Annexure-IX

PROJECT COMPLETION REPORT

1. Title of the project: 'Establishment and evaluation of probiotic potentiality of isolated lactobacilli strains and comparative analysis of their gene expression profile during transit in a stimulated gastrointestinal tract conditions'

2. Principal Investigator(s) and Co-Investigator(s): Dr Raj Kumar Duary

3. Implementing Institution(s) and other collaborating Institution(s): Tezpur University

4. Date of commencement: 10/03/2016

5. Planned date of completion: 31/03/2018

6. Actual date of completion: 31/03/2018

7. Objectives as stated in the project proposal:

- Isolation of lactobacilli from fermented food products (rice beer, misti dahi, milk samples, etc), plant source, feacal sources of human origin (healthy infant and adult) from north-east region of India and identification by both biochemical and molecular techniques.
- Screening of lactobacilli for probiotic attributes as per FAO/WHO (2002) and DBT/ICMR guidelines (Ganguly et al., 2011).
- Identify genes regulation pattern during survival in simulated gastric fluid, intestinal fluid and prebiotic added medium involved in acid, bile, stress and carbohydrate (oligosaccharides) utilization by isolated lactobacilli strains.
- In vitro evaluation of functional properties like, hypocholesterolemic and antioxidative effects of potential putative probiotic lactobacillus strains.

8. Deviation made from original objectives if any, while implementing the project and reasons thereof:

NIL

9. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

- Collection and isolation of samples

Various fermented as well as unfermented samples viz. Curd, Paneer, raw milk, fermented rice beer, fermented soybean, fermented bamboo shoots and pickles etc. were collected from different regions of North - East from in a sterile sample container and were processed further for isolation in the laboratory. Different samples (10 g) were homogenized in 100 mL physiological saline (0.85 % NaCl, w/v), and individual colonies were picked up by streaking the samples in MRS (de Man Rogosa and Sharpe) agar medium and incubating anaerobically at 37 oC. An amount of 0.002 % (w/v) of the phenol red dye (HiMedia, India) was used to select distinct colonies. LAB

isolates were maintained regularly in MRS broth medium at temporary basis and were preserved in 50 % (v/v) glycerol at -80 oC.

- Phenotypic Characterization

The pH of collected samples was immediately noted using pH. Cell motility and morphology were observed in light microscope (Labomed, India). The isolates were Gram stained and catalase tested. Gram positive and catalase negative cultures were further processed. The isolates were also stained via negative staining using Nigrosin dye.

- Acid Tolerance Activity

Acid tolerance activity was determined as demonstrated by Ehrmann et. al. (2002). Fresh (24 hrs) cultures were centrifuged (6000 rpm, 10 min, Eppendorf) and pellets were washed with sterile PBS (pH 6.5). The PBS dissolved pellets were inoculated to different pH in MRS broth tubes and incubated for 0, 30, 60 and 90 minutes. Incubated cultures were serially diluted and their counts (cfu/mL) were taken by using spread plating and anaerobically incubating the cultures in MRS media at 37 oC for 24 hrs.

- Bile Tolerance Activity

MRS broth tubes were prepared separately by adding Ox - bile in different concentrations (0.3, 0.5, 1.0 and 1.5 %) and adjusting to a final pH of 6.5 with 1N HCL or 1N NaOH. Active cultures were centrifuged (6000 rpm, 10 min, Eppendorf) and the pellets were washed three times with sterile PBS (pH 6.5). Serially diluted PBS dissolved pellets were inoculated in different concentrations of bile and incubated for 0, 1, 2, 3, 6, 12, 16 and 24 hrs. The ability of LAB cultures to grow in presence of different concentration of bile was observed by simultaneous plating (24 hrs incubation) after required incubation time. Visible colonies (cfu/mL) were observed in MRS plates by pour plating method within an incubation period of 0, 1, 2, 3, 6, 12, 16 and 24 hrs. A control condition without bile addition was kept accordingly.

- Antimicrobial Activity

The LAB isolates were screened for antimicrobial activity (via well diffusion method) against different pathogen microorganisms as well as some other LAB strains viz. E. coli ATCC 8739, Bacillus cereus ATCC 10876, Staphylococcus aureus ATCC-BAA-976 and Listeria monocytogenes ATCC 13932. The co-culture activities against other LAB cultures like Lactobacillus acidophilus ATCC 4356, L. plantarum ATCC 8014, L. rhamnosus ATCC 7469 and L. gasseri ATCC 19992 were also been checked. An 18-24 hrs grown test culture was plated in respective agar media and wells of approx 8 mm were bored. The cell free supernatant (100-150 µL) of the isolates was added in the wells followed by an incubation period of 12-16 hrs at 37 oC. The diameter of inhibition zone (mm) was measured to confirm resistance potential against the respective test culture.

- Antibiotic Resistance

The freshly grown isolates (18-24 hrs) were plated in MRS agar media and various antibiotic hexadiscs H+14 and H+15 (Ampicillin, Gentamycin, Ciprofloxacin, Penicillin, Streptomycin, Linezolid, Vancomycin etc.) from Himedia, India were placed on smeared plates followed by incubating at 37 oC for 24 hrs. The inhibition zone diameter (mm) was measured after incubation.

- Antioxidative Potential

The antioxidative potential of lysates of homogenized as well as direct culture supernatant was determined by DPPH (2,2- diphenyl-1-(2,4,6-trinitrophenyl) radical scavenging assay as demonstrated by Duan et.al. (2006). Briefly, 2 mL of 0.16 mM DPPH (in methanol) was added to 300 µL of the cell lysate which was made up to 2 mL with distilled water. The lysates were thoroughly mixed and incubated for 30 mins in dark. Sample blank was prepared using methanol in place of DPPH. A control of methanol combined with DPPH was prepared. Absorbance of lysates was determined spectroscopically at 517nm. The scavenging activity (%) of the isolates was determined by the formula;

Scavenging activity (%) = [1-{(A sample-A sample blank)/A control}] x100

- API Sugar Test

The analytical Profile Index (API) of the isolates was observed using rapid API kit (KB009 HiCarbohydrate, HiMedia, India). Active cultures were centrifuged and washed with sterile PBS (pH 6.5). Density and turbidity of the pellet (PBS dissolved) was compared with McFerland solution. 50 µL of the inoculums was added to the respective sugar well in the kit and incubated for 24 -48 hrs. The change in colour was noted down as positive result.

- Bile Salt Hydrolase Activity

The LAB isolates were streaked in MRS agar plates containing 0.5% sodium taurodeoxycholate (TDC) and 0.37% calcium chloride (w/v). Plates were incubated anaerobically for 72 hrs and a positive result was estimated by the presence of precipitated bile acid around the colonies or formation of opaque granular white colonies with silvery shine.

10. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

- Isolation and phenotypic Characterization

A sum total of 102 Lactic acid bacteria (LAB) were isolated from different fermented and unfermented food samples. All the Gram positive, catalase negative bacilli were further

characterized. The gram's staining of the cultures showed small rod shaped gram +ve bacteria (Fig.1)

The short rods were reconfirmed by negative staining.

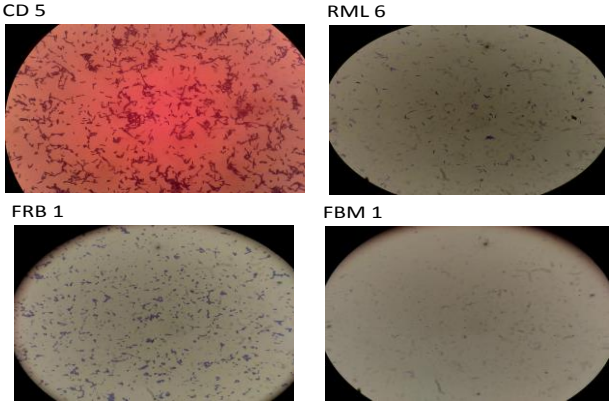


Fig 1: Microscopic analysis of selected isolates

- Acid Tolerance Activity

The potential ability of LAB isolates to survive against different pH ranges were recorded. LAB from fermented milk, bamboo shoot and rice beer were seen to tolerate different pH levels. The growth rate (log cfu/mL) within 24 hrs of incubation has been shown in Table 1. The graph showing acid tolerance level has been shown in Fig. 2.

Table 1: Comparative acid tolerance (Log cfu/mL

pH	CD5				RML6				FRB1				FBM1			
	0 min	30 min	60 min	90 min	0 min	30 min	60 min	90 min	0 min	30 min	60 min	90 min	0 min	30 min	60 min	90 min
2.0	8.75 ± 0.01	8.79 ± 0.1	8.76 ± 0.01	8.78 ± 0.02	6.06 ± 0.02	6.08 ± 0.1	6.24 ± 0.01	6.25 ± 0.02	8.54 ± 0.01	8.67 ± 0.1	8.68 ± 0.01	8.75 ± 0.01	6.06 ± 0.1	6.07 ± 0.01	6.07 ± 0.01	6.16 ± 0.03
2.5	8.93 ± 0.02	9.06 ± 0.01	9.07 ± 0	9.08 ± 0	6.08 ± 0.01	6.08 ± 0.02	6.24 ± 0.02	6.12 ± 0.01	8.87 ± 0.01	8.88 ± 0.02	8.82 ± 0.01	8.93 ± 0.02	6.53 ± 0.01	6.52 ± 0.01	6.54 ± 0.01	6.57 ± 0.01
3.0	8.85 ± 0.01	8.87 ± 0.01	9.04 ± 0.1	9.05 ± 0.1	6.07 ± 0.07	6.08 ± 0.01	6.17 ± 0.08	6.13 ± 0.04	8.94 ± 0.04	8.92 ± 0.06	9.30 ± 0.05	8.85 ± 0.01	7.05 ± 0.03	7.11 ± 0.07	7.10 ± 0.05	7.11 ± 0.0
3.5	9.05 ± 0.02	9.04 ± 0.01	9.02 ± 0.02	9.04 ± 0.02	6.13 ± 0.01	6.16 ± 0.03	6.12 ± 0.02	6.14 ± 0.01	9.04 ± 0.02	9.04 ± 0.02	9.36 ± 0.01	9.05 ± 0.02	8.15 ± 0.01	8.14 ± 0.03	8.33 ± 0.03	8.44 ± 0.01
6.5	9.84 ±	9.87 ±	9.85 ±	9.91 ±	6.84 ±	6.88 ±	6.85 ±	6.87 ±	9.18 ±	9.19 ±	9.44 ±	9.84 ±	8.48 ±	8.45 ±	8.49 ±	8.66 ±

	0.04	0.02	0.01	0.01	0.05	0.02	0.04	0.01	0.02	0.02	0.02	0.04	0.03	0.02	0.01	0.01

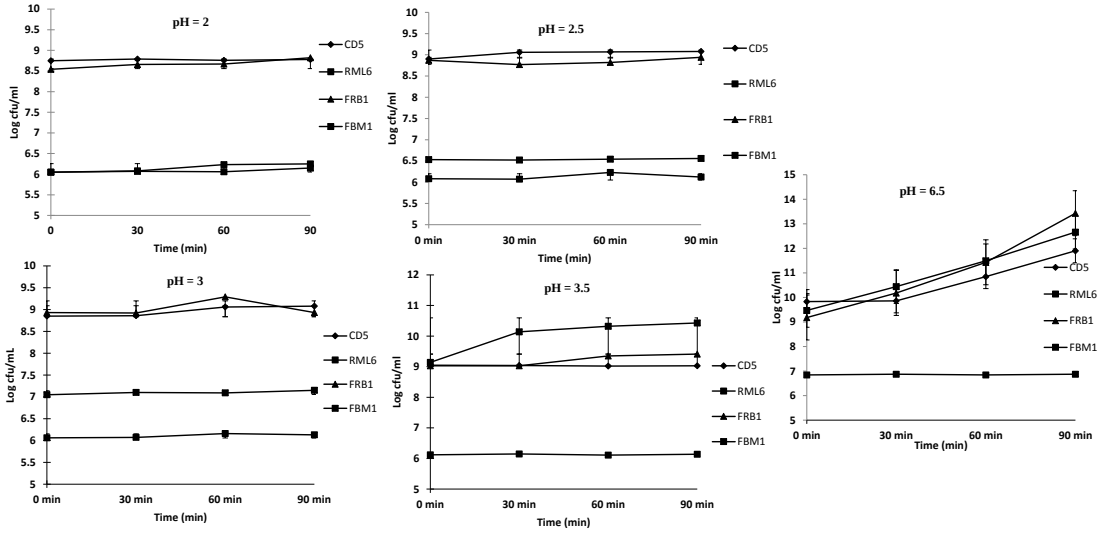


Fig. 2: The survival curve of the isolates at different pH conditions.

All the four isolates (CD5, RML6, FRB1 and FBM1) showed steady tolerance level at different pH conditions.

- Bile Tolerance Activity

A significant survival and growth rate were been observed for the tested isolates in different bile concentrations (Fig 3 and Table 2).

Table 2: Comparative bile tolerance of (Log cfu/mL)

Hr	CD 5				RML 6				FRB 1				FBM 1			
	0.3 %	0.5 %	1 %	1.5 %	0.3 %	0.5 %	1 %	1.5 %	0.3 %	0.5 %	1 %	1.5 %	0.3 %	0.5 %	1%	1.5 %
0	9.05 ± 0.05	9.13 ± 0.03	9.13 ± 0.03	9.09 ± 0.08	9.03 ± 0.13	9.04 ± 0.1	9.05 ± 0.05	9.17 ± 0.04	9.06 ± 0.09	9.14 ± 0.02	9.13 ± 0.03	9.15 ± 0.02	9.14 ± 0.04	9.17 ± 0.12	9. 17 ± 0. 00	9.17 ± 0.01
1	9.15 ± 0.04	9.25 ± 0.04	9.36 ± 0.05	9.28 ± 0.03	9.25 ± 0.01	9.22 ± 0.02	9.26 ± 0.05	9.31 ± 0.02	9.25 ± 0.11	9.44 ± 0.04	9.42 ± 0.04	9.52 ± 0.02	9.21 ± 0.04	9.13 ± 0.02	9.45 ± 0.01	9.46 ± 0.04
2	9.33 ± 0.03	9.43 ± 0.02	9.56 ± 0.03	9.45 ± 0.04	9.47 ± 0.01	9.44 ± 0.02	9.35 ± 0.03	9.59 ± 0.02	9.55 ± 0.03	9.53 ± 0.01	9.68 ± 0.03	9.71 ± 0.01	9.45 ± 0.03	9.27 ± 0.07	9.57 ± 0.01	9.52 ± 0.00

3	9.52 ± 0.02	9.54 ± 0.01	9.63 ± 0.02	9.56 ± 0.01	9.56 ± 0.01	9.56 ± 0.03	9.49 ± 0.01	9.64 ± 0.02	9.63 ± 0.03	9.72 ± 0.02	9.75 ± 0.01	9.82 ± 0.01	9.53 ± 0.04	9.42 ± 0.01	9.66 ± 0.03	9.74 ± 0.02
6	9.55 ± 0.03	9.62 ± 0.01	9.73 ± 0.01	9.63 ± 0.01	9.65 ± 0.01	9.61 ± 0.02	9.65 ± 0.03	9.83 ± 0.02	9.66 ± 0.12	9.83 ± 0.03	9.87 ± 0.02	9.86 ± 0.02	9.6 ± 0.08	9.68 ± 0.17	9.76 ± 0.00	9.86 ± 0.02
16	9.61 ± 0.03	9.67 ± 0.02	9.82 ± 0.01	9.73 ± 0.01	9.71 ± 0.03	9.86 ± 0.03	9.73 ± 0.03	9.81 ± 0.08	9.81 ± 0.03	9.88 ± 0.02	9.33 ± 0.03	9.92 ± 0.02	9.75 ± 0.04	9.74 ± 0.04	9.86 ± 0.04	9.91 ± 0.01
24	9.45 ± 0.05	9.73 ± 0.02	9.85 ± 0.03	9.83 ± 0.02	9.83 ± 0.07	9.96 ± 0.01	9.81 ± 0.01	9.97 ± 0.03	9.92 ± 0.02	9.96 ± 0.04	9.96 ± 0.04	9.63 ± 0.4	9.85 ± 0.02	9.79 ± 0.01	9.95 ± 0.01	9.83 ± 0.21

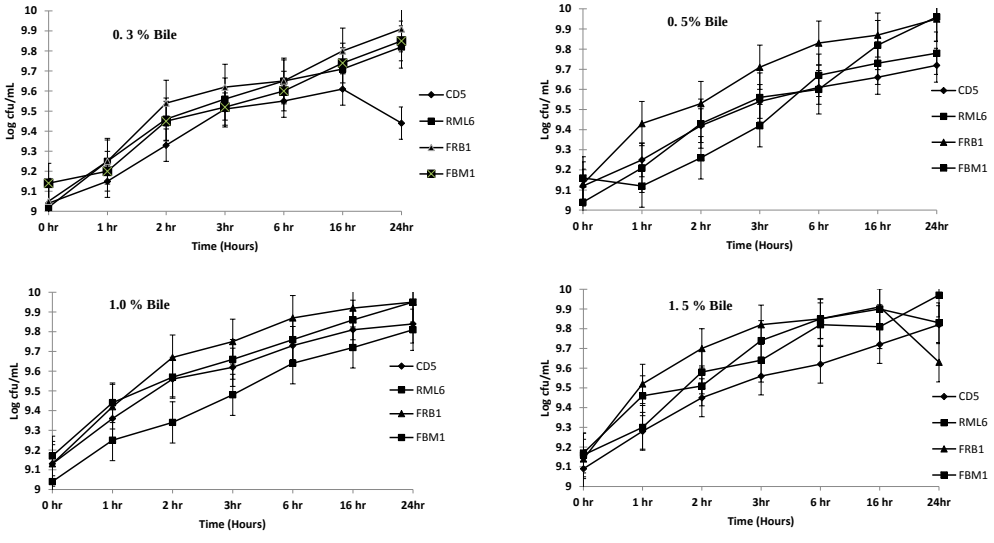


Fig. 3: Survival curve of different isolated against different bile salt concentration in growing media.

All the tested cultures showed surviving and growing in presence of different bile salts concentrations indicating overcoming abilities against toxic effect of bile salts.

- Antimicrobial Activity

The LAB strains showed a significant inhibition against several pathogens (Fig. 4). However, there was no inhibition against other strains of LAB (*Lactobacillus acidophilus* ATCC 4356, *L. plantarum* ATCC 8014, *L. rhamnosus* ATCC 7469) suggesting compatibilities with these. The zone of inhibition within 12-16 hrs against the tested pathogens were shown in Table 3.

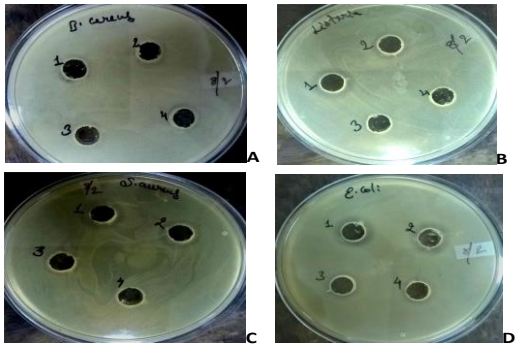


Fig 4 : Agar well assay to confirm antimicrobial potentialities of selected isolates (1 – CD5, 2 – RML6, 3-FRB1, 4-FbM1)

Table 3 : Antimicrobial activity of selected isolates (zone diameter, mm)

Pathogen Microbe	CD 5	RML 6	FRB 1	FBM 1
Staphylococcus aureus ATCC-BAA-976	14±1.0	13±0.5	12±0.5	11±0.5
E. coli ATCC 8739	13±0.5	13±0.2	11±0.3	10±0.1
Listeria monocytogenes ATCC 13932	13±0.3	11±0.1	10±0.1	10±0.05
Bacillus cereus ATCC 10876	12±0.5	11±0.05	10±0.3	10±0.2

- Antibiotic Resistance

The selected isolates were checked for sensitive against different antibiotics (Fig 5). All the isolates were sensitive against the tested antibiotics. The isolates namely CD 5 and FRB1 were found to be most sensitive against Ampicillin and Penicillin (10 mg concentration of both). However, RML6 and FBM1 were most sensitive against Linezolid (30 mg), Ampicillin (10 mg) and Penicillin (10 mg) respectively. The inhibition zone (mm) has been shown in Table 4.

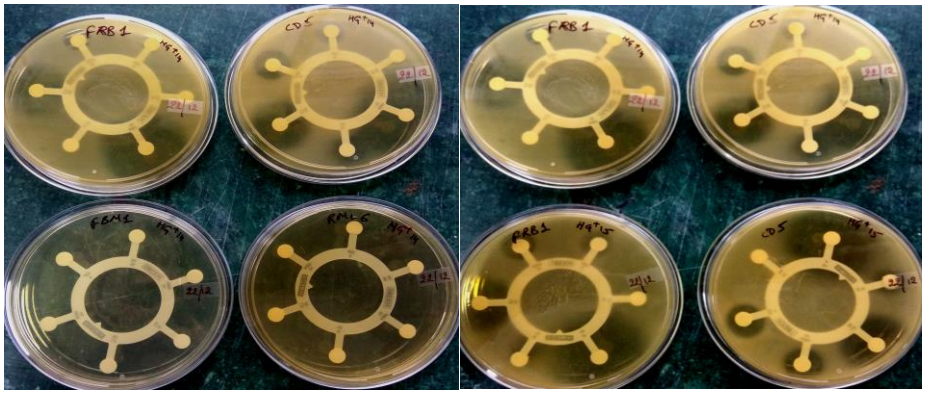


Fig. 5: Antibiotic sensitivity assay for the tested isolates.

Table 4 : Antibiotic activity of selected isolates (zone diameter, mm)

	Hexadisc H^{+14}						Hexadisc H^{+15}					
	S (10)*	LZ (30)	Gen (10)	Cip (5)	VA (30)	Amp (10)	Cip (5)	Gen (10)	LZ (30)	S (10)	P (10)	VA (30)
CD5	10	22	18	12	10	35	15	19	23	10	40	10
RML6	10	19	10	12	11	30	10	11	18	10	19	10
FBM1	10	20	18	10	10	24	10	10	26	11	28	10
FRB1	11	23	17	11	10	32	11	13	21	12	40	10

*Concentration of antibiotic (mg) (S – Streptomycin, LZ – Linezolid, Gen – Gentamycin, VA – Vancomycin, Amp – Ampicillin, Cip – Ciprofloxacin, P- Penicillin)

- Antioxidative Potential

The cell free supernatants were tested for DPPH radical scavenging activity. Radical scavenging activities were found highest in CD5 (85.96±2.0 %) and FRB1 (85.14±1.2 %) isolates of the screened one. However, when cells were homogenized and their supernatant were tested, a significant reduction in the scavenging activity were noted. The comparative DPPH radical scavenging activities are as shown in Table 5.

Table 5: Antioxidative potential of selected isolates

	Cell free supernatant (%)	Homogenized cell free supernatant (%)
CD5	85.96 ± 2.0	11.43 ± .05
RML6	54.82 ± 3.0	10.3 ± 0.1
FBM1	73.2 ± 0.1	9.59 ± 0.01
FRB1	85.14 ± 1.2	10.55 ± 0.1

- API Sugar Test

The sugar fermentation profile of the test isolates were checked using API kit shown in Fig 6

Fig. 6: API kit based fermentation profile of the isolates.

The comparative sugar fermentation profile of the test performance by the isolates is as shown in Table 6.

Table 6: The comparative sugar fermentation profile of the isolates.

Sugar	CD 5	RML6	FRB1	FBM1
Lactose	-	-	-	-
Xylose	+	-	-	-
Maltose	-	-	-	-
Fructose	+	+	+	-
Dextrose	+	+	+	-
Galactose	+	-	-	-
Raffinose	-	-	+	-
Trehalose	+	+	+	-
Melibiose	+	-	-	-
Sucrose	+	+	+	-
L-Arabinose	+	+	+	-
Mannose	+	+	+	+
Inulin	+	+	+	-
Sodium gluconate	-	-	-	-
Glycerol	+	-	-	-
Salicin	+	+	+	+

Dulcitol	-	-	-	-
Inocitol	-	-	-	-
Sorbitol	+	-	-	-
Mannitol	+	+	+	-
Adonitol	-	-	-	-
Erythritol	-	-	-	-
α-Methyl-D- Glucoside	-	-	-	-
Rhamnnose	-	-	-	-
Cellobiose	+	+	+	-
Melezitose	-	-	-	-
α-Methyl-D- mannoside	-	-	-	-
Xylitol	-	-	-	+
ONGP	-	-	-	+
Esculin hydrolysis	+	+	+	-
D- arabinose	+	+	-	-
Citrate utilization	+	-	-	-
malonate utilization	+	-	-	-
sorbose	-	-	-	-

- Bile Salt Hydrolase Activity

Bile salt precipitated, silvery white and colonies were observed as shown in Fig 7.

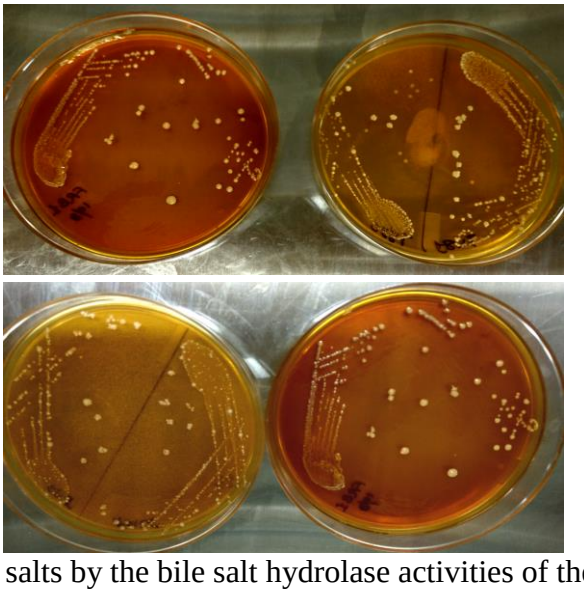


Fig. 7: Precipitation of bile salts by the bile salt hydrolase activities of the isolates.

Among the isolates CD5 and FBM1 showed strong bile salt hydrolase activities as indicated by bile precipitation around the colonies formed.

Table 7 : Bile salt hydrolase activity by selected isolates (+++ : very good precipitation, ++ : good precipitation)

Isolate	MRS (with bile salt)	Control (MRS without bile)
CD5	+++	-
RML6	++	-
FRB1	++	-
FBM1	+++	-

11. Conclusions summarizing the achievements and indication of scope for future work:

The isolation and characterization of LAB from various fermented as well as unfermented food samples was done. A few isolates were purified and were taken further for other biochemical characterization. The above results viz. high DPPH radical scavenging activity, ability to metabolize various sugars, capability to survive at different bile and pH conditions, antibiotic susceptibility and action against pathogens show that north-east fermented food sources is a hub of potential LAB with probiotic properties, which opens a new prospectus to study their action mechanism and survival strategy in various regions of the gastrointestinal tract (GIT).

12. S&T benefits accrued:

Isolates having good survival capacities in high acidic and bile conditions has been identified. They showed good bile salt hydrolase and antioxidant potentialities. The further probiotic potentially establishment of the isolated strains can lead to their application in pharmaceutical supplement formulations.

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year
-	-	-	-	-	-	-

ii. **Manpower trained on the project**

a) Research Scientists or Research Associates: Nil

b) No. of Ph.D. produced: Nil

c) Other Technical Personnel trained: 02

iii. Patents taken, if any: NA

13. Financial Position:

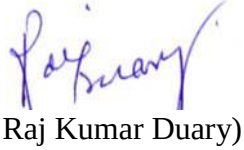
No	Financial Position/ Budget Head	Funds Sanctioned	Expenditure	% of Total cost
I	Salaries/ Manpower costs	2,22,533.00	2,22,000.00	99.8 %
II	Equipment	0.00	0.00	-
III	Supplies & Materials	7,64,967.00	7,64,967.00	100 %
IV	Contingencies	0.00	0.00	-
V	Travel	0.00	0.00	-
VI	Overhead Expenses	62,500.00	62,500.00	100 %
VII	Others, if any	-	-	-
Total				100%

14. Procurement/ Usage of Equipment
a)

No	Name of Equipment	Make/Model	Cost (FE/ Rs)	Date of Installation	Utilization Rate (%)	Remarks regarding maintenance/
NA	NA	NA	NA	NA	NA	NA

b) Plans for utilizing the equipment facilities in future: NA

Name and Signature with Date:



(Dr Raj Kumar Duary)
05/05/2018
(Principal Investigator)

UTILIZATION CERTIFICATE
(Consolidated)

[FOR THE FINANCIAL YEAR 2016-2018 (01.04.2016 to 31.03.2018)]

1. Title of the Project/Scheme: **Establishment and evaluation of probiotics potentiality of isolated lactobacilli strains and comparative analysis of their gene expression profile during transit in a simulated gastrointestinal tract conditions.** (Under DST-SERB: "Empowerment and Equity Opportunities for Excellence in Science" program)
2. Name of the Institution: **Tezpur University**
3. Name of the Principal Investigator: **Dr. Raj Kumar Duary**
4. Science and Engineering Research Board (SERB)
   Sanction order No & date sanctioning the project:
   (First financial sanction order) **SB/EMEQ-361/2014 dated 10 March, 2016**
5. Head of account as given in the original sanction order:

Head	**Amount (in ₹)**
General-A: Manpower, Consumables, National Travel, Contingencies, and Minor Equipments.	₹ 10,00,000 /-
General-B: Overhead Charges	₹ 1,00,000 /-
Total (General A + General B)	₹ 11,00,000/-

6. Amount brought forward from the previous Financial year quoting SERB letter no and date in which the authority to carry forward the said amount was given
   i. **Amount: NIL**
   ii. **Not Applicable**
   iii. **Date: Not Applicable**

7. Amount received during the total financial year (SERB Sanction order no and date)
   i. Amount: ₹ **10,50,000** /-
   ii. Order No: **SB/EMEQ-361/2014**
   iii. Date: **10.03.2016**

8. Total amount that was available for expenditure (excluding commitments) during the financial year (Sr. No. 6 + 7) **₹ 10,50,000 /-**

9. Actual Expenditure (excluding commitment): **₹ 10,49,467 /-**

10. Balance amount available at the end of the financial year: ₹ **533 /-**

11. Unspent balance refunded, if any (details of cheque no. etc): **NIL**

12. Amount to be carried forward to the next financial year: **NIL**

UTILIZATION CERTIFICATE

Certified that out of **₹ 10,50,000.00** of grants-in-aid sanctioned during the year **2016-2018** in favour of **Tezpur University** vide SERB order No. **SB/EMEQ-361/2014** dated **10/03/2016** and **₹ 0.00** on account of unspent balance of the previous year, a sum of **₹ 10,49,467.00** has been utilised for the purpose of **project entitled "Establishment and evaluation of probiotics potentiality of isolated lactobacilli strains and comparative analysis of their gene expression profile during transit in a simulated gastrointestinal tract conditions."** for which it was sanctioned and that the balance of **Rs. 533.00** remaining unutilized at the end of the year will be adjusted towards the grants-in-aid payable during the next year *i.e.* **NA**.

Signature of PI: Date: 24/11/2020	Signature of the Finance Officer: Date:	Signature of the Registrar: Date:

Assistant Professor
Department of Food Engg. & Technology
Tezpur University
Napaam, Tezpur- 784028, Assam

Finance Officer
Tezpur University

Registrar
Tezpur University

Annexure-II

REQUEST FOR CONSOLIDATE INSTALMENT WITH UP-TO-DATE STATEMENT OF EXPENDITURE

1. SERB Sanction Order No and date : **SB/EMEQ-361/2014 dated 10 March, 2016**
2. Name of the PI : **Dr. RAJ KUMAR DUARY**
3. Total Project Cost : **Rs. 11,00,000.00**
4. Revised Project Cost (if applicable) : **NA**
5. Date of Commencement : **March 10, 2016**
6. Statement of Expenditure
   (month wise expenditure incurred during current financial year April, 2016 to March, 2018)

Month & year	Expenditure incurred (Rs.)
April, 2016 - March 2017	**5,70,559.00**
April, 2017 - March 2018	**4,78,908.00**

7. Grant received in each year
   a. 1st Year : **Rs. 6,00,000.00**
   b. 2nd Year : **Rs. 4,50,000.00**
   c. 3rd Year : **NA**
   d. Interest, if any: **NIL**
   e. Total (a+b+c+d): **Rs. 10,50,000.00**

Annexure-II

Statement of Expenditure (Consolidated)

1st April 2016 to 31st March 2018

Sr No (I)	Sanctioned Heads (II)	Total Fund Received (2016-17) (III) In ₹	Expenditure Incurred		Total Expenditure till 31st March 2018 (VI = IV + V) In ₹	Balance as on 31st March 2018 (VII = III – VI-V) In ₹	Requirement of Funds upto 31st March 2019 In ₹	Remarks (if any)
			1st Year 2016-17 (IV) In ₹	2nd Year 2017-18 (V) In ₹				
1.	**Manpower costs**	**2,22,533.00**	1,38,000.00	84,000.00	2,22,000.00	533.00	NA	
2.	**Consumables**	7,64,967.00	4,01,309.00	3,63,658.00	7,64,967.00	0.00	NA	
3.	**Travel**	**0.00**	0.00	N/A	0.00	0.00	NA	
4.	**Contingencies**	**0.00**	0.00	N/A	0.00	0.00	NA	
5.	**Others (Tax)**	NIL	NIL	N/A	NIL	NIL	NA	
6.	**Overhead**	62,500.00	31,250.00	31,250.00	62,500.00	0.00	NA	
7.	**Total**	**10,50,000.00**	**5,70,559.00**	**4,78,908.00**	**10,49,467.00**	**533.00**	**NIL**	

Name of Principal Investigator: Dr. RAJ KUMAR DUARY

Signature of PI: Date: 24/11/2020 Assistant Professor Department of Food Engg. & Technology Tezpur University Napaam, Tezpur- 784028, Assam	Signature of the Finance Officer: Date: 25/11/2020 Finance Officer Tezpur University	Signature of the Registrar: Date: Registrar Tezpur University