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Probiotic Properties Profiling of Isolated Lactic Acid Bacteria from the Intestine of *Channa punctata*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RAN, Saifullah and FKS designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors MAAM, MSH and MA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study was conducted for isolation, identification and probiotic profiling of *Lactobacillus spp.* from the intestine of indigenous and non-indigenous *Channa punctata* species.
Study Design: *Lactobacillus spp.* from the intestine of *Channa punctata* fish was isolated and cultured as well as screened for probiotic properties and profiling.
Place and Duration of Study: The research work was carried out in Biotechnology Laboratory, Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Jessore-7408, Bangladesh, from July to October, 2015.
Methodology: Lactic Acid Bacteria (*Lactobacillus spp.*) were isolated from the intestine of dissected *Channa punctata* by using MRS agar medium following spread plate method. Isolated bacteria were subjected to gram staining, microscopic observation and biochemical tests example catalase for identification of *Lactobacillus spp*. Thereafter, bile salt tolerance test, phenol tolerance test and antibiotic sensitivity test were done for probiotic profiling.

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Results: Two Lactic Acid Bacteria were isolated from indigenous and non-indigenous *Channa punctata* species where total bacterial cell was counted as 2.1×10^{10} and 1.9×10^{9} cfu/gm. Both the fish intestines contain bacteria having gram positive, non-sporulating, catalase negative rods which were confirmed to be *Lactobacillus*. They were able to survive in bile salt and phenol at 0.1, 0.2, and 0.3% concentration. The *Lactobacillus* isolated from indigenous *Channa punctata* was resistant to Azithromycin, Cefuroxime, Ciprofloxacin, Tetracycline, Ampicillin, Erythromycin, Vancomycin, Chloramphenicol and Co-trimoxazole while *Lactobacillus* from non-indigenous *C. punctata* showed almost same resistant except Cefotaxime and Gatifloxacin. They are claimed as probiotics.
Conclusion: Thus, the study claimed that the isolated, counted and identified *Lactobacillus spp*. are belong to probiotic that can exert beneficial health action for human.

Keywords: Probiotics; LAB; Channa punctata; bile salt and phenol tolerance; antibiotic sensitivity.

1. INTRODUCTION

Lactic acid bacteria (LAB) strains are normal intestinal microbiota, some of them can be used as probiotics for human and animals [1]. Probiotics is defined as live microorganism which, when administered in sufficient amounts, bestow a health benefit on the host [2]. Usually, log 6–log 7 of probiotic bacteria per ml/gm of the ingested product at the time of consumption are considered adequate for exertion of health benefiting actions, including preventing growth of pathogenic bacteria, production of antimicrobial agents [3-5]. Besides this, some probiotics contribute to develop herd performance and their health management [6,7]. The genus *Lactobacillus* is an important lactic acid bacterial (LAB) group belonging to probiotics.

Channa punctata of *chhanadae* families (Snakehead fish) is a popular and most widespread fish in fresh water in Southeast Asia. It is one of the common staple food fish in Thailand, Cambodia, Vietnam and other South East Asian countries where they are extensively cultured. This fish has different beneficial effects, including wound healing and remedies, inhibition of pathogenic microorganisms, enzymatic contribution to digestion and increased immune response [8]. In the aquaculture environment the first study on the screening of probiotic bacteria was reported in 1980s [9].

To find out probiotic one has to evaluate few characteristics such as phenol and bile salts tolerance and also antibiotic susceptibility since the bacterium has to survive in the diversity offered by adverse intestinal environment to stressful condition and also to prevail when traditional antibiotics are in action simultaneously [10,11]. The aim of this study was the isolation, identification and probiotic profiling of *Lactobacillus spp.* from the intestine of indigenous and non-indigenous *Channa punctata* species. To our knowledge, probiotic strains from the intestine of the fish have not yet studied to date in Bangladesh. It is quite understandable that occurrence of *Lactobacillus* in the sources belong to this region has to be well reported. The claimed probiotics could aid in future research of bio-therapeutics for sure.

2. MATERIALS AND METHODS

2.1 Sample Collection

The 5 samples of indigenous *Channa punctata* fish was collected from the local canal of Horinar Bil, Jessore and 5 non-indigenous species were collected from the central pond of Jessore University of Science and Technology (JUST), Jessore, Bangladesh. For the taxonomical confirmation we took the samples to the responsible scientist on duty Freshwater Fish Breeding Center, Chachra. All the fishes were weighted more than 100 gm as well measured as 10 to 15 cm in length. At first, whole body of fishes were clearly washed with 70% alcohol and dissected. Thereafter, the intestines were collected and washed with 0.85% saline water and distilled water for 2 to 3 times and took 1 gm from small parts of intestine. It was then homogenized with the clean mortar and pestle under sterile condition [12].

2.2 Isolation of Lactobacillus Bacteria

By following the technique of serial dilution, 0.1 ml of the homogenized intestine sample was spread over prepared plate count agar (PCA) (Merck) and incubated at 37°C for 72 hours to count the colony forming unit (CFU). Rest of the homogenate was poured into MRS broth medium through filtration. It was incubated for 24 to 48 hours at 37°C. After that serial dilution was done

with 0.1 ml of culture. Sample culture was then spread on MRS agar and incubated at 37°C for 72 hours [13]. White colony was observed and from this single colony subculture was done on new MRS agar for 3 or 4 times to find axenic culture. Isolated pure bacterial single colonies were stored for further study on agar slants.

2.3 Identification of Lactobacillus Bacteria

The gram staining technique with microscopic observation of the morphological characteristics and classical biochemical test sets were applied for the identification of bacterium. The slide was examined under light microscope with a magnification of 1000X [14].

2.4 Probiotic Profiling

2.4.1 Bile salt tolerance

Testing bile salt tolerance of the isolate was on the carts. The test was executed in MRS broth which included 0.1, 0.2 and 0.3% (w/v) Oxgall bile salt (Sigma, USA). Duplicate bottles of MRS broth containing filtered different concentrations of bile salt were inoculated by 30 µl of cultured strain and incubated at 30°C. Growth rate was assessed by measuring the optical density by spectrophotometer at 600 nm after 2 and 4hrs incubation [15].

2.4.2 Phenol tolerance test

For the determination of phenol tolerance of isolated LABs test tube containing MRS broth were adjusted with different concentration (0.1-0.3%) of phenol. After sterilization, each test tube was inoculated with 1% (v/v) fresh overnight culture of LABs and incubated at 37°C for 24 h. After 24 h of incubation their growth were absorbance at 620 nm of cell concentration by Spectrophotometer [16].

2.4.3 Antibiotic sensitivity test

In this study antibiotic sensitivity test was done according to disk diffusion method. Here 13 antibiotic discs were used, i.e. Ampicillin (AMP), Azithromycine (AZM), Cefotaxime(CTX), Cefuroxime (CXM), Chloramphenicol (C), Ciprofloxacin (CIP), Co-trimoxazole (COT), Erythromycin (E), Gatifloxacin (GAT), Gentamicin (GEN), Nalidixic acid (NA), Tetracycline (TE) and Vancomycin (VA). Where, 100 µl of MRS broth containing isolated LABs ($Isolate_1$ and $Isolate_2$) were spread on Nutrient Agar medium containing plates prior to placing antibiotic discs on them. Then it was incubated overnight at 37°C for 24 hrs. Resistance and susceptibility were estimated by measurement of zone of inhibition [17].

2.5 Statistical Analysis

Each experiment was run in triplicate and mean values were calculated with SPSS version 11.0 was used for the data analysis.

3. RESULTS

3.1 Total Bacterial Count in Intestine

The counted colony that has been estimated was 2.1×10^{10} and 1.9×10^{9} cfu/gm from the intestines of indigenous and non-indigenous *C. punctata* species respectively.

3.2 Identification of Lactobacillus

Isolated bacteria from both the fishes showed white to yellowish colony on MRS agar (Fig. 1a). Both the fish intestines contain bacteria having gram positive, non-sporulating, catalase negative rods which were confirmed to be *Lactobacillus* [18]. Thus, LAB isolated from indigenous *C. punctata* was denoted as $Isolate_1$ and isolate from Non-indigenous *C. punctata* was $Isolate_2$.

3.3 Probiotic Profiling

3.3.1 Bile salt tolerance

Both the LABs ($Isolate_1$ and $Isolate_2$) showed significant proliferation at 0.1, 0.2 and 0.3% bile salt concentration after 2 and 4 hrs of growth, however, their growth get down with the increase of bile salt concentration. The trending pattern showed resemblance even after 6, 8 and 12 hrs of incubation with enhanced proliferation (Fig. 2).

3.3.2 Phenol tolerance test

Both the LABs exhibited cabalistic proliferation at 0.1, 0.2 and 0.3% phenol concentration after 2 and 4 hrs of growth, however, their growth descended with the increase of phenol concentration. The trending pattern showed resemblance even after 6, 8 and 12 hrs of incubation with enhanced proliferation (Fig. 3).

3.3.3 Antibiotic sensitivity test

Both the bacterial strains were resistant to numerous antibiotics. $Isolate_1$ was resistant to Azithromycin, Cefuroxime, Ciprofloxacin and Tetracycline; while moderately resistant to Ampicillin, Erythromycin and Vancomycin; intermediate resistant to Chloramphenicol and Co-trimoxazole; and susceptible to Cefotaxime, Gatifloxacin, Gentamicin and Nalidixic Acid. $Isolate_2$, moreover, showed resistance to Cefuroxime, Ciprofloxacin and Vancomycin. It was moderately resistant to Ampicillin, Azithromycin, Gatifloxacin and Tetracycline; intermediate resistant to Cefotaxime, Chloramphenicol, Co-trimoxazole and Erythromycin and susceptible to Gentamicin and Nalidixic Acid. The details of the results have been given in Table 1.

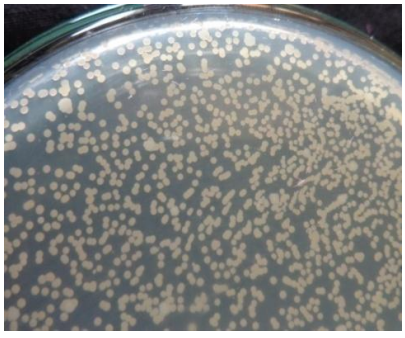
4. DISCUSSION

This work provides evidences of the amplitude of *Lactobacillus* in the intestine of two *C punctata* species for the very beginning. Total bacterial cell was counted as 2.1×10^{10} and 1.9×10^{9} cfu/gm. Other studies reported almost similar result like Allameh et al. [19] founded bacterial load of $1.5x10^{5}$ cfu/g in the intestine of *Channa striatus* while Al-harbi and Uddin [20] reported $1.6x10^{8}$ cfu/g population in the intestine of *oreochromis niloticus*. *Lactobacilli have* been isolated from the intestines of *Hypophthalmichthys molitrix*, *Oreochromis mossambicus*, and black tiger shrimp [21,22]. Bucio et al. [23] reported the presence of *Lactobacilli* in the intestines of freshwater fishes from a river and from a farm with a recirculation system.

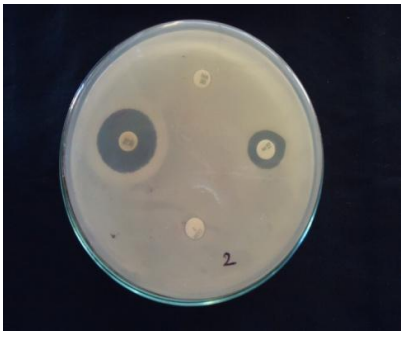
Table 1. Antibiotic sensitivity test results on isolated LAB

Antibiotics	Concentration (µg/disc)	Zone Inhibition (in mm)		Sensitivity LAB	
		$Isolate_1$	$Isolate_2$	$Isolate_1$	$Isolate_2$
Ampicillin (AMP)	25	8	6	MR	MR
Azithromycin (AZM)	30	03	08	R	MR
Cefotaxime (CTX)	30	17	10	S	IR
Cefuroxime (CXM)	30	0	0	R	R
Chloramphenicol (C)	5	15	13	IR	IR
Ciprofloxacin (CIP)	30	0	0	R	R
Co-trimoxazole (Cot)	30	I3	15	IR	IR
Erythromycin (E)	30	8	13	MR	IR
Gatifloxacin (GAT)	10	22	7	S	MR
Gentamicin (GEN)	30	27	23	S	S
Nalidixic Acid (NA)	30	20	22	S	S
Tetracycline (TE)	25	0	9	R	MR
Vancomycin (VA)	30	7	0	MR	R

"R" Indicates resistant, "MR" Indicates moderately resistant, "IR" Indicates Intermediate Resistant, "S" indicates susceptible



a. Isolated LAB on MRS agar plate, Isolate from *C. punctata*



b. Antibiotic sensitivity test results on isolated LAB

Fig. 1. Isolated LAB on MRS agar medium and antibiotic sensitivity test result

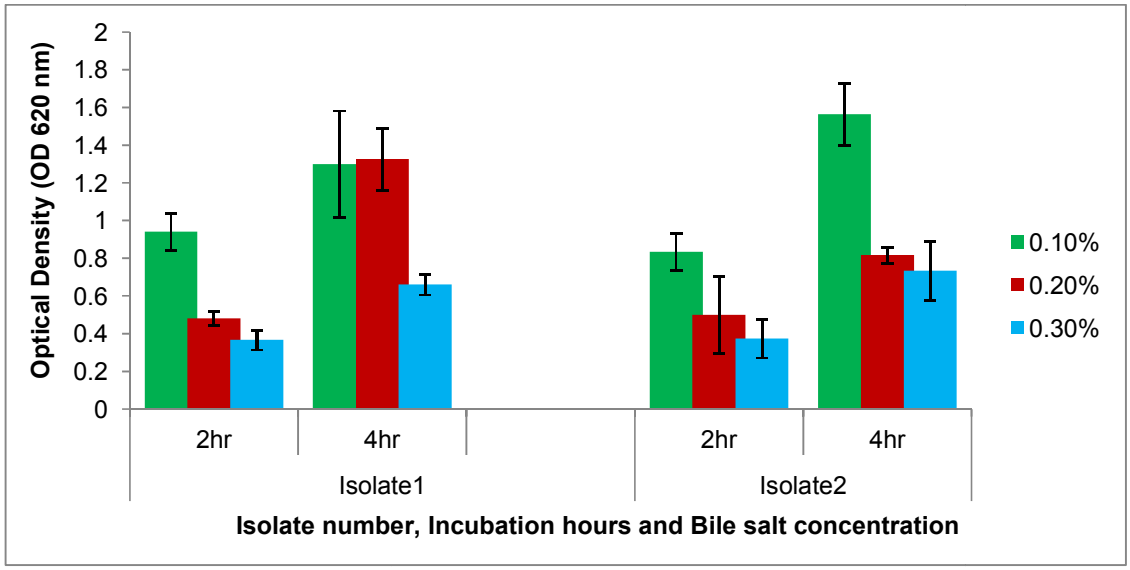


Fig. 2. Bile salt tolerance test result

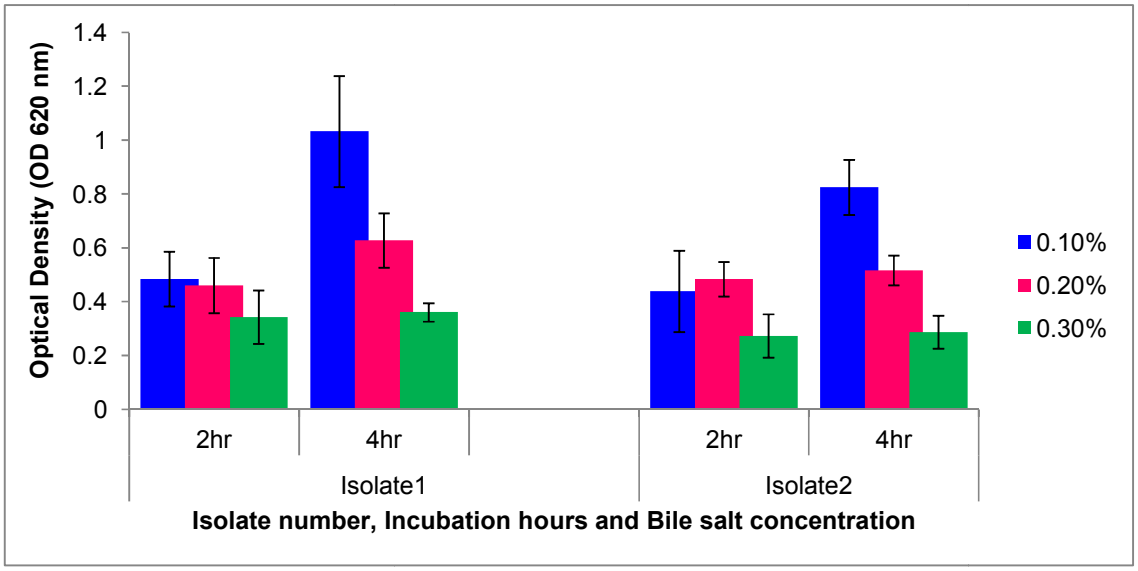


Fig. 3. Phenol salt tolerance test result

On the other hand, both the LABs were tested for their bile salt tolerance against three concentrations i.e. 0.1, 0.2 and 0.3%. Although an increase in bile salt quantity decreased the proliferation rate of the two isolated LABs, it is quite substantive that they could survive different bile salt concentrations. This is another notable probiotic feature since the fish intestine possesses variable bile salt concentration [24]. Survival of *Lactobacillus* at 0.1 to 0.4% bile salt concentration has been reported similar by many other workers as well [10,15]. Tolerance of these two probiotics to bile salt and acidic environment denotes they are endurable under stress.

Moreover, probiotic bacteria showed their ability to grow at different phenol concentrations. In this study, all probiotic isolates were able to tolerate 0.1-0.3% phenol concentration. Survival of Lactobacillus at 0.1 to 0.4% bile salt concentration has been reported by other worker as well [25]. Abdullah-Al-Mamun et al. [26] reported similar result for phenol tolerance. Tolerance of these two probiotics to phenolic

environment proves they are viable under adverse condition.

Almost similar results have been described by many scientists for Antibiotic sensitivity test like Allameh et al. [19] reported antibiotic susceptibility profiles showed that this strain was resistant to Streptomycin, intermediate to Amoxicillin and Kanamycin and sensitive to Gentamycin, Tetracycline, Chloramphenicol, and Ampicillin. Kim and Austin [27] founded the antibiotic susceptibility of Carnobacterium strains. They reported resistance to Ampicillin, Gentamycin, Kanamycin, Streptomycin and Penicillin G but sensitivity to Chloramphenicol, Tetracycline and Co-trimaxazole. They also claimed that antibiotic resistant probiotic is gainful in the case of antibiotics administration to fish and the establishment of the beneficial microorganisms in the intestine for prolonged periods.

5. CONCLUSION

In this study two LABs ($Isolate_1$ and $Isolate_2$) were isolated from *Channa punctata* and identified as *Lactobacillus* successfully. The total bacterial cell was counted as 2.1×10^{10} and 1.9×10^{9} cfu/gm. They were able to survive in bile salt and phenol at 0.1, 0.2, and 0.3% concentration. The *Lactobacillus* ($isolate_1$) isolated from indigenous *Channa punctata* was resistant to Azithromycin, Cefuroxime, Ciprofloxacin, Tetracycline, Ampicillin, Erythromycin, Vancomycin, Chloramphenicol and Co-trimoxazole. $Isolate_2$ from non-indigenous *C. punctata* showed almost same resistant surplus to Cefotaxime and Gatifloxacin. They are claimed as probiotics. Therefore, 16s rRNA assay can be used for further accurate identification of the lactic acid bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chou LS, Weimer B. Isolation and characterization of acid- and bile-tolerant isolates from strains of Lactobacillus acidophilus. J Dairy Sci. 1999;82(1):23-31. Available:http://dx.doi.org/10.3168/jds.S0022-0302(99)75204-5
2. Ganguly NK, Bhattacharya SK, Sesikeran B, et al. ICMR-DBT Guidelines for Evaluation of Probiotics in Food. The Indian Journal of Medical Research. 2011;134(1):22-25.
3. Tsai CC, Lin PP, Hsieh YM. Three Lactobacillus strains from healthy infant stool inhibit enterotoxigenic Escherichia coli grown *in vitro*. Anaerobe. 2008;14(2):61-67. Available:http://dx.doi.org/10.1016/j.anaerobe.2007.11.003
4. Wagner RD, Johnson SJ, Kurniasih Rubin D. Probiotic bacteria are antagonistic to *Salmonella enterica* and *Campylobacter jejuni* and influence host lymphocyte responses in human microbiota-associated immunodeficient and immunocompetent mice. Mol Nutr Food Res. 2009;53(3):377-388. Available:http://dx.doi.org/10.1002/mnfr.200800101
5. Gillor O, Etzion A, Riley MA. The dual role of bacteriocins as anti- and probiotics. Appl Microbiol Biotechnol. 2008;81(4):591-606. Available:http://dx.doi.org/10.1007/s00253-008-1726-5
6. Vasconcelos JT, Elam NA, Brashears MM, Galyean ML. Effects of increasing dose of live cultures of *Lactobacillus acidophilus* (Strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (Strain NP 24) on performance and carcass characteristics of finishing beef steers. J Anim Sci. 2008;86(3):756-762. Available:http://dx.doi.org/10.2527/jas.2007-0526
7. Sargeant JM, Amezcua MR, Rajic A, Waddell L. Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of weaned domestic ruminants: A systematic review. Zoonoses Public Health. 2007;54(6-7):260-277. Available:http://dx.doi.org/10.1111/j.1863-2378.2007.01059.x
8. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews. 2000;64(4):655-671. Available:http://dx.doi.org/10.1128/MMBR.64.4.655-671.2000
9. Dopazo CP, Lemos ML, Lodeiros C, Bolinches J, Barja JL, Toranzo AE. Inhibitory activity of antibiotic-producing marine bacteria against fish pathogens. J Appl Bacteriol. 1988;65(2):97-101.

Available:http://dx.doi.org/10.1111/j.1365-2672.1988.tb01497.x
10. Cebeci A, Gürakan C. Properties of potential probiotic *Lactobacillus plantarum* strains. Food Microbiology. 2003;20(5): 511-518.
Available:http://dx.doi.org/10.1016/S0740-0020(02)00174-0
11. Pan X, Wu T, Zhang L, Song Z, Tang H, Zhao Z. *In vitro* evaluation on adherence and antimicrobial properties of a candidate probiotic *Clostridium butyricum* CB2 for farmed fish. J Appl Microbiol. 2008;105(5):1623-1629.
Available:http://dx.doi.org/10.1111/j.1365-2672.2008.03885.x
12. Saikot FK, Zaman R, Khalequzzaman M. Pathogenecity test of Aeromonas isolated from Motile Aeromonas Septicemia (MAS) infected Nile Tilapia on Some freshwater fish. Science International. 2013;1(9):325-329.
13. Paludan-Müller C, Henrik Huss H, Gram L. Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. International Journal of Food Microbiology. 1999;46(3):219-229.
Available:http://dx.doi.org/10.1016/S0168-1605(98)00204-9
14. Collins C. Antimicrobial sensitivity testing. Collins and Lyne's Microbiological Methods. London, Arnold Publisher; 2004.
15. 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(1–4):188-191.
Available:http://dx.doi.org/10.1016/j.aquaculture.2008.03.014
16. Roy RP, Bahadur M, Barat S. Isolation, identification and antibiotic resistance of *Aeromonas SPP.* And *salmonella SPP.* from the fresh water loach, *Lepidocephalichthys guntea* and water of Terai River Lotchka, West Bengal, India. Zoologica Poloniae. 2013;58(1-2):5-17.
17. Hoque M, Akter F, Hossain K, Rahman M, Billah M, Islam K. Isolation, identification and analysis of probiotic properties of *Lactobacillus spp.* from selective regional yoghurts. World Journal of Dairy & Food Sciences. 2010;5(1):39-46.
18. Pyar H, Peh K. Characterization and identification of *Lactobacillus acidophilus* using biolog rapid identification system. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(1):189-193.
19. Allameh SK, Daud H, Yusoff FM, Saad CR, Ideris A. Isolation, identification and characterization of *Leuconostoc mesenteroides* as a new probiotic from intestine of snakehead fish (*Channa striatus*). African Journal of Biotechnology. 2012;11(16):3810-3816.
Available:http://dx.doi.org/10.5897/AJB11.1871
20. Al-Harbi AH, Uddin N. Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. Aquaculture. 2005;250(3–4):566-572.
Available:http://dx.doi.org/10.1016/j.aquaculture.2005.01.026
21. Hagi T, Tanaka D, Iwamura Y, Hoshino T. Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. Aquaculture. 2004;234(1–4):335-346.
Available:http://dx.doi.org/10.1016/j.aquaculture.2004.01.018
22. Thillaimaharani K, Logesh A, Sharmila K, Magdoom BK, Kalaiselvam M. Studies on the intestinal bacterial flora of Tilapia *Oreochromis mossambicus* (Peters, 1852) and optimization of alkaline protease by Virgibacillus pantothenticus. Journal of Microbiology and Antimicrobials. 2012;4(5):79-87.
Available:http://dx.doi.org/10.5897/JMA11.112
23. Bucio A, Hartemink R, Schrama JW, Verreth J, Rombouts FM. Presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system. Food Microbiology. 2006;23(5):476-482.
Available:http://dx.doi.org/10.1016/j.fm.2005.06.001
24. Lars A. Lactic acid bacteria. Lactic Acid Bacteria, CRC Press. 2011;1-16.
Available:http://dx.doi.org/10.1201/b11503-2
25. Sathyabama S, Vijayabharathi R, Bruntha devi P, Ranjith kumar M, Priyadarisini VB. Screening for probiotic properties of strains isolated from feces of various human groups. Journal of Microbiology. 2012; 50(4):603-612.
Available:http://dx.doi.org/10.1007/s12275-012-2045-1
26. Abdullah-Al-Mamun M, Hasan MJ, Al Azad S, Uddin MG, Shahriyar S, Mondal KJ.

Evaluation of potential probiotic characteristics of isolated lactic acid bacteria from goat milk. British Biotechnology Journal. 2016;14(2):1-7. Available:http://dx.doi.org/10.9734/BBJ/2016/26397

27. Kim DH, Austin B. Characterization of probiotic carnobacteria isolated from rainbow trout (*Oncorhynchus mykiss*) intestine. Letters in Applied Microbiology. 2008;47(3):141-147.

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