British Microbiology Research Journal
17(2): 1-7, 2016, Article no.BMRJ.28820
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Activity of *Lactobacillus spp* and *Lactococcus spp* Isolated from Various Parts of Pebbly Fish, *Alestes baremoze*

Charles D. Kato[1,2], Carol E. Kahuma[1], Victoria T. Namulawa[3] and Nasser Kasozi[4*]

[1]School of Bio-security, Biotechnical and Laboratory Sciences, College of Veterinary Medicine, Animal Resources and Bio-security, Makerere University, P.O.Box 7062, Kampala, Uganda.
[2]Faculty of Biomedical Sciences, Kampala International University, Western Campus, P.O.Box 71, Bushenyi, Uganda.
[3]Aquaculture Research and Development Center, National Agricultural Research Organisation, P.O.Box 530, Kampala, Uganda.
[4]Abi Zonal Agricultural Research and Development Institute, National Agricultural Research Organisation, P.O.Box 219, Arua, Uganda.

Authors' contributions

This work was carried out in close collaboration between all authors. Authors CDK, VTN and NK proposed, designed and supervised the study. Authors CDK and CEK performed the experiments. Author CDK performed the statistical analysis. Authors CDK and NK wrote the final version of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/28820
Editor(s):
(1) Alok K. Upadhyay, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.
Reviewers:
(1) Fakruddin, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.
(2) Paul Yahaya Idakwo, University of Maiduguri, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/16285

Original Research Article

Received 7th August 2016
Accepted 9th September 2016
Published 21st September 2016

ABSTRACT

The study sought to identify microbial communities and potential probiotics in one of the popular fish species, *Alestes baremoze*. A total of 15 samples were collected from Lake Albert and swabs of the skin, gills and gut were made, and cultured on general purpose and selective media. The bacteria isolated were confirmed using morphological and biochemical tests while probiotic screening was done using the agar spot method. The prevalent potential pathogenic bacteria were

**Corresponding author: E-mail: kasonax@yahoo.com;*

Staphylococcus aureus (100%) and *Escherichia coli* (67.7%). The highest total microbial load was generally recorded for samples from the skin. *Staphylococcus spp* had the highest total microbial load recorded from all the samples (skin, $8.50\pm22.1x10^2$ cfu/g and gills, $8.00\pm24.3x10^2$ cfu/g). When the effect of potential probiotic genera (*Lactobacillus spp* and *Lactococcus spp*) isolated from skin, gills and gut was investigated, *Lactobacillus spp* showed the highest overall activity against all test pathogens. The highest activity for *Lactobacillus spp* was demonstrated against E. *coli* (15.4±0.19 mm) and *S. aureus* (14.0±0.17 mm). The highest activity for *Lactococcus spp* was demonstrated against *S. aureus* (8.7±0.12 mm) and *E. coli* (7.2±0.1 mm). Furthermore, no probiotic activity was recorded against *Streptococcus spp* and *Pseudomonas spp.* No significant ($p> 0.05$) differences in antimicrobial activity were noted using Lactic acid bacteria isolated from the different fish body parts. Based on the positive results from this study, *Lactobacillus spp*. should be further characterised and studied in challenge experiments in fish to explore its probiotic effects.

Keywords: Alestes baremoze; Lake Albert; lactic acid; pebbly fish; probiotics.

1. INTRODUCTION

In order to improve aquaculture production, there is need to control fish reproduction, have sound knowledge on the biology of farmed fish, on technology and development of specific feeds. However, a number of challenges to improve productive, feasible, and sustainable aquaculture in present super intensive systems still exist. Fish diseases remain a major challenge for the aquaculture industry with significant consequences on production and trade in many countries [1-4]. Although vaccines are continually developed and marketed, they cannot be used as a universal disease control measure in aquaculture. Control of pathogens in fish farms has been routinely achieved by the administration of antimicrobial agents [5]. However, excessive use of antibiotics has been associated with the emergence of antibiotic resistance with significant public health consequences [6,7]. Currently, the modern aquaculture industry requires practices that maintain a healthy environment, like the use of probiotic microbes [8].

A number of probiotic organisms have been evaluated for use in aquaculture, with results indicating improved resistance against diseases, reduction in fish mortality and increased survival [9,10]. Most studies on aquatic diseases in Uganda have concentrated on identifying common potential pathogens affecting fish species [11,12]. Preventive strategies against aquatic diseases are limited yet disease incidences are on the rise in hatcheries and grow-out systems [12]. The addition of substantial amounts of antibiotics and chemotherapeutics remains the method of choice for disease control in Uganda. As new technologies continue to be developed for the culture of new species such as the anticipated *A. baremoze* aquaculture, generating knowledge on probiotics will be of significant practical importance in developing biological disease control strategies as opposed to the chemical-based ones.

It has been reported that some species of lactic acid bacteria (LAB) are not pathogenic and have not been found to cause infectious disease in fish [9]. Therefore, the aim of this study was to understand the probiotic properties of *Lactococcus spp*, and *Lactobacillus spp* against several fish pathogens by examining their antibacterial properties. This study will generate basic information on potential probiotics that could be used as a guide in the development of probiotic technologies for *Alestes baremoze* culture.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

A total of 15 fresh samples of *Alestes baremoze* (40.8 ±1.82 cm SL) were collected from Lake Albert and transported in an icebox to the Microbiology laboratory at Makerere University. Immediately upon arrival, fish were placed on a clean, sterile aluminum foil surface, sterile swabs were soaked in saline solution and swabbed along the skin surface and gills at various positions and labelled accordingly. Thereafter, using a sterile scalpel blade, an incision was made along the ventral surface running from the mid-section up to the anal opening, to reveal the gut, which was also cut and swabbed as described above.

2.2 Enumeration, Isolation and Identification of Pathogenic Bacteria

Ten serial dilutions of the original stock culture from the gills, skin and gut were prepared. Each dilution was plated on solidified freshly prepared nutrient agar and spread using a sterile glass rod. The inoculated agar was incubated at 37°C for 24 hours after which the developed colonies were counted. Counts within 30-300 colony forming units (cfu) were reported as total viable count (TVC). Distinct colonies from each plate were then picked using a sterile wire loop and sub cultured onto freshly prepared nutrient agar and incubated at 37°C for 24 hours to obtain pure cultures. Characterization of pure isolates was performed using colonial characteristics, gram staining and biochemical methods according to Cheesbrough [13] and Mac Faddin [14].

2.3 Isolation, Selection and Identification of Selected LAB

Using serial dilution (up to 10^6 CFU/ml, NS), 0.1 ml of homogenized samples from the gills, skin and gut were spread on tryptic soy agar (TSA) (Merck) and incubated at 30°C for 48 hours. The prepared samples were then immersed in de Man Rogosa and Sharpe (MRS) (Merck) broth and incubated at 30°C for 24 hours. After pipetting, 0.1 ml of the cultured broth was transferred to MRS agar containing bromo-cresol purple (0.17 g/L). The plates were incubated at 30°C for 48 hours under anaerobic condition (Oxoid anaerobic gas pack jar). Yellow colonies were sub-cultured three times on new MRS agar to obtain single pure colonies that were identified using gram staining and catalase reaction using 3% hydrogen peroxide.

2.4 Probiotic Characteristics

2.4.1 Antimicrobial activity

Antibacterial activity of the strains was tested using the agar spot method. This was done using cell-free cultured broth of the individual selected colonies as described by Schillinger and Lücke [15]. In this method, overnight cultures of *Lactobacillus spp* and *Lactococcus spp* were spotted onto the surface of MRS agar plates (1.2% w/v agar, 0.2% w/v glucose) and incubated anaerobically for 24 hours at 37°C. The indicator organisms (*Staphylococcus spp, Streptococcus spp, Proteus spp, Pseudomonas spp and E. coli*) were then inoculated into 7 ml of soft agar medium (nutrient broth containing 0.7% w/v) to a final concentration of approximately 107 colony forming unit (cfu)/ml and the soft media was poured on the plates. After 24 hours of incubation at the optimal growth temperature for the indicator strains, inhibition halos were measured and the width of the clear zones calculated by subtracting diameter of the spot from the diameter of the clear zone without pathogen growth.

2.5 Data Analysis

All data from the microbial and probiotic examination was entered in Microsoft Excel sheets and later transferred to Graph pad version 6.0 for statistical analysis. Microbial load from the skin, gills and gut were compared using a One-way ANOVA. Significant differences in antibacterial activity across the different pathogenic bacteria were analyzed using a One-way ANOVA set at significance level of ($p< 0.05$). Multiple comparisons between groups (fish surface and pathogenic bacteria strains) were done using Tukey`s multiple comparison test, differences were taken as significant at $p< 0.05$.

3. RESULTS

3.1 Total Microbial Load

The result of the total viable bacterial count of the fresh fish samples expressed in colony forming unit per gram (cfu/g) is shown in Table 1. The data showed 100% growth across all samples (N= 15). The highest total microbial load was generally recorded for samples from the skin. *Staphylococcus spp* had the highest total microbial load recorded from all the samples (skin, $8.50\pm22.1x10^2$cfu/g and gills, $8.00\pm24.3x10^2$ cfu/g) followed by *Lactobacillus spp* (skin, $6.00\pm16.7x10^2$ cfu/g, gills, $4.00\pm10.9x10^2$ cfu/g, gut, $2.50\pm9.9x10^2$ cfu/g) respectively. *Staphylococcus spp, Lactobacillus spp,* and *Lactococcus spp* were significantly highest on the skin ($8.50 \pm22.1x10^2$ cfu/g, $6.00\pm16.7x10^2$ cfu/g, $2.80\pm9.9x10^2$ cfu/g) respectively compared to the gills and gut. *Micrococcus spp* was only detected in gut ($1.00\pm24.9x10^3$ cfu/g).

3.2 Frequency of Occurrence of the Various Isolates

The frequencies of the isolated bacteria per fish body part are indicated in Table 2. *Staphylococcus aureus* and *E. coli* were only

Table 1. Total microbial load across fish parts

Organism	Skin (cfu/g)	Gills (cfu/g)	Gut (cfu/g)	p value
Staphylococcus aureus	850 ±22.1	800±24.3	ND	0.001*
Escherichia coli	120±3.41	90±1.95	ND	0.001*
Micrococcus spp	ND	ND	1000±24.9	< 0.0001*
Lactobacillus spp	600±16.7	400±10.9	250±9.9	0.001*
Lactococcus spp	280±9.9	140±4.6	70±1.98	0.001*

**indicates significant differences across fish parts. ND: Not detected*

Table 2. Incidences of isolates per fish body part

Organism	Skin (N=15)	Gills (N=15)	Gut (N=15)	P value
Staphylococcus aureus	15 (100%)	15 (100%)	0	<0.0001*
Escherichia coli	10 (67.7%)	8 (53.3%)	0	0.0004*
Micrococcus spp	0	0	8 (53.3%)	<0.0001*
Lactobacillus spp	10 (67.7%)	12 (80%)	15 (100%)	0.06
Lactococcus spp	8 (53.3%)	10 (67.7%)	15 (100%)	0.06

isolated from the skin and gills, while *Micrococcus spp* was only detected in the gut for 8 fish (53.3%). For *Lactobacillus spp* and *Lactococcus spp*, no significant differences were noted across the different fish body parts.

3.3 Probiotic Activity

The study examined the antibacterial activity of the isolated *Lactococcus spp* and *Lactobacillus spp* against selected pathogenic bacteria, as a measure of probiotic activity (Table 3). The probiotic activity of the genera isolated was tested against five fish pathogens (*Staphylococcus aureus, Streptococcus spp, Proteus spp, Pseudomonas spp* and *E. coli*). *Lactobacillus spp* showed the highest overall activity against all test pathogens as compared to *Lactococcus spp.* The highest activity for *Lactobacillus spp* was demonstrated against *E. coli* (15.4±0.19 mm) and *S. aureus* (14.0±0.17 mm). No significant (p> 0.05) differences in antimicrobial activity were noted using *Lactobacillus* spp isolated from the different fish body parts. The highest activity for *Lactococcus spp* was demonstrated against *S. aureus* (8.7±0.12 mm) and E. *coli* (7.2±0.1 mm). No significant (p> 0.05) differences in antimicrobial activity were noted using *Lactococcus spp* from the different fish body parts. Furthermore, no probiotic activity was recorded against *Streptococcus spp* and *Pseudomonas spp.*

4. DISCUSSION

Control of pathogens in fish farms has been routinely achieved by the administration of antimicrobial agents [5]. However, excessive use of antibiotics has been associated with the emergence of antibiotic resistant with significant

Table 3. Antimicrobial activity of *Lactococcus spp* and *Lactobacillus spp* against fish pathogens

Lactococcus					
Pathogenic organism	**Skin (mm)**	**Gills (mm)**	**Gut(mm)**	**Total**	**P value**
S. aureus	9±0.17	11±0.19	6±0.17	8.7±0.12	0.08
Streptococcus spp	0	0	0	0	N/A
Proteus spp	7±0.11	4.5±0.16	3.5±0.10	5.0±0.16	0.06
Pseudomonas spp	0	0	0	0	N/A
E. coli	8±0.19	6.5±0.11	7±0.10	7.2±0.1	0.08
Lactobacillus					
Pathogenic organism	**Skin (mm)**	**Gills (mm)**	**Gut(mm)**	**Total**	**P value**
S. aureus	17±0.22	15±0.16	11±0.16	14.0±0.17	0.18
Streptococcus spp	12±0.18	9±0.19	5±0.16	8.7±0.16	0.20
Proteus spp	10±0.19	8±0.21	6±0.16	8.0±0.14	0.15
Pseudomonas spp	9±0.19	7.5±0.11	6±0.19	7.5±0.18	0.08
E. coli	17±0.20	16±0.19	13±0.19	15.4±0.19	0.12

public health consequences [6,7,16]. Currently, modern aquaculture industry requires practices that maintain a healthy environment, like the use of probiotic microbes [17,8].

The current study showed that bacterial load varied across the three segments of the skin, gills and gut with the skin having the highest number of bacteria. These results are not surprising since aquatic environment is rich in pathogenic organisms and the skin acts as the first line of defense against the invasion of environmental pathogens. Our study revealed that *S. aureus* (100%) and *E coli* (60%) were more commonly isolated. Similar studies have demonstrated the presence of *S. aureus* [18] and *E. coli* in fish [19]. Since *S. aureus* is an indicator of hygiene and sanitary conditions, the presence of this organism might point to unhygienic condition during processing and storage. Although recent investigations have shown that *E. coli* and fecal coliform bacteria can be found in unpolluted warm tropical waters and that *E. coli* can survive indefinitely in this environment [20], they are particularly useful as indicators of fecal contamination and poor handling of seafood. However, these pathogenic bacteria were absent in the gastrointestinal tract as compared to the Lactic acid bacteria (LAB) and *micrococcus spp.* Lactic acid bacteria were present in all the three segments of the skin, gills and gut. Similarly, Ringø and Gatesoupe [21] reported that LAB are part of the normal microbiota of *Cyprinidae*, *Escocidae* and *Percidae*, and that lactic acid bacteria are present in several fish species at larval, fry and fingerling stages.

As compared with other bacteria, *Micrococcus spp* and *Lactobacillus spp* were predominantly highest in the gastrointestinal tract of *A. baremoze*. It could be that these bacteria strains are normal inhabitants of the digestive tract, and therefore develop mechanisms to survive in this environment like adhering to the exposed surface of the epithelial cells. In addition, the nutrient composition may favour their presence in the gut. According to Ringø and Gatesoupe [21] when nutrient supply is limited in the gastrointestinal tract, the composition of the microbiota may be affected by competition for this nutrient. Lactic acid bacteria are nutritionally demanding, requiring carbohydrates, amino acids, peptides, nucleic acid derivatives and vitamins. *Lactobacilli* rely on other microorganisms to act on complex molecules to provide certain nutrients. It seems entirely possible that competition for nutrients plays a role in the composition of the gastrointestinal tract microbiota.

Lactic acid bacteria are generally considered to be non-pathogenic though genus *Lactobacillus* has been reported to cause disease [21]. In this study, Lactic acid bacteria are purely considered as a potential probiotic. The inhibitory effect of lactic acid bacteria against fish pathogen is not limited to strains isolated in fish. It is generally considered that Gram-positive bacteria including lactic acid bacteria are numerically dominant members of the normal microbiota in the gastrointestinal tract of endothermic animals at their early life stage. Most probiotics suggested as biological control agents in aquaculture belong to the lactic acid bacteria [10,4,22]. The probiotic genera isolated from this study were *Lactococcus spp and Lactobacillus. Lactococcus spp* did not show any inhibitory effect against *Streptococcus spp* and *Pseudomonas spp,* whereas *Lactobacillus spp* showed inhibitory effects against all the potential pathogenic bacteria used (*S. aureus, Streptococcus spp, Klebsiella spp, Proteus spp, Pseudomonas spp* and *E. coli*). This probiotic activity of *Lactobacillus spp* has already been demonstrated in a number of studies in fish [23-26]. Similar trends have been reported by Balcázar et al. [6] who found that the growth of *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Yersinia ruckeri* and *Vibrio anguillarum* was inhibited by *Lactococcus lactis.* The colonization of the fish digestive tract by bacteria capable of producing lactic acid fermentation may inhibit the proliferation of putrefactive microbes in that site, thus protecting the host from diseases caused by toxins generated by proteolytic bacteria.

5. CONCLUSION

In conclusion, prevalent micro-organisms were isolated from the skin, gills and gut of *A. baremoze* included: *Staphylococcus spp, E. coli, Micrococcus spp, Lactobacillus spp* and *Lactococcus spp*. of these organisms, *Staphylococcus spp* and *E. coli spp* were identified as potential pathogens while *Lactobacillus spp* and *Lactococcus spp* were identified as potential probiotics. Probiotic activity was highest for *Lactobacillus spp.* Future studies characterizing the observed probiotic species would be important to aid their use in aquaculture. Depending on the ability of *Lactobacillus spp*. to suppress pathogens (*S. aureus*, *Streptococcus spp, Proteus spp*, *Pseudomonas spp* and *E. coli*) growth under in vitro conditions, it should be further studied in challenge experiments in fish to observe its potential probiotic effects in situations directly relevant to aquaculture conditions.

ACKNOWLEDGEMENTS

This study was supported by the grant from the Competitive Grant Scheme (CGS Project ID/no: CGS/4/32/14) of the National Agricultural Research Organisation (NARO) implemented by Abi ZARDI under the Agricultural Technology and Agribusiness Advisory services project (ATAAS) funded by the World Bank.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Austin B, Austin DA. Bacterial fish pathogens: disease of farmed and wild fish. 4th ed. UK: Springer Science & Business Media; 2007.
2. Gram L, Melchiorsen J, Spanggaard B, Huber I, Nielsen TF. Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. Applied and Environmental Microbiology. 1999;65(3):969-73.
3. Tellez-Bañuelos MC, Santerre A, Casas-Solis J, Zaitseva G. Endosulfan increases seric interleukin-2 like (IL-2L) factor and immunoglobulin M (IgM) of Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromona hydrophila*. Fish & Shellfish Immunology. 2010;28(2):401-5.
4. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews. 2000;64(4):655-71.
5. Holmström K, Gräslund S, Wahlström A, Poungshompoo S, Bengtsson BE, Kautsky N. Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science & Technology. 2003;38(3): 255-66.
6. Balcázar JL, Vendrell D, de Blas I, Ruiz-Zarzuela I, Muzquiz JL, Girones O. Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquaculture. 2008; 278(1):188-91.
7. De La Peña AM, Espinosa-Mansilla A. Analysis of antibiotics in fish samples. Analytical and Bioanalytical Chemistry. 2009;395(4):987-1008.
8. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L. Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture. 2008;274(1):1-14.
9. Gatesoupe F. The use of probiotics in aquaculture. Aquaculture. 1999;180(1): 147-65.
10. Irianto A, Austin B. Probiotics in aquaculture. Journal of Fish Diseases. 2002;25(11):633-42.
11. Akoll P, Konecny R, Mwanja WW, Nattabi JK, Agoe C, Schiemer F. Parasite fauna of farmed Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) in Uganda. Parasitology Research. 2012; 110(1):315-23.
12. Walakira J, Akoll P, Engole M, Sserwadda M, Nkambo M, Namulawa V, et al. Common fish diseases and parasites affecting wild and farmed Tilapia and catfish in Central and Western Uganda. Uganda Journal of Agricultural Sciences. 2014;15(2):113-25.
13. Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press; 2006.
14. Mac Faddin JF. Biochemical tests for identification of medical bacteria. Williams & Wilkins Co.; 1976.
15. Schillinger U, Lücke FK. Antibacterial activity of Lactobacillus sake isolated from meat. Applied and Environmental Microbiology. 1989;55(8):1901-6.
16. Alderman D, Hastings T. Antibiotic use in aquaculture: Development of antibiotic resistance–potential for consumer health risks*. International Journal of Food Science & Technology. 1998;33(2):139-55.
17. Joerger R. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poultry Science. 2003; 82(4):640-7.
18. Ibrahim B, Sheshi M. Isolation and identification of bacteria associated with fresh and smoked fish (*Clarias gariepinus*) In Minna Metropolis, Niger State. Nigeria. Journal of Applied & Environmental Microbiology. 2014;2(3):81-5.
19. Mandal SC, Hasan M, Rahman MS, Manik MH, Mahmud ZH, Islam MS. Coliform bacteria in Nile Tilapia, *Oreochromis niloticus* of shrimp-Gher, pond and fish market. World Journal of Fish and Marine Science. 2009;1(3):160-6.
20. Huss HH. Assurance of seafood quality. Food & Agriculture Org. 1994;334.

21. Ringø E, Gatesoupe FJ. Lactic acid bacteria in fish: A review. Aquaculture. 1998;160(3):177-203.
22. Hamid THTA, Khan AJ, Jalil MF, Azhar S. Isolation and screening of lactic acid bacteria, *Lactococcus lactis* from *Clarias gariepinus* (African catfish) with potential use as probiotic in aquaculture. African Journal of Biotechnology. 2014;11:29.
23. 23. Kim PI, Jung MY, Chang YH, Kim S, Kim SJ, Park YH. Probiotic properties of *Lactobacillus* and *Bifidobacterium* strains isolated from porcine gastrointestinal tract. Applied Microbiology and Biotechnology. 2007;74(5):1103-11.
24. Nayak S. Probiotics and immunity: A fish perspective. Fish & Shellfish Immunology. 2010;29(1):2-14.
25. Nikoskelainen S, Ouwehand A, Salminen S, Bylund G. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. Aquaculture. 2001;198(3):229-36.
26. Suzer C, Çoban D, Kamaci HO, Saka Ş, Firat K, Otgucuoğlu Ö, et al. Lactobacillus spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata* L.) larvae: Effects on growth performance and digestive enzyme activities. Aquaculture. 2008; 280(1):140-5.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16285