



INV THE CHARACTERIZATION OF LACTIC ACID BACTERIA FOR THEIR POTENTIAL USE AS PROBIOTICS, ISOLATED FROM INTESTINE OF LABEO ROHITA (ROHU) FROM CHANDRAPUR DISTRICT.

S.B.Page¹, P.H.Kumbhare²

¹Department of Microbiology, Centre for Higher Learning and Research, S.P.College, Chandrapur (M.S), India

²Department of Microbiology, Guru Nanak College of Science, Ballarpur, Dist.-Chandrapur (M.S.), India

Email:sunilpage@gmail.com¹, dr.kumbhareph@yahoo.com²

ABSTRACT

It has been reported by many investigators that, the lactic acid bacteria act as potential probiotics which activate the immune system of host animal also improve microbial balance of gastrointestinal tract. The investigation was carried out to evaluate the probiotic potential of the lactic acid bacteria isolated from intestinal tract of a fresh water fish, *Labeo rohita* (Rohu), from 'Ramala Lake', of Chandrapur city.

The isolated lactic acid bacteria have been characterized and identified and then tested for by morphological, cultural and biochemical properties and tested for probiotic potential by acid tolerance and bile salt tolerance studies.

In our present investigation, two lactobacillus species isolated from the gut of *Labeo rohita* fish fulfill the required criteria of probiotics. The isolated bacteria tolerate high acidity between Ph 3 to 5 and bile salt concentration up to 0.3 %.

Keywords: Lactic acid bacteria, Probiotics, *Labeo rohita*, Acid tolerance, Bile salt tolerance

INTRODUCTION

The centrally placed "Ramala lake" of Chandrapur city is the main source of fresh water fishes for whole Chandrapur district of Maharashtra state .The fish market occupies most of Rohu (*Labeo rohita*) fishes are brought from the Ramala lake .

The term Probiotics ("for life") are environmental friendly microorganisms generally described as health promoting bacteria. According to WHO probiotics are define as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host".

Probiotic concept introduced in the early 20 th century by Elie Metchnikoff, gained much popularity recently with considerable and a significant advances in functional and health food market across the world. India is also fast emerging as a potential market for probiotic foods.

Lactic acid bacteria are well known microorganisms that have probiotic properties. Lactic acid bacteria are normal microflora of gastrointestinal tract of healthy animals like mammals and aquatic animals with no harmful effects (Salminen et al., 2004; Lara -Flores, 2011).

The colonization of the intestine by probiotic bacteria which prevents harmful bacteria from growth by competition exclusion and by the production of organic acid and antimicrobial compounds (P. Muthukumar et al., 2015)

The acid tolerance and bile salt tolerance are fundamental probiotic properties that indicate the ability of probiotic microbes to survive the intestinal tract, specially, acidic conditions of stomach and presence of bile salt in the intestine (Hyronimus et al., 2000, Erkila and Petaja, 2000). Lactic acid bacteria are normal native

microbiota of aquatic animals from temperate regions (Ringo, 2004).

The present study is based on the study of probiotic properties of Lactic acid bacteria isolated from the intestine of Rohu (*Labeo rohita*) which is normal edible fish in the local market of Chandrapur district, Maharashtra state, of India.

MATERIALS AND METHODS

1) Collection of the fresh water fish and Isolation of Lactobacillus Species:

The Rohu fish (*Labeo rohita*) was collected from centrally located freshwater reservoir namely Ramala lake of Chandrapur city from where edible fresh water fishes are supplied to common fish market of Chandrapur district, Maharashtra state.

The collected fish was brought to the laboratory in transparent plastic jar containing lake water. The body surface of fish was cleaned thoroughly with distilled water, then disinfected with alcohol (70%); dissected under aseptic condition. Intestine taken out and washed three times with normal saline (NaCl 0.85%) and homogenized with a mechanical homogenizer (Rangpipat et al. 2008). The pieces of gastrointestinal tract were homogenized with sterile distilled water and centrifuged for 10 minutes at 13,000 rpm. One ml of supernatant was serially diluted with sterile distilled water in 6 test tubes. Among serially diluted samples 10⁻⁵ and 10⁻⁶ sample selected and 0.1 ml of diluted sample was inoculated on lactic acid bacteria selective agar (Himedia, M 1072). Then incubated at 37°C for 48 hours. The isolated colonies developed on lactic acid bacteria selective agar were picked up and cultivated on slants of selective agar to obtain pure culture (Balcazar J.L et. al. (2008).

2) Identification of Lactobacillus Species:

Selective colonies were characterized and identified following Bergey's Manual of systematic bacteriology (Whitman et al., 2009) for their morphology, gram staining, motility test, cultural and Biochemical tests (Ghosh et al., 2002). Two types of lactobacillus species were isolated from the gut of *Labeo rohita* fish collected from Ramala lake.

3) Study of probiotic characters of lactic acid bacteria isolated from intestine of *Labeo rohita* :

In vitro tests was carried out to screen out potential probiotic characters of isolated two lactobacillus species. These tests were based on the gut environment of fish which they mimic under in vitro conditions for screening potential probiotic strains.

1. Acid tolerance (Resistance to gastric acidity)
2. Bile salt resistance.

The acid tolerance test was carried out as per the method of Dhanasekaran et al., (2008, 2010), whereas the bile salt resistance was determined by a method given by Salminen et al., (2004)

RESULTS AND DISCUSSION

The isolated and homogenized gut sample from *Labeo rohita* fish was serially diluted with sterile distilled water in 6 test tubes up to 10⁻⁶ dilution and the diluted sample from 10⁻⁶ dilution was inoculated on the Lactic acid selective agar medium (Himedia-M1072). After incubation the colony characteristics developed were noted and prepared the slant culture.

The morphological identification of isolated Lactobacillus species was done by studying Gram staining, motility test, and the Biochemical identification by IMViC test and sugar fermentation. (Table-1). Two lactobacillus species were identified based on cultural, morphological and biochemical characterization.

Dhanasekaran et al., (2008, 2010) reported similar findings and noted, maximum population of lactobacillus species in the gut of fresh water fishes.

In the present study the identified, Lactobacillus species-1 was found to be Gram positive, having long rods, colony appearance yellow on selective media, ferment glucose, lactose and sucrose with acid and gas production, show Indole test positive, MR test positive, VP test positive and CU test positive whereas Lactobacillus species-2 was found to be Gram positive, having short rods, colony appearance white on selective media, ferment glucose, lactose and sucrose with acid and gas production, show Indole test positive, MR test positive, VP test positive and CU test positive.

Further probiotic characteristic of isolated lactobacillus species was done by acid tolerance test and bile salt tolerance test.

1. Acid tolerance test:

The isolated lactobacilli species gave promising results to in vitro selection probiotic criteria such as pH and bile salt tolerance tests.

The much more growth was observed at pH 3, pH 4 and highest at pH 5 and growth decreases after pH 6. (Fig.- 1). One of the most important criteria for the probiotics organism is the potential viability at low pH. In this study, survival and growth at low pH confirm that these lactobacilli species survive under harsh condition of stomach, hence serve to possess probiotics properties.

2. Bile salt tolerance:

Bile salt tolerance to various detrimental concentrations ranging from 0.0%, 0.15% and 0.30% at 2 hrs incubation period recorded for lactobacillus species 1 and 2. Result shows activity as well as growth in all three concentrations for 2 hrs incubation period (Fig.- 2).

Bile salt tolerance is required for probiotics bacteria to grow and survive in fish intestine (Salminen et al., 2004). The probiotics that have capacity to tolerate low pH and bile salt indicate that they are capable of inhabit and survive under stress conditions.

In the present study, both strain of Lactobacilli isolated show acid and bile salt tolerance capacity and appear to have high potential of probiotics properties.

Table 1. Identification and Characterization of Lactobacillus species isolated from the gut of *Labeo rohita*

Characteristics		
1) Colony appearance	Yellow	White
2) Grams staining	Gram positive long rods	Gram positive, short rods
3) Motility test	Non motile	Non motile
IMViC Test		
4) Indole test	+	+
5) Methyl red test	+	+
6) V P test	+	+
7) CU test	+	+
Sugar Fermentation		
8) Glucose	A +	G +
9) Fructose	A +	G +
10) Sucrose	A +	G +
Species Identified	Lactobacillus species-1	Lactobacillus species-2

[A-Acid production; G-Gas production]

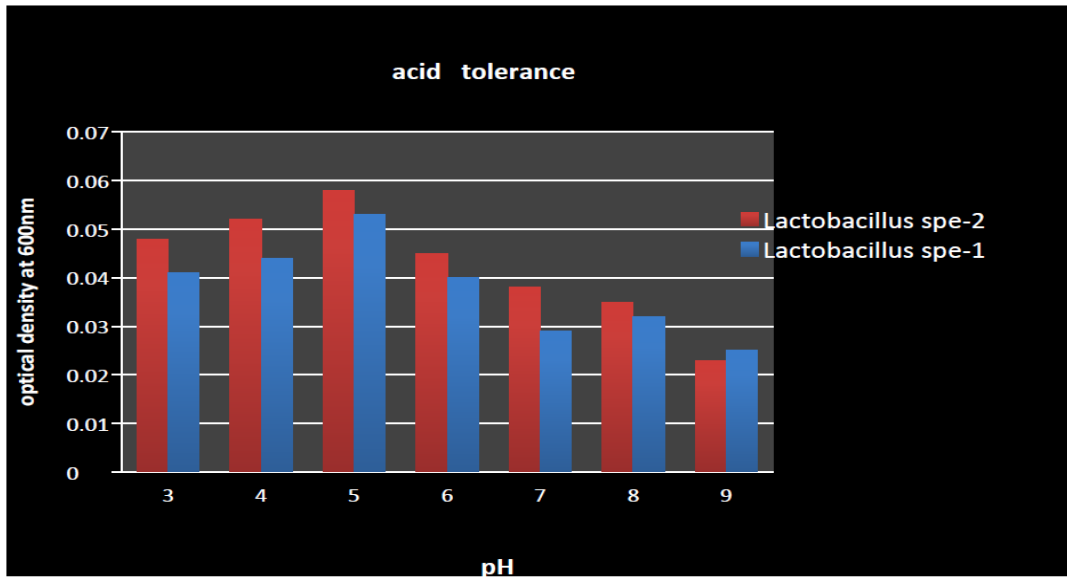


Fig.1 Acid tolerance of selected *Lactobacillus* species

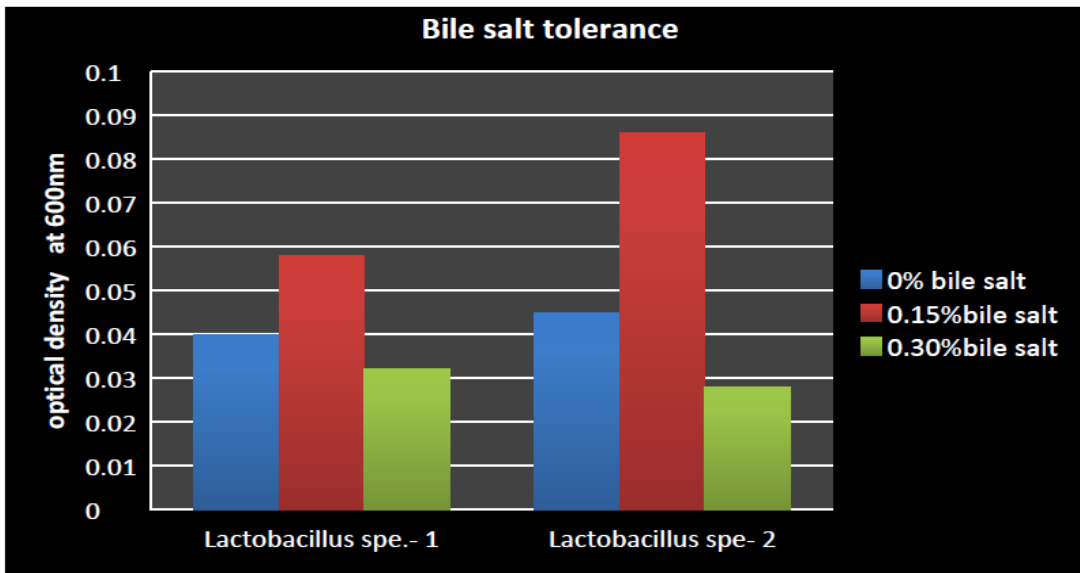


Fig.2 Bile salt tolerance of selected *Lactobacillus* species

CONCLUSION

In the present study, the *Lactobacillus* species-1 and isolated from the gut of *Labeo rohita* fish collected from Ramala lake of Chandrapur city show high acid and bile salt tolerance capacity and appears to have high potential of probiotics properties.

BIBLIOGRAPHY

- Balcazar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuola, I., Muzquiz, J. L., and Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal Microbiota of fish. *Aquaculture*, 278, 188-191.
- Dhanasekaran, D., Saha, S., Thajuddin, N., Panneerselvam, (2008). Probiotic effect of *Lactobacillus* isolates against bacterial pathogens in *Charis orientalis*, *Med. Biol.*, 15(3): 97-102.
- Dhanasekaran, D., Subhasish Saha, N. Thajuddin, M., Rajalakshmi, A(2010). Probiotic effect of *Lactobacillus* isolates against bacterial pathogens in fresh water fish. *J.Coast. Dev.*,13(2): 103-112.
- Erkkila, S.Petaja, E.(2000). Screening of commercial meat starter cultures at low PH in

the presence of bile salts for potential probiotic use. *Journal meat sci.*, 55:297-300.

5. Ghosh, K., Sen, S. K., Ray, A. K. (2002). Characterization of bacilli isolated from the gut of rohu, *Labeo rohita* fingerlings and its significance in digestion. *J. Appl. Aquacult.*, 12(3): 33-42.

6. Hyronimus, B., Le Marrec, C., Hadi Sassi, A., Deschamps, A. (2000). Acid and bile tolerance of spore-forming lactic acid bacteria. *Int. J. Food Microbiol.*, 61: 193-197.

7. Lara-Flores, M. (2011). The use of probiotic in aquaculture: an overview. *International Research Journal of Microbiology*, 2, 471-478.

8. P. Muthukumar and C. Kandeepan ; Isolation, Identification and characterization of probiotic organism from intestine of fresh water fishes (2015) *Int. J. Current. Microbiology App. Sci.* 4 (3): 607-616.

9. Rengpipat, S., Rueangruklikhit, T., and Piyatiratitivorakul, S. (2008). Evaluation of lactic acid Bacteria as probiotic for juvenile seabass (*Latescalcalifer*). *Aquaculture Research*, 39, 134-143.

10. Ringo, E. 2004. Lactic acid bacteria in fish and fish farming in: *Lactic acid bacteria microbiological and funtional aspects*, 3rd edn. . p. 581-610.

11. Salminen, S., Wright, A. V., Ouwehand, A. (2004). *Lactic acid bacteria* (Vol. 1). Marcel Dekker, Inc., New York.

12. Whitman, W. B., De Vos, P., Garrity, G. M., Jones, D., Noel, R., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K. H. (2009). *Bergey's manual of systematic bacteriology*. 2nd edn, Vol. 3