



CHARACTERIZATION OF INTESTINAL PROBIOTIC BACTERIA OF ROHU FOR AQUACULTURE PRACTICES

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ABSTRACT: In the present study, the probiotic microorganisms isolated from the intestine of Rohu (*Labeo rohita*) collected from centrally located Ramala lake.

The microorganisms are studied for its morphological, biochemical, cultural and probiotic characters i.e Acid tolerance and Bile salt tolerance.

The microbial strains which satisfy probiotic characteristics are further identified at molecular level by 16SrRNA Sequencing and found they are isolate 1 is *B. coagulans NTUIOB YUN 2* and isolate 2 is *B.coagulans SS1a*.

These two strains are studied for in vivo study to check probiotic efficiency by studying significant results to use in Aquaculture practices.

Key words: - Probiotics; Rohu; Bacillus coagulans; 16S rRNA Sequencing.

INTRODUCTION :

Modern days busy lifestyle and exposure to high doses of drugs and antibiotics also exposure of variety of microorganisms our digestive system disturbed and also distorts our normal inhabitant gut microflora. The urgent need of modern days is to reestablish this disturbed microflora by the use of ecofriendly, safe, cost effective probiotics.

Probiotics are helpful bacteria that are normal inhabitant of intestine and help to keep our gut healthy and fit. In recent study deals with the potential use of probiotic bacteria which are isolated from the gut of the fresh water fish, Rohu (*Labeo rohita*) which is normal inhabitant of centrally located Ramala Lake, a prime source of fish. The Indian major carp Rohu (*Labeo rohita*) is the most popular in the region and intensively cultured because of its high growth rate and consumer demand.

The study deals with the isolated microorganism whether satisfy the probiotics characteristics identified at molecular level can be used for Aquaculture practices.

METHOD:

1. Collection of fresh water fish *Labeo rohita* (Rohu) and its Maintenance: The fingerlings of *Labeo rohita* having size (10-12 cm (l) × 5-6 cm (w)), and weight, 100 ± 0.10 gm. were selected for the study. The fingerlings of *Labeo rohita* were maintained in the laboratory for further study in a transparent rectangular, (50 (l) × 30 (w) × 15 (d) cm.), glass container having 25 Litre water capacity.

2. Isolation of Intestinal Bacteria from the *Labeo rohita*: Preparation of Intestinal Extract or Inoculum (Ghosh K. et al., 2010).

3. Characterization of isolated bacterial species from the Intestinal Extract of *Labeo rohita* by Morphological, Biochemical and Cultural characteristics.

The isolated bacterial species obtained from the Intestinal Extract of *Labeo rohita* on agar plate were further characterized by studying their; Morphological, 3.2 Biochemical and 3.2 Cultural characteristics.

In this study the selective media for probiotic lactic acid bacteria (e.g. Lactobacillus Selective

agar medium, (Hm. - M1072) was used for the cultivation. The Isolates 1 to 2 were cultivated on the selective media and after incubation observed for the development of colonies. This media selectively grow the probiotic lactic acid bacteria and inhibit the growth of other bacteria.

Following three tests were carried out for Probiotic Characterization of Bacterial Isolates 1 to 2 from the intestine of

Labeo rohita.

A. Acid Tolerance test (Resistance to gastric acidity) Dhanasekaran et al. 2008, 2010)

B. Bile Salt Tolerance test (Salminen et al 2004) .

4. In Vivo study of probiotic efficiency of isolate 1 and 2 .

RESULTS:

The results show that the microbial strains isolated on selective media are further observed for their morphological, biochemical characteristic shows the isolate 1 and isolate 2 is Gram positive with large rods and endospore former also they are Indole test, Methyl red test, Vogues Proskaur test and Citrate utilization test are positive. Further their sugar utilization of Glucose, Lactose, and Mannitol shows both strains are acid and gas positive. Catalase test for these strains are positive for both.

They are further identified at molecular level by 16S rRNA Sequencing and found they are isolate 1 is *B. coagulans*

NTUIOB YUN2 and isolate 2 is *B.coagulans* SS1a.

The strains are studied for probiotic characteristics like acid tolerance and Bile salt tolerance and found that they are both acid and bile salt tolerant.

These probiotic strains for aquaculture practices further studied by In vivo study by comparison with control diet (CD) and Basal Diet (BD) with probiotic feed culture (PFC) and found significant increase in size and weight.

CONCLUSION:

Therefore the use of probiotic instead of antibiotic treatment is better initiative to control the diseases of aquatic fishes and to improve health and immunity.

The intestinal tract of a fresh water fishes such as, *Labeo rohita*, *Catla catla*, *Cirrhina mrigala* etc. could be the best source for the isolation of potential probiotic bacteria for sustainable use to control the infectious diseases in fishes in an aquaculture.

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Table3: Morphological, Biochemical Characterization of Isolated Bacterial Species (i.e. Isolate-1 to 2) from the intestine of *Labeo rohita*

Isolated Bacterial Species	Gram Staining	Shape	Motility test	Endospore staining	IMViC Test					Sugar Fermentation Test		
					I	MR	VP	C	Catalase	Glu	Lac	Man
IS-1	Positive	LargeRods	Active Motile	Endospore former	+	+	+	+	+	+	+	+
IS-2	Positive	LargeRods	Active Motile	Endospore former	+	+	+	+	+	+	+	+

Fig. Isolated Colonies of bacterial species from the intestine of *Labeo rohita* fish on agar plate. (C= Controlplate)

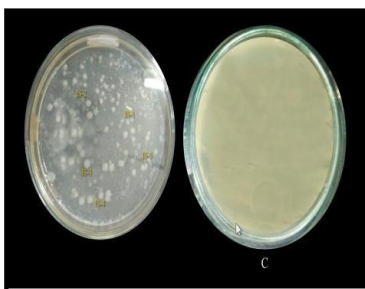


Fig. Nutrient agar slant cultures of isolated bacterial species (Isolates 1 to 2) from the intestine of *Labeo rohita*



Fig. Biochemical Characterization of Isolate 1 to 2 by IMViC test.

(I-Indole, MR-Methyl Red,VP- Vogues Proskaur-Citrate ; G = Glucose, L= Lactose, M = Mannitol)

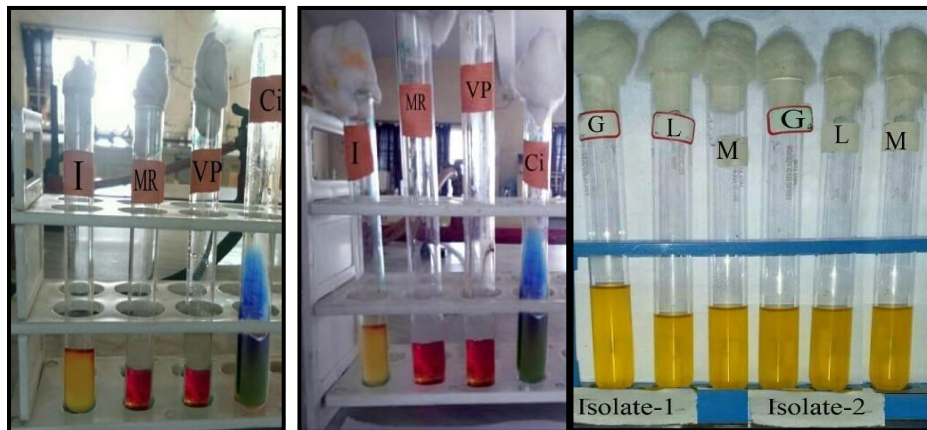


Fig. A) Aligned Sequence Data of Sample (1325bp): (Isolate-1)

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 CTGTAAGATCGGGATAACGCCGGGAAACCGGGGCTAATACCGGATAGTTTTTCTCCGCATGGAGGAAAAAGGAAAGACGGCT
 TTTGCTGTCACTTACAGATGGCCCGCGCGCATTAGCTAGTTGGTGGGTAACGGCTACCAAGGCAACGATGCGTAGCCGACC
 TGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCAACTCTACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGAC
 GAAAGTCTGACGGAGCAACGCCGCGTGAGTGAAGAAGGCCTTCGGGTCTGTAATACTGTTGCCGGGAAGAACAAGTGCCGTT
 CGAACAGGGCGCGCCTTGACGGTACCCGGCCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCA
 AGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCAGGCGGCTTCTTAAGTCTGATGTGAAATCTTGCGGCTCAACCGCAAGCG
 GTCATTGGAAACTGGGAGGCTGAGTGCAGAAGAGGAGAGTGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA
 ACACCAGTGGCGAAGGCGGCTCTCTGCTGTAAGTACGCTGAGGCGGAAAGCGTGGGGAGCAACAGGATTAGATACCTGT
 GTAGTCCACGCCGTAACAGTATGAGTCTAAGTGTAGAGGTTTCCGCCCTTAGTGTGCTGACGCTAACGCATTAAGCACTCCGCT
 GGGGAGTACGGCCGCAAGGCTGAACTCAAAGGAATTGACGGGGGCCGCACAAGCGTGGAGCATGTGGTTAATTGAAGCA
 ACGCGAAGAACCTTACCAGGCTTGTACATCCTCTGACCTCCCTGGAGACAGGGCCTTCCCTTCGGGGGACAGAGTGACAGGTGG
 TGATGGTTGTCTGACGCTGCTGCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGACCTTAGTGCCAGCATTAG
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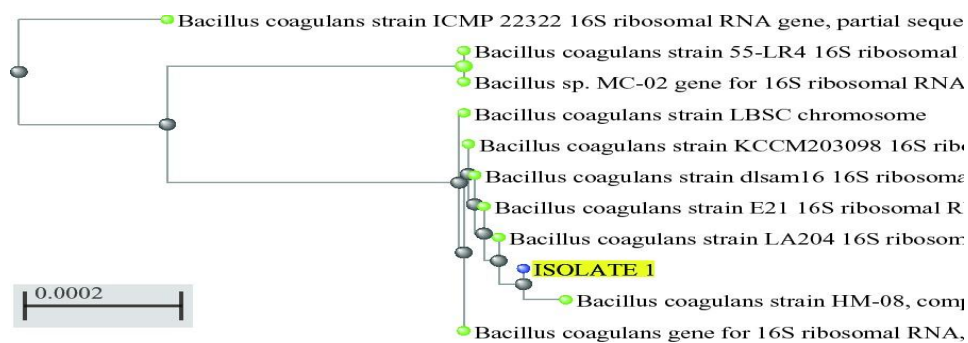


Fig.A) Aligned Sequence Data of Sample (~1321bp) of Isolate-2

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CGCGCGGGGCACCCCTGCGACATCCGCATCACGCCCTACTCCGGGGCTAATACCGGATAGTTTTTCTCCGCATGGAGGA
AAAAGGAAAGACGGCTTTTGTCTGACTTACAGATGGGCCCGCGGCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCAA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCAAACTCTACGGGAGGCAGCAGTAGGGA
ATCTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCTGAGTGAAGAAGGCCTTCGGGTCGTAAAACTGTGTCGGGGGA
AGAACAAGTGCCGTTTGAACAGGGCGGCGCTTACGGTACCCGGCCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTGCCGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTTCTTAAGTCTGATGTGAAATCTTGC
GCTCAACCGCAAGCGGTATTGAAACTGGGAGGCTTGAAGTGCAGAAGAGGAGAGTGAATTCCACGTGTAGCGGTGAAATGCG
TAGAGATGTGGAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAAGTACGCTGAGGCGGAAAGCGTGGGGAGCAAACA
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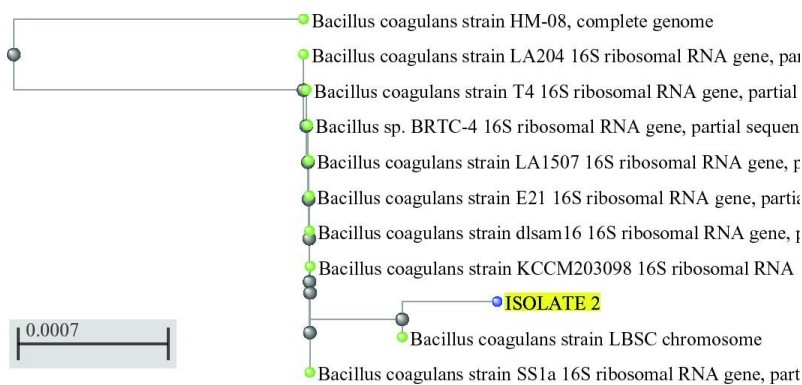
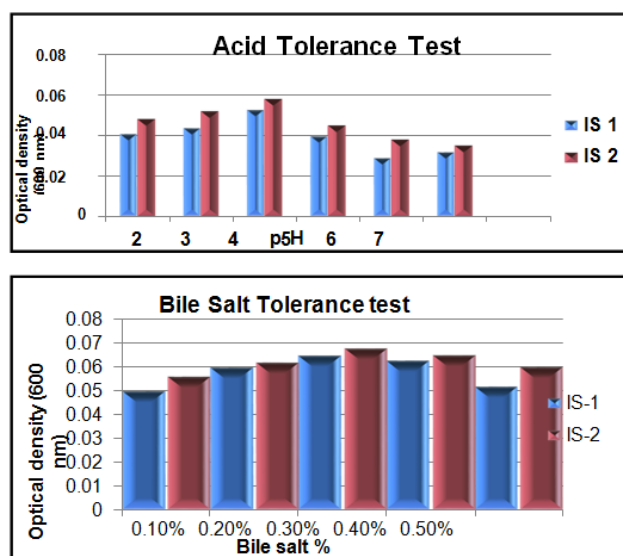


Fig. Probiotic Characterization of Bacterial Isolates 1 to 2 from the Intestine of *Labeo rohita*



These probiotic strains for aquaculture practices further studied by In vivo study by comparison with control diet (CD) and Basal Diet (BD) with probiotic feed culture (PFC) and found significant increase in size and weight.

Weight and Size measured every week up to 60 days	Control Diet (CD)		Basal diet (BD) + Probiotic Feed Culture (PFC)							
	T1 (Basal Diet (BD))*		T2 BD + 1.0 ml. PFC		T3 BD + 2.0 ml. PFC		T4 BD + 3ml. PFC		T5 BD + 4 ml. PFC	
	Wt (gm.)	Size (mm.)	Wt (gm.)	Size (mm.)	Wt (gm.)	Size (mm.)	Wt (gm.)	Size (mm.)	Wt (gm.)	Size (mm.)
1st week	12.0	72	12.3	70	14.7	76	15.33	79	17.55	85
2nd week	15.5	76	17.5	79	19.6	85	23.5	105	25.8	115
3rd week	15.6	76	17.9	79	20.0	86	23.7	105	26.2	116
4th week	15.9	77	18.2	79	20.3	86	23.9	106	26.6	117
5th week	16.0	77	18.7	80	20.7	87	24.6	107	27.2	118
6th week	16.1	78	19.2	80	21.3	87	25.3	108	27.9	119
7th week	16.3	78	19.7	81	21.6	87	26.0	109	28.3	119
8th week	16.5	79	20.3	81	22.3	88	26.5	110	28.9	120
9th week	17.2	79	20.9	82	23.0	89	27.0	111	29.2	120

Weight and Size measured every week up to 60 days	Control Diet (CD)		Basal diet (BD) + Probiotic Feed Culture (PFC)							
	T1 (Basal Diet (BD))*		T2 BD + 1 ml. PFC		T3 BD + 2 ml PFC		T4 BD + 3 ml. PFC		T5 BD + 4 ml. PFC	
	Wt. (gm.)	Size (mm.)	Wt. (gm.)	Size (mm.)	Wt. (gm.)	Size (mm.)	Wt. (gm.)	Size (mm.)	Wt. (gm.)	Size (mm.)
1st week	15.0	75	13.5	82	15.8	72.2	17.5	87	16.6	79
2nd week	16.5	77	14.6	89	16.6	73.9	19.0	99	17.5	86
3rd week	16.9	78	16.2	93	17.6	82.3	23..5	110	18.3	92
4th week	17.2	82	17.7	96	19.3	86.8	25.2	121	20.2	105
5th week	17.6	84	19.3	98	21.0	87.5	27.6	135	23.2	117
6th week	18.1	86	21.9	101	21.8	88.6	29.8	142	24.6	121
7th week	18.3	89	22.2	107	23.6	91.2	31.9	145	25.3	123
8th week	18.5	91	22.9	110	25.0	93.0	32.0	150	26.9	127
9th week	19.0	94	23.2	115	26.7	96.0	35.0	153	27.1	129

Fig.In- Vivo Study of Probiotic Efficiency of Identified *B.coagulans* strainSS1a (Isolate 1,2) (Feeding Pattern)



Fig. Experimental Setup for In- Vivo study of Probiotic Efficiency of Identified *B.coagulans* strainSS1a(Isolate 1-2) (Feeding Pattern)



Fig. Evaluation of Results of *B.coagulans* strain NTUIOB YUN2 and SS1a (Isolate -1,2)