacteria isolated from fish intestine. Systematic and Applied Microbiology. **24**, 183–191.

Rodgers, S., 2001.Preserving non-fermented refrigerated foods with microbial cultures: a review. Trends Food Science Technology. **12**: 276-284.

Rodriguez, J.M., Martinez, M.I. and Kok, J. 2002. Pediocin PA-1, awide spectrum bacteriocin from lactic acid bacteria. Crit. Rev. Food Sci.Nutr. **42:** 91–121.

Ross, R.P., Morgan, S. and Hill, C. 2002. Preservation and fermentation: past, present and future. International Journal of Food Microbiology **79**: 3 – 16

Sakata, T., Sugita, H., Mitsuoka, T., Kakimota, D. and Kadota, H. 1980. Isolation and distribution of obligate anaerobic bacteria from the intestines of freshwater fish. Bull. Japan Society of Science of Fish, **46**: 1249-1255.

Salminen, S. and Von Wright,A. 1998. Lactic acid bacteria microbiology and functional aspects. 2nd Edn.In Azizpour, K. 2009. Biochemical characterization of lactic acid bacteria isolated from rainbow trout (Onchorhynchus mykiss) of west Azarbaijn, Iran. Research Journal of Biological Sciences **4(3)**: 324-326.

Schroder, K., Clausen, E., Sandberg, A.M., and Raa, J., 1980. Psychrotrophic *Lactobacillus plantarum* from fish and its ability to produce antibiotic substances. In:Connell, J. J. (Ed.), Adv. Fish. Sci. Technol. Fishing News Books, London. pp. 480-483.

Seema, N. P., 2000. Studies on Lactic Acid Bacteria from Tropical Fish and Shellfish. Ph.D(Microbiology)Thesis- Cochin University of Science and Technology

Seppola, M., Olsen, R.E., Sandaker, E., Kanapathippillai, P., Holzapfel, W.H., and Ringo, E., 2006. Random amplification of polymorphic DNA (RAPD) typing of *Carnobacteria* isolated from hindgut chamber and large intestine of Atlantic cod*(Gadus morhua* L.). Systematic Applied Microbiology. **29**: 131-137.

Sharpe, M.E., 1979. Identification of the lactic acid bacteria. In: Skinner, F.A. and Lovelock, D.W. (Eds.), Identification methods for microbiologists. Soc. Appl. Bacteriol. Technical Series no. 14, Academic Press, London, pp. 246-255.

Snoeks, J., 2005. The Global Invasive Species Database. Retrieved on 25th Oct 2011 at http://www.discoverlife.org/mp/20o?search=Lates+niloticus&flags=HAS:.

Stiles, M.E. and Holzapfel, W.H., 1997. Lactic acid bacteria of foods and their current taxonomy. International Journal of Food Microbiology. **36:** 1-29.

Strom, E. and Olafsen, J.A., 1990. The indigenous microflora of wild-captured juvenile cod in net-pen rearing. In: Leroi, F., 2010.Occurrence and role of lactic acid bacteria in seafood products. Food Microbiology.**27 (6)**: 698-709.

Tadesse, G., Ephraim, E. and Ashenafi, M. 2005. Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some foodborne pathogens and effect of growth medium on the inhibitory activity. Internet Journal of Food Safety **5**: 13-20.

Toranzo, A.E., Novoa, B., Baya, A.M., Hetrick, F.M., Barja, J.L., and Figueras, A.,1993. Histopathology of rainbow trout, *Onchorhynchus mykiss* (Walbaum), and striped bass, *Morone saxatilis* (Walbaum), experimentally infected with *Carnobacterium piscicola*. Journal of Fish Diseases. **16**: 261-267.

Tramer, J., Fowler, G. G. 1964. Estimation of nisin in foods.Journal of Science Food Agriculture.**15**: 522-528.

Truelstrup Hansen, L., Gill, T., Drewes Rontved, S., and Huss, H.H., 1996.Importance of autolysis and microbiological activity on quality of cold-smocked salmon. Food Research International. **29**: 181-188.

Vandamme, E. J. 1984. Biotechnology of Industrial Antibiotics. New York: Marcel Dekker.

Vandamme, P., Pot, B., Gillis, M., deVos, P., Kersters, K. and Swings, J., 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiology Reviews. **60**: 407-438.

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.

Varadaraj, M.C., Devi, N., Keshava, N. & Manjrekar, S.P. (1993). Antimicrobial activity of neutralized extracellular culture filtrates of lactic acid bacteria isolated from a cultured Indian milk product ('dahi'). International Journal of Food Microbiology, **20:** 259-267

Wallbanks, S., Martinez.Murcia, A.J., Fryer, J.L., Phillips, B.A., and Collins, M.D., 1990. 16S rRNA sequence determination for members of the genus Carnobacteriumand related lactic acid bacteria and description of *Vagococcus salmoninarum* sp. nov. International Journal of Systematic Bacteriology.**40:** 224-230.

Wan, J., Hickey, M. W. and Coventry, M. J. 1995. Continuous production of bacteriocins, brevicin, nisin and pediocins, using calcium alginate-immobilized bacteria. Journal of Applied Bacteriology.**79**: 671-676.

Wang W, Ye B, Yang L, Li Y, Wang Y. 2007. Risk assessment on disinfection by-products of drinking water of different water sources and disinfection processes. Env. Intl **33**: 219–25.

Welker, T.L and Lim, C., 2011. Use of Probiotics in Diets of Tilapia. Journal of Aquaculture Research Development. **S1**: 014

Wessels, S. and Huss, H.H., 1996. Suitability of *Lactococcus lactis* subsp. *Lactis*ATCC 11454 as a protective culture for lightly preserved fish products. Food Microbiology.**13**: 323-332.

Williams, A.M., Fryer, J.L., and Collins, M.D., 1990.*Lactococcus piscium* sp. nov. A new *Lactococcus* species from salmonid fish. FEMS Microbiology, Letters **68:** 109-114.

Wolf, C. E., Gibbons, W. R. 1996. Improved method for the quantification of the bacteriocin nisin. Journal of Applied Bacteriology. **80:** 453-457.

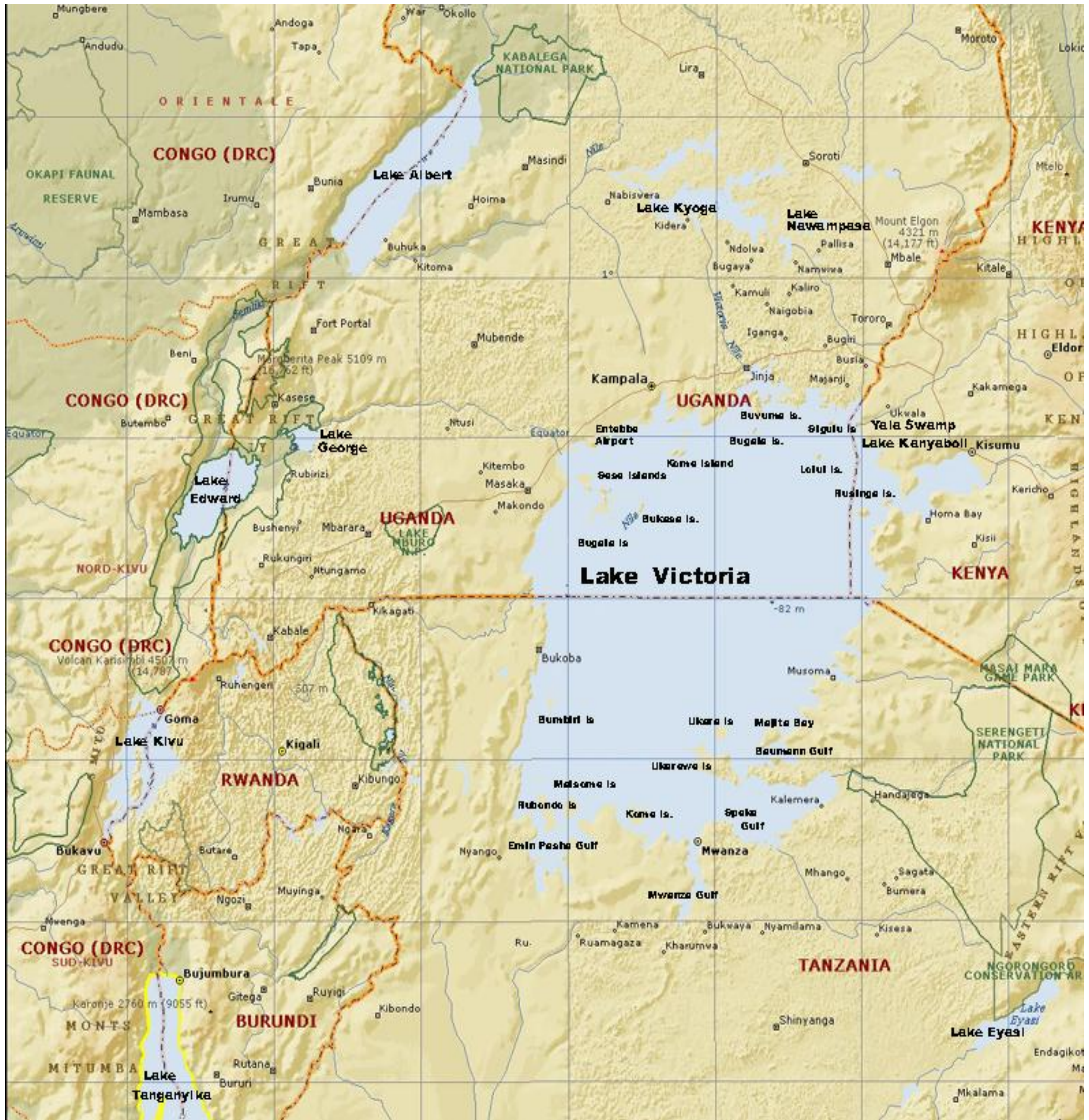
Wong, J. M., de Souza, R., Kendall, C. W., Emam, A. and Jenkins, D. J. 2007. Colonic health: Fermentation and short chain fatty acids. Journal of clinical gastroenterology. **40(3)**: 235-243.

Yang, G.M., Bao, B.L., Peatman, E., Li, H.R., Huang, L.B., and Ren, D.M., 2007. Analysis of the composition of the bacterial community in puffer fish Takifuguobscurus. Aquaculture **262:** 183-191.

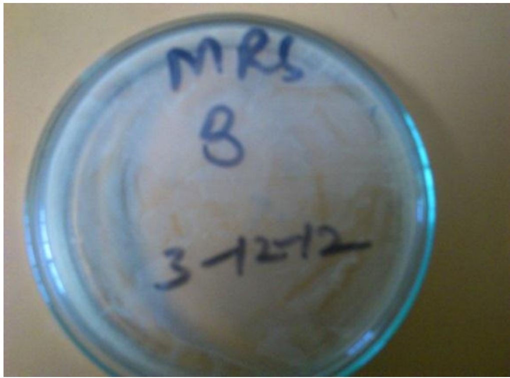
Yuk, H.G., Tocmo, R., Krizman,K., Khoo, W.J., Phua, K.L. and Kim, M. 2014. Listeria monocytogenes in Vacuum-Packed Smoked Fish Products: Occurrence, Routes of Contamination, and Potential Intervention Measures. Comprehensive Reviews in Food Science and Food Safety. **13**.

7.0 APPENDICES

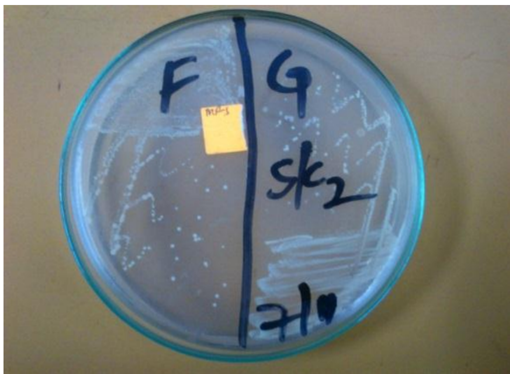
Appendix 1: Map of Lake Victoria



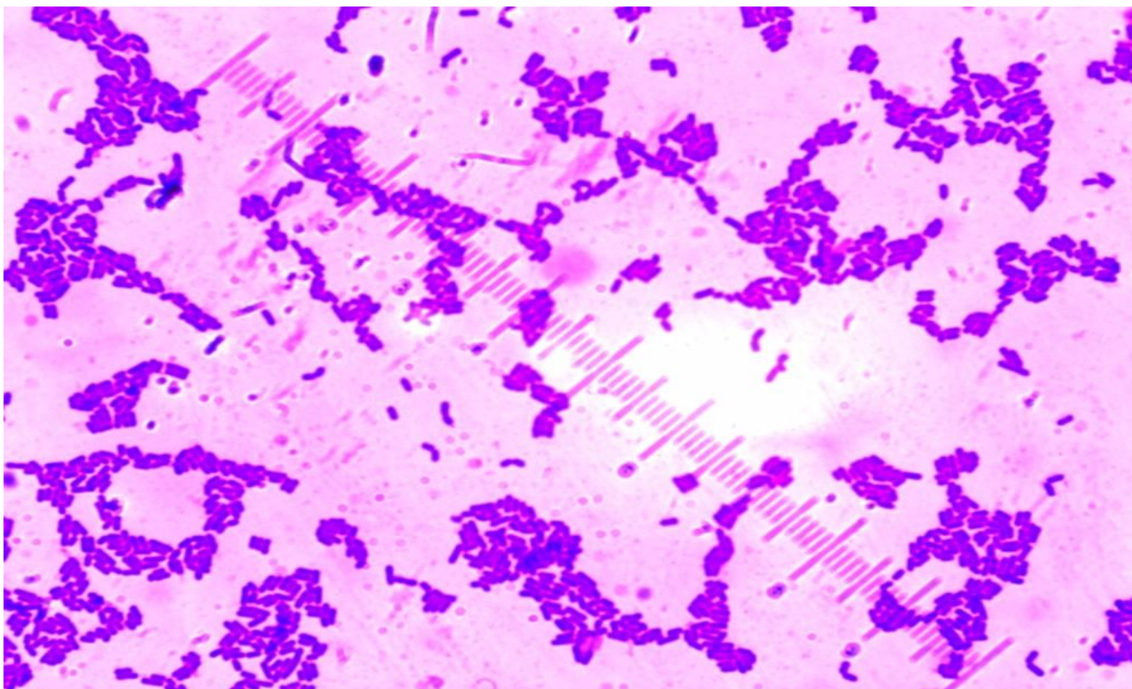
Appendix 2: Concentrated LAB culture on MRS agar



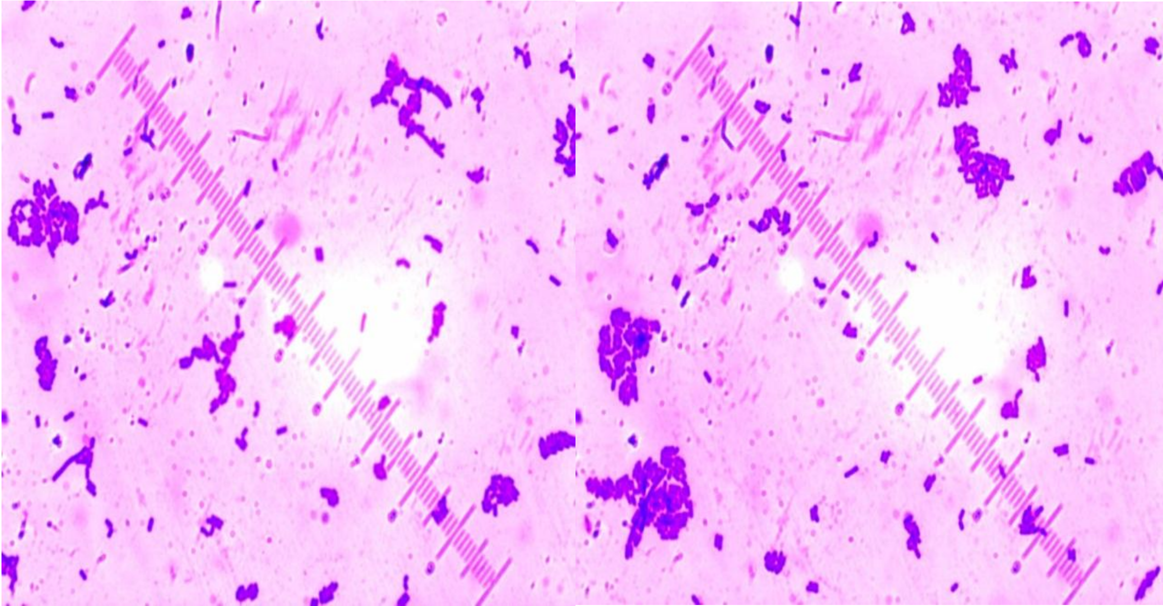
Appendix 3 Streaked purified colonies of LAB G



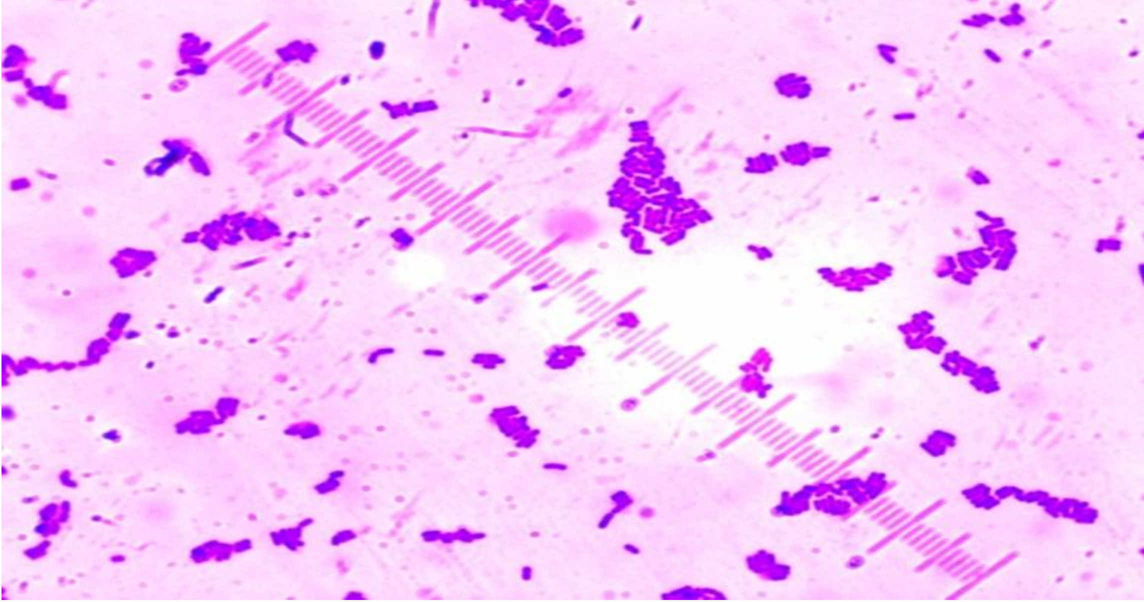
Appendix 4: Photos of LAB B under microscope



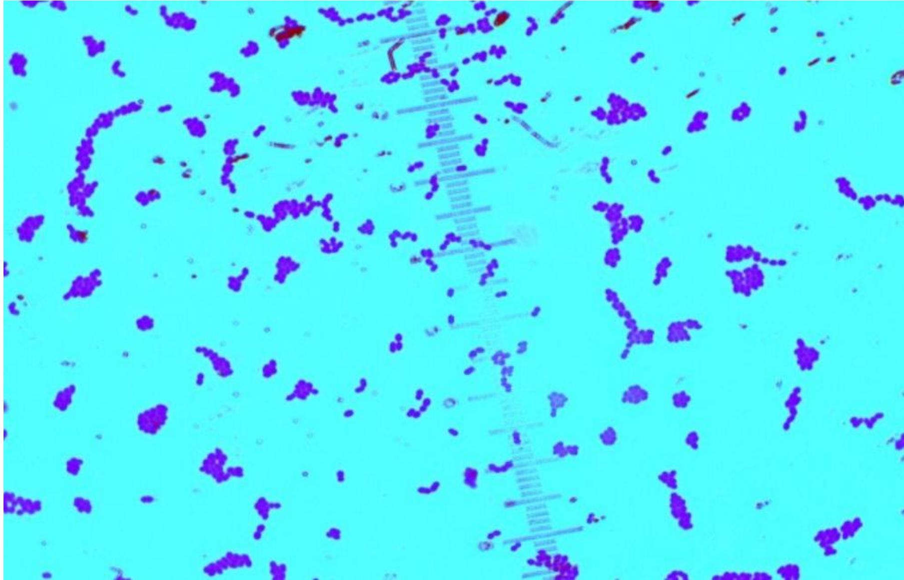
Appendix 5: Photos of LAB E under microscope



Appendix 6: Photos of LAB G under microscope



Appendix 7: Photos of LAB Mi under microscope

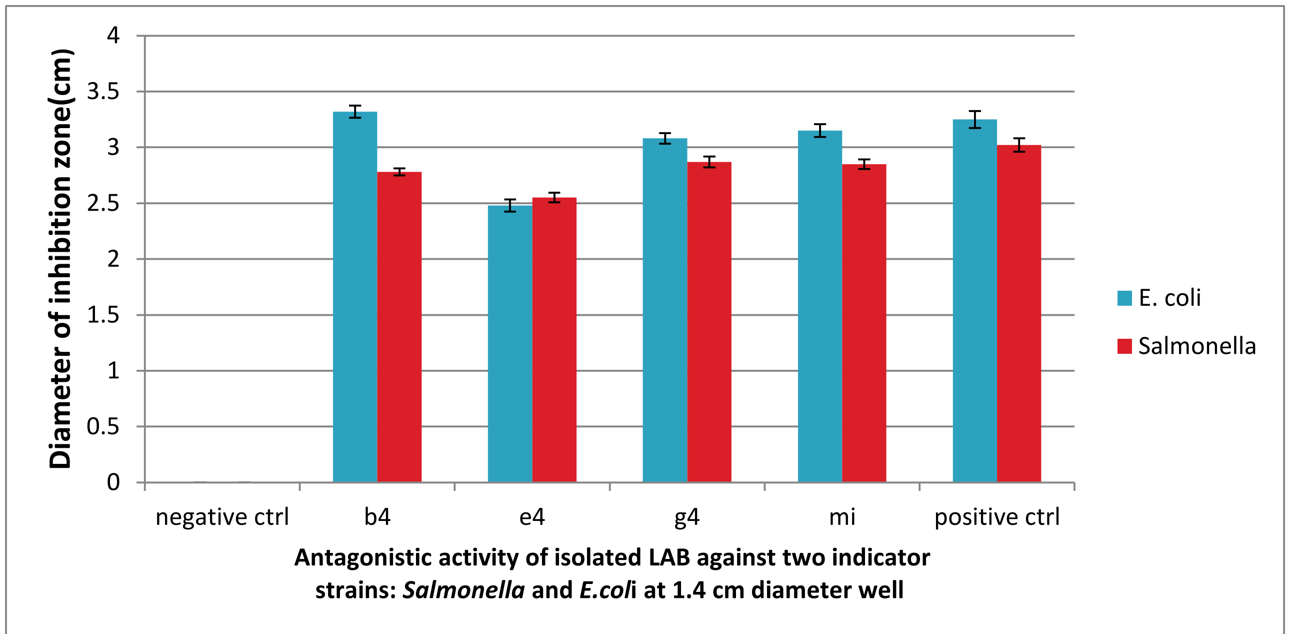


Appendix 8a

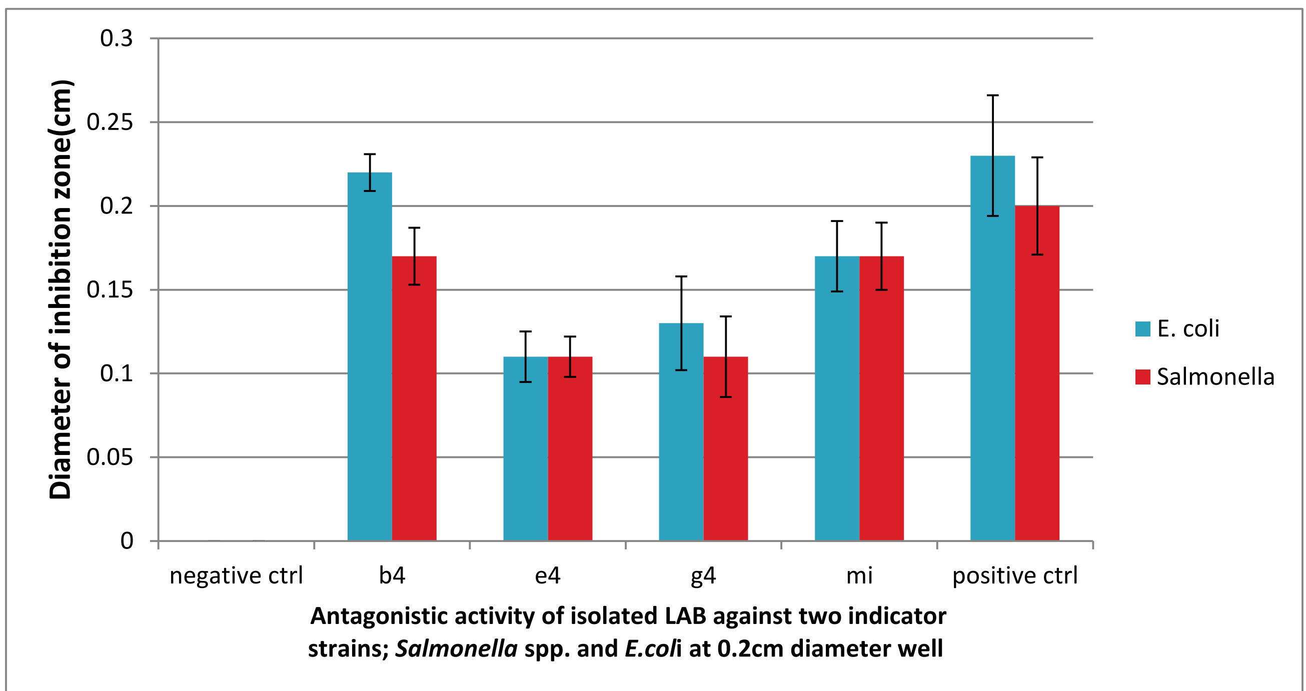
Appendix 8b

Photos of James and Maxwel during laboratory work

Appendix 9



Appendix 10



Illustrated in Appendix 9 and 10, isolates B4, G4, E4 and Mi4 showed antagonistic activity for both indicator strains (*E. coli* and *Salmonella sp*.) at different concentrations (well diameters of 1.4 and 0.2cm with 2 and 0.3ml respectively).

Appendix 11**:** LAB Counts and means for wet and dry seasons

Size	**Sample**	**Wet(April to June 2011)**			**Dry(July to Sept. 2011)**		
		Length(cm)	Wt(g)	Cfu/g	Length(cm)	Wt(g)	Cfu/g
Small(1 to 999g)	A1	37	520	6700	37	450	8600
	A2	42	750	7400	40	680	7300
	A3	35	420	10000	36	420	6400
	B1	35	420	10000	33	380	3500
	B2	38	540	28000	34	440	5800
	B3	35	410	12000	35	410	4200
	C1	43	860	6500	42	760	4500
	C2	38	680	3600	38	640	5000
	C3	44	940	20000	39	940	8600
	D1	46	940	5400	43	600	9000
	D2	45	500	6000	46	820	4000
	D3	45	620	7600	46	620	6500
	E1	36	540	8700	38	540	4300
	E2	41	730	23000	42	680	4400
	E3	42	850	40000	41	850	6400
	F1	43	910	7400	40	720	6700
	F2	39	550	6900	40	620	5400
	F3	40	610	8000	40	610	7500
	G1	37	640	4000	38	630	3500
	G2	42	760	7600	42	660	23000
	G3	43	900	10000	43	900	6700
	H1	35	440	9000	36	420	5000
	H2	40	580	7600	40	460	7000
	H3	43	920	11000	40	920	8300

Appendix 11: LAB Counts and means for wet and dry seasons (continued)

Size	Sample	Wet(April to June 2011)			Dry(July to Sept. 2011)		
		Length(cm)	Wt(g)	Cfu/g	Length(cm)	Wt(g)	Cfu/g
Medium(1000 to 1999g)	A4	46	1400	46000	46	1500	22000
	A5	45	1300	13000	46	1400	7500
	A6	49	1600	21000	46	1600	23000
	B4	44	1300	12000	44	1200	12000
	B5	46	1550	14000	46	1350	14000
	B6	45	1400	15000	45	1400	17000
	C4	43	1020	30000	44	1000	4000
	C5	47	1500	9000	45	1200	15000
	C6	48	1720	70000	45	1720	26000
	D4	46	1600	14000	45	1400	14000
	D5	45	1010	9200	47	1300	3600
	D6	47	1730	45000	48	1730	20000
	E4	48	1800	9800	47	1460	7800
	E5	44	1360	32000	44	1160	24000
	E6	48	1720	35000	45	1720	16000
	F4	43	1200	12000	44	1100	9800
	F5	47	1700	7900	47	1500	32000
	F6	45	1300	15000	43	1300	14000
	G4	46	1420	34000	46	1300	22000
	G5	49	1600	31000	49	1400	21000
	G6	47	1750	23000	46	1750	16000
	H4	45	1440	9000	46	1300	62000
	H5	48	1900	54000	49	1700	4600
	H6	44	1320	23000	45	1320	14000

Appendix 11: LAB Counts and means for wet and dry seasons (continued)

Size	Sample	Wet(April to June 2011)			Dry(July to Sept. 2011)		
		Length(cm)	Wt(g)	Cfu/g	Length(cm)	Wt(g)	Cfu/g
Large(2000 to 4500g)	A7	56	4200	67000	57	3900	46000
	A8	52	3800	73000	53	3800	52000
	A9	50	2300	38000	47	2300	34000
	B7	58	4300	150000	59	4000	30000
	B8	50	2200	38000	52	2150	18000
	B9	60	4420	80000	52	4420	43000
	C7	53	3100	73000	54	2800	36000
	C8	51	2200	5000	52	2300	8000
	C9	55	3200	68000	56	3200	48000
	D7	49	2000	5400	49	2100	11000
	D8	54	3700	16000	54	3700	9000
	D9	51	2600	70000	57	2600	50000
	E7	52	3200	8700	52	3100	100000
	E8	53	2800	23000	52	2700	15000
	E9	61	4100	70000	55	4100	56000
	F7	51	2300	53000	53	2200	28000
	F8	54	2600	6900	52	2400	8000
	F9	57	3000	42000	55	3000	86000
	G7	50	2640	33000	52	2100	17000
	G8	52	3760	86000	50	2000	18000
	G9	53	3840	45000	55	3840	65000
	H7	54	3600	24000	53	2800	19000
	H8	53	3100	120000	53	2700	130000
	H9	59	4100	130000	56	4100	56000
mean counts of LABs (cfu/g)		30213^{a}			21749^{b}		

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

Appendix 12: Authors own publication

a) Refereed journals

1. Obar, J. A., Shitandi. A. A., Mahungu. S. M. and Agasa, L. O. 2015. The Abundance of Lactic Acid Bacteria in the Gastrointestinal Tract of Lake Victoria Nile Perch. Food Science and Quality Management, 42: 2224-6088.

b) Manuscript under review

1. Anti-bacterial properties of Lactic Acid Bacteria from Lake Victoria Nile Perch (*Lates niloticus)*

c) Conference papers: both of the papers mentioned above were presented in international conferences as mentioned below

1. Anti-bacterial properties of Lactic Acid Bacteria from Lake Victoria Nile Perch (*Lates niloticus)*. 8th Egerton University international conference: 26th – 28th March 2014.

2. The Abundance of Lactic Acid Bacteria in the Gastrointestinal Tract of Lake Victoria Nile Perch. Maseno University Annual Interdiscipliary Research Conference, 30th June – 1st July, 2014