

Progress Report for R&D Projects

[Final Report]*

Section-A: Project Details

A1. Project Title: Comparative metagenome of human gut of North and North-eastern region of India

A2. DBT Sanction Order No. & Date: BT/536/NE/TBP/2013 dt. 07 August, 2014

A3. Name of Principal Investigator:

Parent Institute at NER

1. Dr. Raj Kumar Duary

Assistant Professor, Dept. of FET, Tezpur University

Collaborating Institute

2. Dr. Sunita Grover

Principal Scientist and Head,

Dairy Microbiology Division, ICAR-National Dairy Research Institute (ICAR- NDRI),

Karnal-132001

Name of Co-PI/Co-Investigator:

Parent Institute at NER

3. Dr. SougataSaha

Assistant Professor, Dept. MBBT, Tezpur University

Collaborating Institute

4. Dr. Virender Kumar Batish

Emeritus Scientist, MBU, Dairy Microbiology Division, ICAR-NDRI

5. Mrs. Rashmi H. M.

Scientist, MBU, Dairy Microbiology Division, ICAR-NDRI

A4. Institute:

Parent Institute at NER: Tezpur University

Collaborating Institute: ICAR-National Dairy Research Institute (ICAR-NDRI)

A5. Address with Contact Nos. (Landline & Mobile) & Email:

Parent Institute at NER:

Telephone: 03712-275709, +91-9957669564; Fax: +91-3712-267009;

E-mail: rkduary@gmail.com

Collaborating Institute:

Telephone: 0184-2259100 Fax: 0184-2250042 E-mail: sungro@gmail.com

A6. Total Cost: Rs. 64.15 lakhs

A7. Duration: 3 years from the date of sanction order

A8. Approved Objectives of the Project:

1. Generation of comprehensive catalogue of the gut microbes in healthy human gut from North Indian and North-East Indian population using high-throughput 16S rRNA sequencing of metagenome isolated from fecal samples.
2. Comparison and statistical analysis of 16S rDNA high-throughput sequencing data in related with the food habit and life style in these two populations by 16S metagenomic sequencing analyses.
3. Analysis of SCFAs (Short chain fatty acids) in the fecal samples of both population and study their correlation with the identified microbiome by 16S rDNA high-throughput sequencing data and physiological condition.

A9. Specific Recommendations made by the Task Force (if any):

- Possibilities of using the facilities of National Institute of Biomedical Genomics (NIBMG) at Kalyani, West Bengal, India.

As advised, we have done 40 of our samples sequenced from NATIONAL INSTITUTE OF BIOMEDICAL GENOMICS (NIBMG) under collaboration with Dr. ArindamMaitra,

In-charge of Core Technology Research Initiative (CoTeRI) using 454 GS FLX System (ROCHE).

- Diet of the people should be defined in terms of amount, quantity and the number of times taken.

The groups have been categorized based on diet from each age group based on amount and number of times of intake in both vegetarian and non-vegetarian group. This information has been included in questionnaires during collection of samples.

- Develop the database and it should be available at DBT website

The developed database of human gut genome from both populations will be got uploaded at DBT website besides submission at NCBI GenBank/EMBL (public platform) gene bank.

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period

Objective 1:

Generation of comprehensive catalogue of the gut microbes in healthy human gut from North Indian and North-East Indian population using high-throughput 16S rRNA sequencing of metagenome isolated from fecal samples.

NDRI Part:

a. Generation of comprehensive catalogue of the gut microbes in healthy human gut from North Indian Population

i. Study population and faecal biospecimen collection from North India

The study population size of 40 subjects (**Table 1**), 10 vegetarian and 10 non vegetarian under the age group of 20-30 and similarly 10 vegetarian and 10 non vegetarian under the age group of 20-30 after following the inclusion and exclusion criteria as laid down in the proforma. All study participants provided informed consent. The average subject age was 25 ± 2.55 years (mean \pm s.d.) in 20-30 age group and 55 ± 2.82 years (mean \pm s.d.) in 50-60 age group.

Table 1 : Study population comprising of vegetarian and non-vegetarian groups from north-India

S. No.	Age Group (20-30 yrs)		Age Group (50-60 yrs)	
1	1GB23V	5RK29NV	11DV50V	28H59NV
2	2SK24V	7MP24NV	16RS55V	29RP55NV
3	3MK23V	8RK25NV	22RMC57V	32MS55NV
4	4LB25V	9RPP28NV	24RK52V	33SP57NV
5	12NN29V	10MK27NV	25ID55V	34SK51NV
6	15G22V	14K25NV	27GS59V	36JS55NV
7	19NT29V	23SSK27NV	30SG57V	37RS52NV
8	20RS26V	35CB24NV	31SSC59V	38HS55NV
9	21S23V	43GL20NV	41BS59V	39RS53NV
10	26AM25V	44SK22NV	42DR55V	40SS51NV

V : Vegetarian; NV : Non-vegetarian

ii. DNA Extraction and analysis of quantity, purity and integrity

Different protocols which included Freeze-Thawing, Triton X Method, physical lysis (Sonication), Guanidine isothiocyanate, TE Lysis and Zymo-Research DNA MiniPrep Kit were tested for extraction of DNA from faecal samples. The quantity and purity of DNA extracted with each method was assessed by spectrophotometric method using Synergy H1 Multi-Mode Reader (BioTek, Winooski,VT). The integrity and DNA shearing was assessed by agarose gel electrophoresis as well. Amongst these, DNA extraction using Zymo-Research DNA MiniPrep Kit yielded high quality and large quantity of metagenomic DNA in the range of 20–30µg/250mg of faecal sample, which was sufficient for downstream analysis such as Amplicon library construction or sequencing. Hence, Zymo-Research DNA MiniPrep Kit was selected for isolation of DNA from all faecal samples as shown in **Fig. 1**.

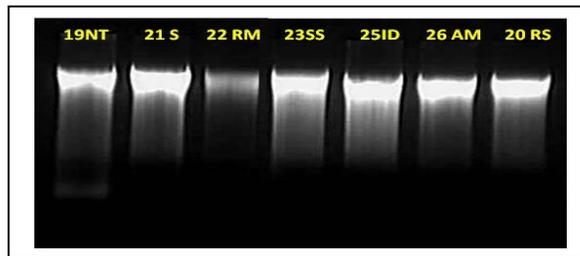
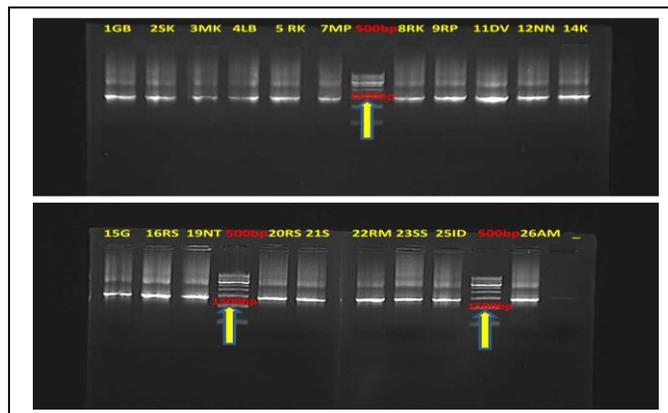


Fig. 1. Genomic DNA extracted using Zymo Kit

iii. Evaluation of PCR amenable metagenomic DNA from faecal Biosamples using 16S rRNA gene amplification

The metagenomic DNA extracted from all the 40 samples were evaluated for their suitability for PCR by 16S rDNA gene amplification using universal primers 16S 27F 5'CCAGAGTTTGATCMTGGCTCAG 3' and 16S R 5' CGGTTACCTTGTTACGACTTCACC 3'. The DNA obtained from all the 40 samples gave PCR amplicons of size ~1.5 kb as shown in

Fig.



2.

Fig. 2. PCR amplification of 16S rRNA gene using metagenomic DNA

iv. Amplicon Library Preparation for 454 pyrosequencing

The quantity and quality of each individual DNA preparation was again confirmed with NanoDrop and the amplicons from all the 40 samples were prepared by using 20ng of template metagenomic DNA. PCR amplification of the bacterial 16S rRNA gene hypervariable V3–V5 region using the universal bacterial primers 357F (5'-CCTACGGGAGGCAGCAG -3') and 926R (5'-CCGTCAATTCMTTTRAGT -3') incorporating the 454 universal adapters and multiplex identifiers at the 5' end of the reverse primer was carried out.

v. Purification, Digital Quantification and fluorescence-based size characterization of Amplicon Libraries

All the prepared amplicons from each samples were successfully purified by using Qiagen MiniElute PCR purification kit. The purified amplicons were quantified by using Qubit® 2.0 Fluorometer and the size of amplicons from each sample was characterized on a Bioanalyzer 2100 (using a DNA1000 LabChip (Agilent)). After determination of quantity and the size of the amplicons, the concentration of amplicons from each samples was calculated in molecules/μl. The quantified molecules were diluted separately and pooled in equal concentration to get the final concentration of 1×10^9 molecules/μl in Pool 1 (amplicons from 10 vegetarian samples under age group 20-30 years). Similarly, the second sets of 10 amplicons from non-vegetarian population were pooled in equal concentration to get the final concentration of 1×10^9 molecules/μl in Pool 2.

vi. Emulsion PCR, Bead enrichment/Recovery and Sequencing

Emulsion PCR was carried out with the pooled amplicon libraries Pool 1 and Pool 2 separately in the separated 96 well PCR plates. After emulsion PCR, the amplified beads were recovered and enriched using Bead Recovery Reagents Kit. A total of 2.2 and 2.4 million bead were recovered from Pool 1 and Pool 2 amplicon libraries respectively. Finally, the sequencing was carried out with 2,000,000 beads each from P1 and P2 in the region 1 and region 2 respectively of the PicoTiterPlate (PTP) according to standard Roche/454 protocols using the GS Titanium Sequencing Kit (Roche Diagnostics) and the GS Junior device.

vii. Metagenomic data generation of gut microbiome of healthy North Indian population of different age and diet groups

The sequencing was carried out with 2,000,000 beads each pool in the region 1 and region 2 respectively of the PicoTiterPlate (PTP) according to standard 454 GS-FLX (Roche) protocols

using the GS Titanium Sequencing Kit (Roche Diagnostics) and the GS Junior device. After quality analysis, 554 and 428 Mb data was obtained with average Q value of 34. The 16S rDNA high-throughput sequencing metagenomic data was subjected to QIIME analysis for comparative and statistical analysis of bacterial diversity and taxonomic composition of bacteria in North Indian gut with different age (20-30 & 50-60 years) and food habit (Veg & Non-veg)

Table 2: Metagenomic data generated from North Indian population

Sequencing Report							
SL. No.	DVD Barcode#	Run ID	Region Name	Avg Q Value	Median Read Length (bp)	Data Size (Mb)	QC Status
1	TU_040815_1	TU_P1_P2_24JUL2015	Region1	34	542	554	PASS
			Region2				
2	TU_040815_2	TU_P3_P4_30JUL2015	Region1	33	543	428	PASS
			Region2				

Tezpur University

- b. Generation of comprehensive catalogue of the gut microbes in healthy human gut from North-East Indian Population**
- i. Study population and faecal biospecimen collection from North-East India**

The same population size was selected and similar criteria as mentioned in earlier was followed for the collection of faecal biospecimen collection from North-East India (Table 3).

Table 3: Study population comprising of vegetarian and non-vegetarian groups from North-East India

S. No.	Age Group (20-30 yrs)		Age Group (50-60 yrs)	
1	TURP26V1	TURP26NV1	TURP60V1	TURP50NV1
2	TURP20V1	TURP26NV2	TURP60V2	TURP51NV1
3	TURP20V2	TURP26NV3	TURP50V1	TURP51NV2

4	TURP20V3	TURP26NV4	TURP50V2	TURP54NV1
5	TURP20V4	TURP25NV1	TURP50V3	TURP55NV1
6	TURP20V5	TURP22NV1	TURP51V1	TURP55NV2
7	TURP23V1	TURP30NV1	TURP55V1	TURP55NV3
8	TURP23V2	TURP30NV2	TURP55V2	TURP59NV1
9	TURP23V3	TURP30NV3	TURP55V3	TURP60NV1
10	TURP21V1	TURP29NV1	TURP52V1	TURP58NV1

V: Vegetarian; NV : Non-vegetarian

ii. Metagenomic DNA extraction, Amplicon Library Preparation and characterization for 454 pyrosequencing, Emulsion PCR, Bead enrichment/Recovery and Sequencing

The same SOP's (Standard Operating Procedures) were followed for the metagenomic DNA extraction, amplicon library preparation and characterization for 454 pyrosequencing, Emulsion PCR, Bead enrichment/Recovery and Sequencing of samples collected from the North-East India.

Metagenomic data generation from TUPart

After quality analysis, the 16S rDNA high-throughput sequencing metagenomic data was subjected to QIIME analysis for comparative and statistical analysis of bacterial diversity and taxonomic composition of bacteria in North-East Indian gut with different age (20-30 & 50-60 years) and food habit (Veg & Non-veg)

Objective 2

Comparison and statistical analysis of 16S rDNA high-throughput sequencing data in related with the food habit and life style in these two populations by 16S metagenomic sequencing analyses.

The taxonomic data from QIIME analysis has been subjected to statistical analysis for the comparison of most abundant phyla, family and genera present in the gut microbiota of North and North-East Indian population.

i. Comparative analysis of major gut microflora at phylum level

The major gut microbial phyla such as Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria were compared for their abundance in the gut microbiota of North and North-East

population. The data representing percentage abundance of each phyla has been analysed using one way ANOVA and results have been represented in **Fig.3**.

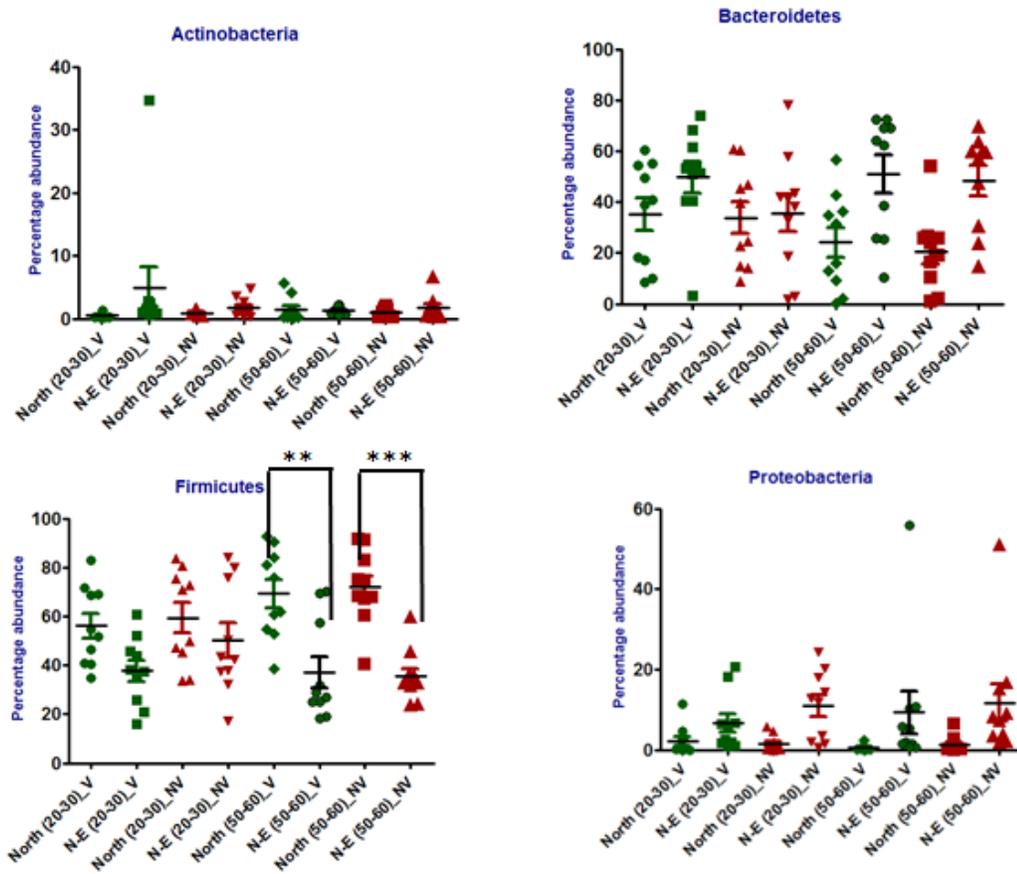


Fig. 3: Comparative analysis of major gut microflora at phylum level (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria)

The data presented in **Fig. 3** clearly indicate the higher abundance of *Bacteroidetes* and *Proteobacteria* and lower abundance of *Firmicutes* in the gut microbiota of North-East Indian (NE) population compared to North (N) Indian population. However, the higher abundance of *Firmicutes* was observed in the North-Indian population in all age and diet groups. The higher abundance of *Firmicutes* in North India population was significant at the age of 50-60 compared to 20-30 age group in both vegetarian and non-vegetarian diet groups. However, no significant difference in the abundance of *Actinobacteria* was observed between the two different population groups (N and NE) of different age and diet.

ii. Comparative analysis of major gut microflora at family level

For comparison of gut microbial abundance at family level, a total of 11 major families, including *Bifidobacteriaceae* and *Coriobacteriaceae* of phylum Actinobacteria, *Bacteroidaceae* and *Prevotellaceae* of Bacteroidetes phylum, *Lactobacillaceae*, *Streptococcaceae*, *Clostridiaceae*, *Ruminococcaceae*, *Lachnospiraceae* and *Veillonellaceae* of phylum Firmicutes and the family *Enterobacteriaceae* from the phylum proteobacteria were selected. The data on percentage abundance of each family in the gut microbiota of North and North-East Indian population was compared analysed by ANOVA to determine the significant difference in the aforesaid families (Fig. 4).

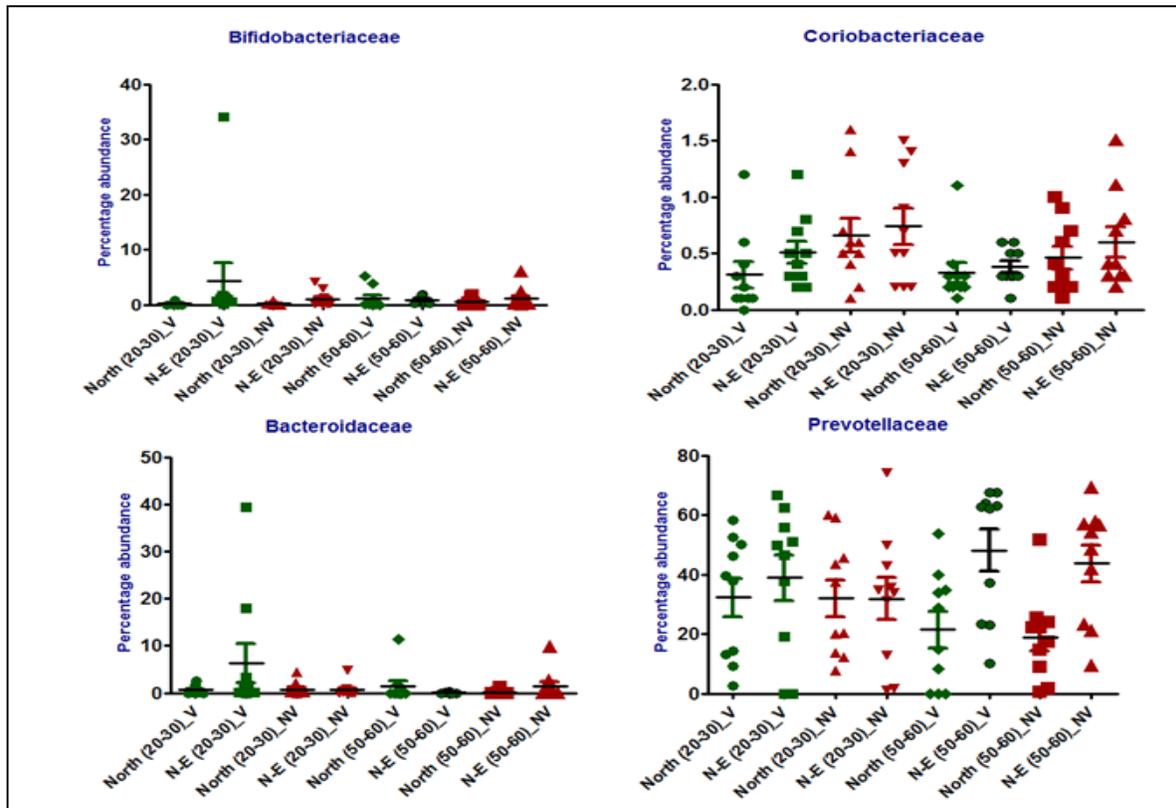


Fig.4: Comparative analysis of major gut microbial families (*Bifidobacteriaceae*, *Coriobacteriaceae*, *Bacteroidaceae* and *Prevotellaceae*)

Among the families of *Bifidobacteriaceae* and *Coriobacteriaceae* of phylum Actinobacteria, non-significant higher abundance of family *Coriobacteriaceae* was reported in the north-east (NE) population in all age and diet groups. Similarly in the families of *Bacteroidaceae* and *Prevotellaceae* of Bacteroidetes phylum, higher abundance of *Prevotellaceae* was observed in the NE population.

Under the phylum *Firmicutes*, comparatively lower abundance of *Lactobacillaceae* was observed in the NE population and no significant difference was observed in the abundance of *Streptococcaceae* between different population groups. In other families of the phylum *Firmicutes* viz. *Clostridiaceae* and *Veillonellaceae*, no significant trend of decline or increase was observed between North and North-Eastern population groups. However the families *Ruminococcaceae*, *Lachnospiraceae* showed their increased abundance in the gut microbiota of north Indian population in all diet and age groups and the increased abundance of *Lachnospiraceae* was significant in the population age group of 50-60 under vegetarian category (Fig. 5). In the phylum of Proteobacteria, comparatively higher abundance of *Enterobacteriaceae* was observed in the North-Eastern population.

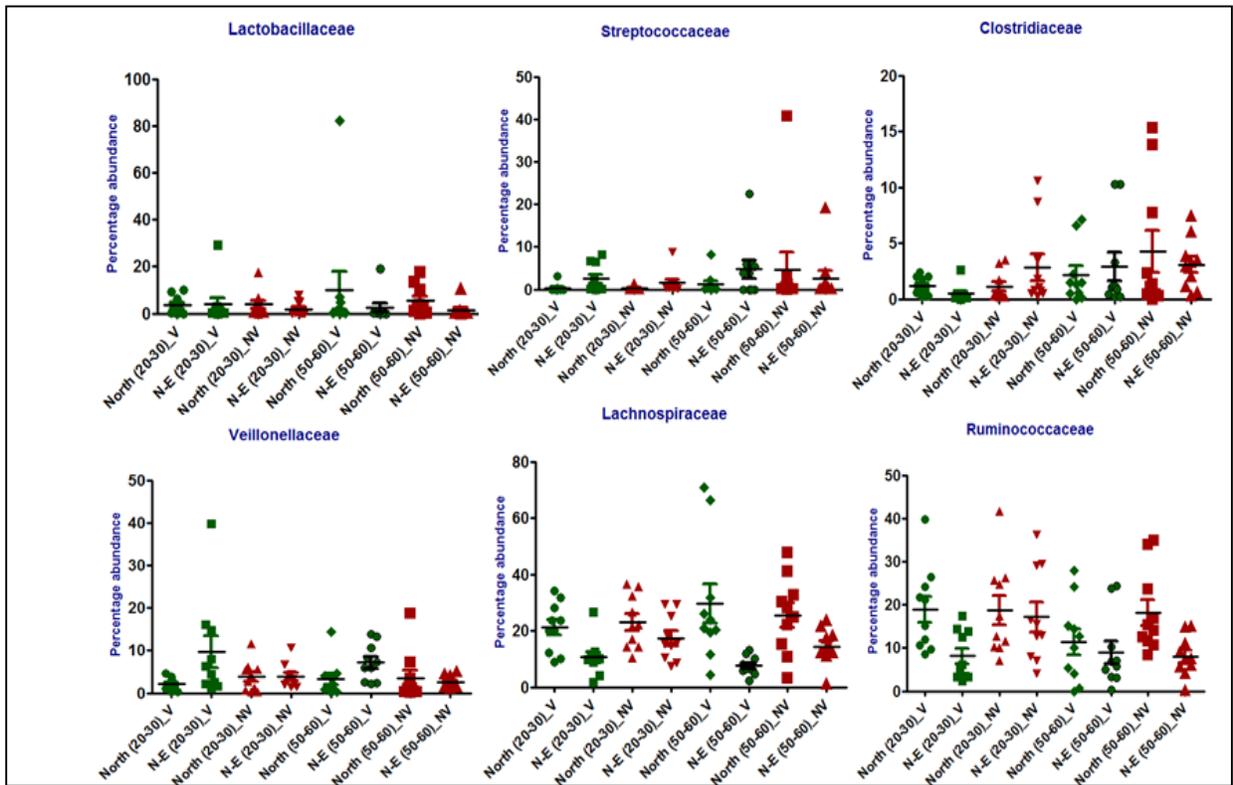


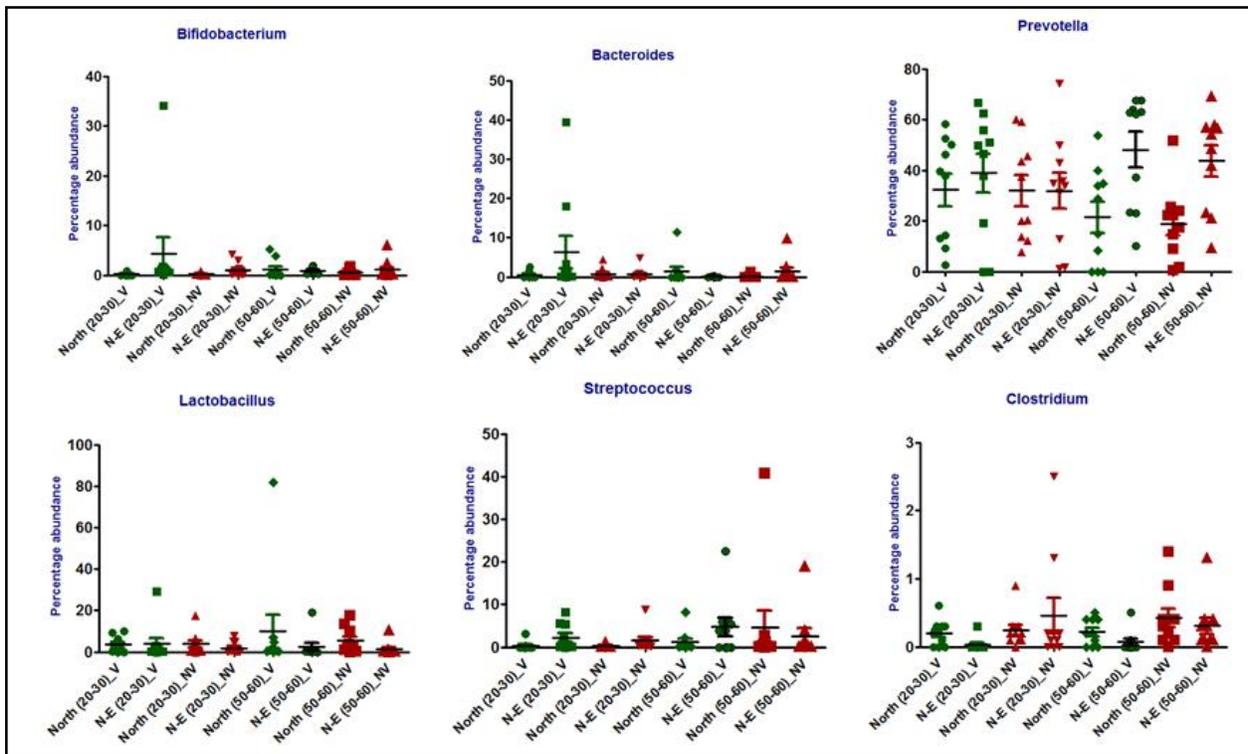
Fig.5: Comparative analysis of major gut microbial families of phylum Firmicutes

iii. Comparative analysis of major gut microflora at genus level

Like family *Bifidobacteriaceae*, no difference in the abundance of genus *Bifidobacteria* was observed between the different population groups and also no significant difference in the genera of *Bacteroides* was observed between the different population groups although higher abundance of *Bacteroidetes* phylum was reported in North-East population. However, the higher abundance

Prevotella under the phylum Bacteroidetes was observed in the NE population. From this, it can be concluded that, the higher abundance of Bacteroidetes in NE population may be due to the higher abundance of *Prevotellaceae* at family level and *Prevotella* at genus level. However, the genera *Lactobacillus* and *Streptococcus* under respective families of phylum Firmicutes showed no significant trend of either decline or increase between different population groups and in the family of *Clostridiaceae* under Firmicutes comparatively higher abundance of clostridium genera was observed in the non-vegetarian population (Fig. 6)

Fig.6: Comparative abundance of *Bifidobacterium*, *Bacteroides*, *Prevotella*, *Lactobacillus*, *Streptococcus* and *Clostridium* in North and North-East Indian gut.



In the family of *Lachnospiraceae*, higher abundance of genera *Blautia* and *Coprococcus* was observed in the north-Indian population and in *Blautia*, the abundance was significant in vegetarian population under the age group 50-60. However, in case of *Coprococcus*, their higher abundance in north Indian population was significant in all diet groups under the age 50-60. Again the higher abundance of butyrogenic genera *Faecalibacterium* was observed in the north Indian population compared to north-east India and their abundance was significant in the vegetarian population under the age group 50-60 (Fig. 7). However, in the genera of Ruminococcus, Megamonas and

Megasphaera no significant difference in their abundance was observed between the different population groups.

In case of *Catenibacterium* genus of family *Erysipelotrichales*, decreased abundance was observed in the north-east Indian population. However their decreased abundance was again significant at the age group of 50-60 in both diet groups.

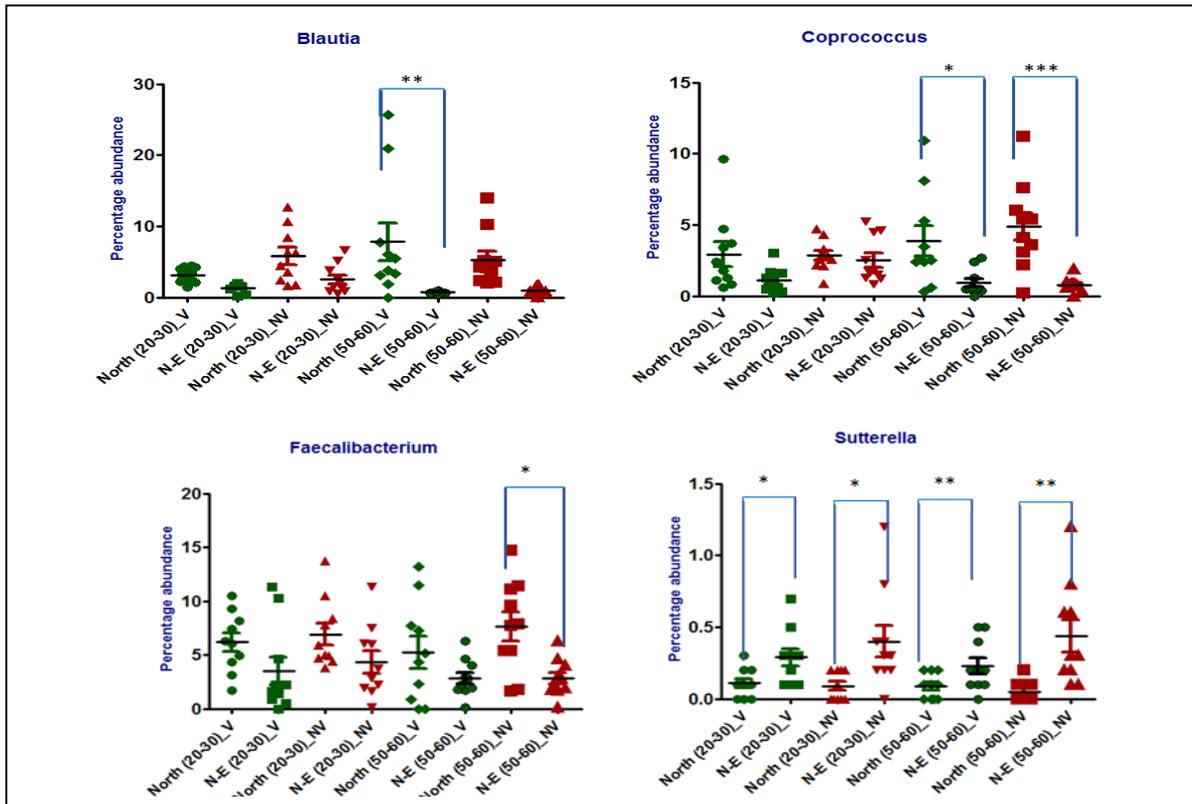


Fig.7: Comparative abundance of *Blautia*, *Coprococcus*, *Faecalibacterium* and *Sutterella* in North and North-East Indian gut of different age and diet

Among proteobacteria, significant higher abundance genus *Sutterella* of *Alcaligenaceae* family was observed in all the north-Indian population groups (**Fig. 7**).

Objective 3

- c. Analysis of SCFAs (Short chain fatty acids) in the fecal samples of both population and study their correlation with the identified microbiome by 16S rDNA high-throughput sequencing data and physiological condition.
- i. **GC based analysis of faecal butyrate, acetate and propionate**

Short chain fatty acid profiling of different age groups from faecal samples was carried out using GC (Gas Chromatography) method (Fig. 14). Initially, the standard curves of acetate, propionate and butyrate were prepared for quantification of SCFAs. The concentrations for acetate, propionate and butyrate used for the preparation of standard curve of each were in the range of 1.0 to 60 mM; 0.312 to 20 mM and 0.3125 to 20 mM respectively. The standards curves were constructed using the values obtained from the area under the peaks at particular retention times. Across the two age groups, although the contents of acetate, propionate and butyrate decreased, there was non-significant decline which might be due to low sample size. The diet also demonstrated non-significant decline in SCFA profile.

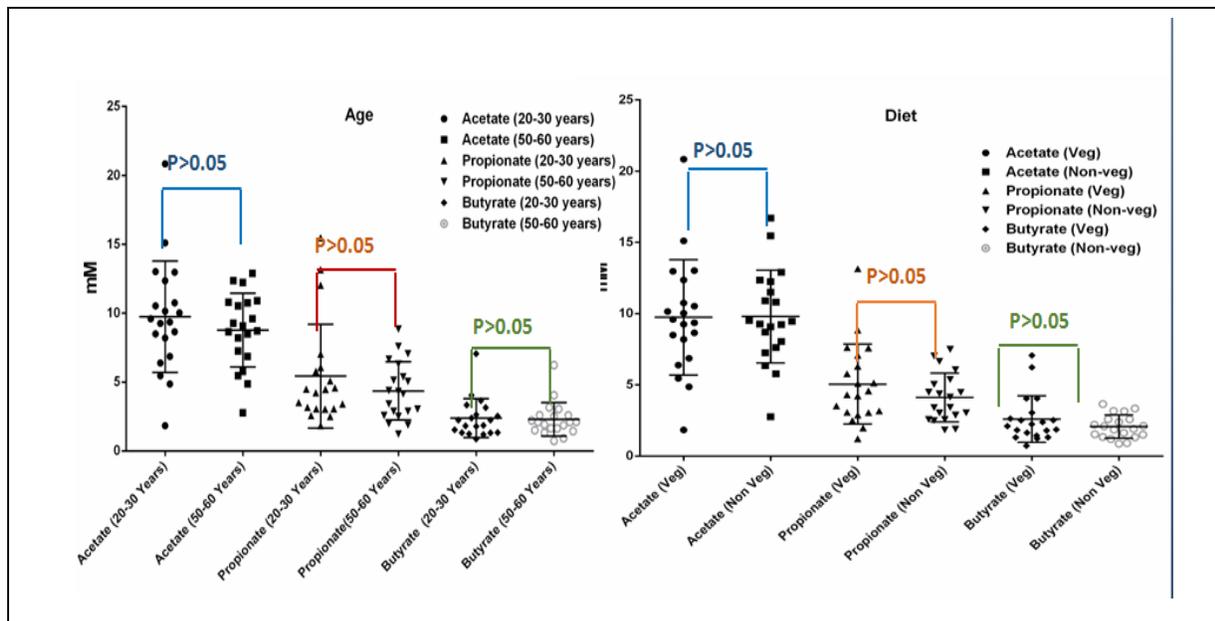


Fig. 8: SCFAs profiling in different age groups (A) and dietary habits (B)

ii. NMR based analysis of faecal butyrate, acetate and propionate.

Similarly the faecal butyrate, acetate and propionate concentration was also compared between different age groups and no significant difference observed in SCFA composition between population of different age (20-30 & 50-60) groups

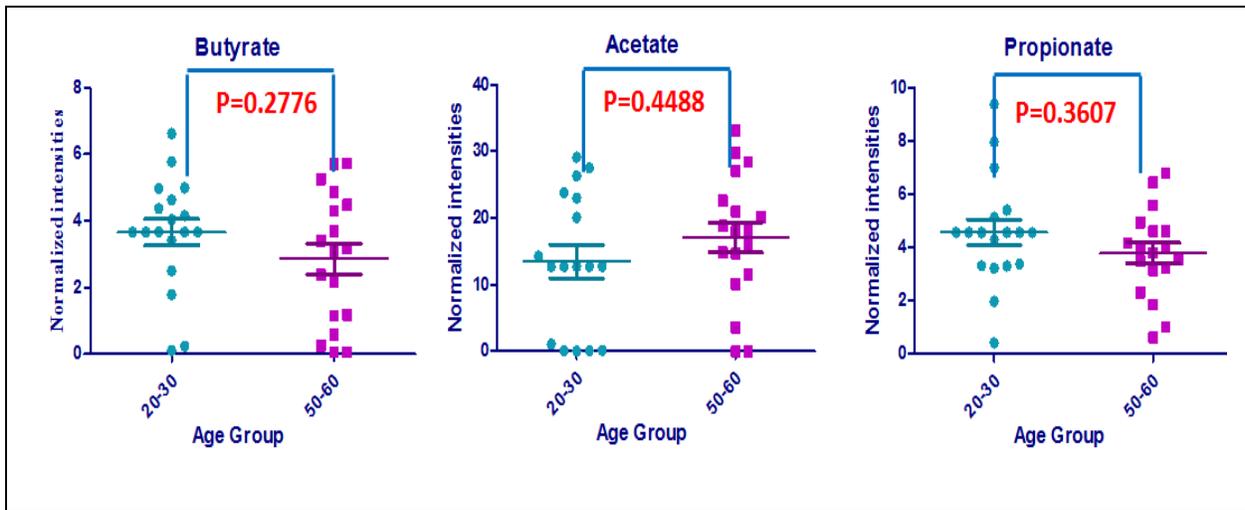


Fig. 9: NMR based analysis of SCFAs in different age groups

Further the NMR spectra was analyzed to identify key metabolites that vary significantly between different age groups (**Fig. 10**).

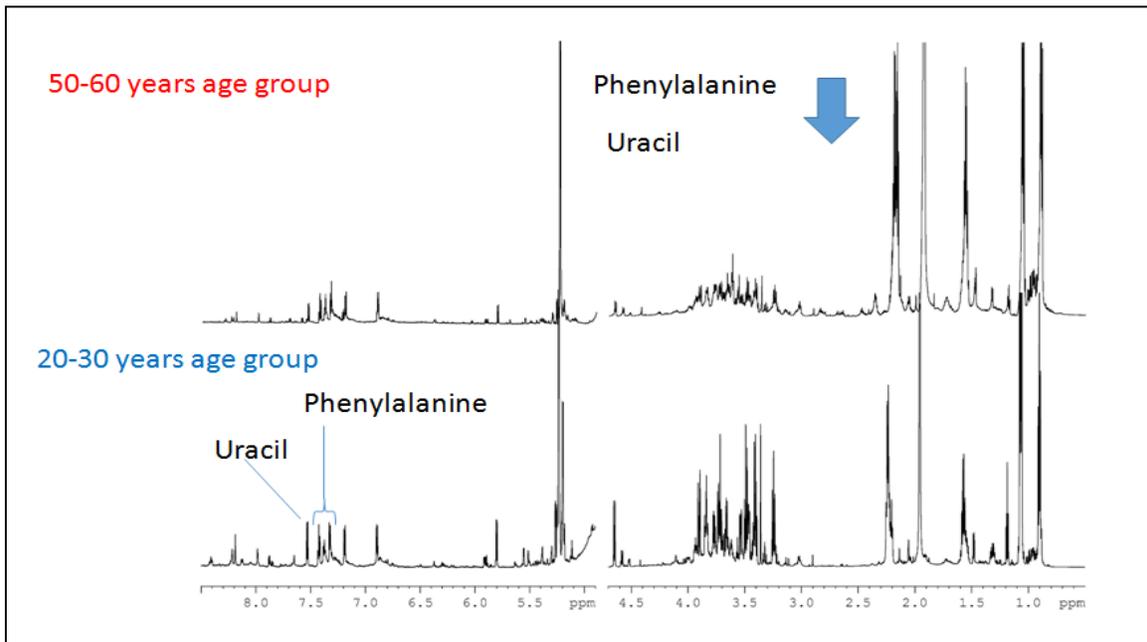


Fig.10 : NMR spectrum of metabolites from faecal samples

On analysis, phenylalanine and uracil were found significantly decreased in 50-60 years of age group compared to 20-30 years of age group. Besides glycolate was marginally unregulated in 50-

60 years of age group compared to 20-30 years of age group, though it is statistically not significant (Fig.10).

B2. Summary and Conclusions of the Progress made so far (minimum 100 words, maximum 200 words).

The metagenomic data on gut microbiota of from North and North-East Indian population of different age and diet groups was analyzed using QIIME (Quantitative Insights into Microbial Ecology). Diversity analysis revealed that Gut microbial richness decreases with ageing both Vegetarian and Non-Vegetarian population. Comparative analysis of metagenomic data at phylum level showed higher abundance of *Bacteroidetes* and *Proteobacteria* and lower abundance of *Firmicutes* in the gut microbiota of NE-Indian population. However no significant difference in the abundance of *Actinobacteria* was observed between the two different population groups of different age and diet. At family level, higher abundance of *Prevotellaceae* and *Enterobacteriaceae* and lower abundance of *Lactobacillaceae* was observed in the NE population. The families *Ruminococcaceae* and *Lachnospiraceae* showed their increased abundance in the N-Indian gut. At genus level, higher abundance of genera *Blautia* and *Coprococcus* and *Faecalibacterium* was observed in the North Indian population compared to NE India. However, no significant difference in the abundance of *Ruminococcus*, *Megamonas* and *Megasphaera* was observed between the different population groups. Among proteobacteria, significant higher abundance genus *Sutterella* and *Alcaligenaceae* family was observed in all the North-Indian population groups. The data pertaining to gut microbiota of N and NE-Indian population of different age and diet groups revealed the impact of diet and age on the gut microbiota. However, in analysis of SCFAs (Short chain fatty acids) in the fecal samples by both GC and NMR, no significant difference was observed in the composition of acetate, butyrate and propionate between population groups of different age and diet.

B3. Details of New Leads Obtained, if any:

This is the first report on the comparative metagenome of gut microbial populations with different age from Vegetarian and Non-Vegetarian diet of both North and North-East populations. A relative higher abundance of *Bacteroidetes* and *Proteobacteria* phylum and lower abundance of *Firmicutes* phylum in the gut microbiota of NE-Indian population. Interestingly, a lower abundance

of *Lactobacillaceae* was observed in the NE population as compared with North one. While, the SCFAs contents (acetate, butyrate and propionate) were more or less similar in both the studied groups. The study showed there is paradigm shift in the microbiota of the gut of these dietary differentiated peoples (North and North East-Indian population), since there is vast variation in the dietary intake of these two extreme part of our country.

B4. Details of Publications & Patents, if any:

Article:

Preeti Sarkar, Rashmi H Mallapa, Sougata Saha, Virender Kumar Batish, Sunita Grover and Raj Kumar Duary. Dietary spectrum on human gut microbiome and health interrogation- An integrative view. (Communicated)

Rashmi H Mallapa, Preeti Sarkar, Arindam Maitra, Sougata Saha, Virender Kumar Batish, Sunita Grover and Raj Kumar Duary. Comparative Analysis of Gut microbiome of North and North-East Indian population of different age groups based on dietary interventions. (Under preparation)

Conference Presentation

- "Comparative Analysis of Gut microbiome of North Indian Vegetarian and Non-Vegetarian population of different age groups" for presentation at International Conference on Microbiome Research (ICMR) 2018 from 19th - 22nd November 2018 at Hyatt Regency Pune, India ICMR-2018HM0046.
- "Comparative Analysis of Gut microbiome of North Indian and North-East Indian Vegetarian and Non-Vegetarian population of different age groups" In: 9th India Probiotic Symposium "Probiotics through the lifespan" Date: 24-25 November, 2018 Venue: Amity University, Kolkata, India.
- Dietary interventions on gut microbiome-a metagenomics study. In: National conference cum workshop organizing organized by the Food Engineering and Technology Dept, Tezpur University, 27 to 28th March, 2015 on "Innovative prospects in food processing: integration of engineering and biological sciences" IPFP-2015.

Invited Speaker:

- On "Milk based dietary interventions on gut microbiome affecting human health" under the theme "Functional Dairy Foods for Better Health" organized at Faculty of Dairy Technology, WBUAFS, Mohanpur Campus, Nadia, West Bengal on 24th September, 2017.

Section-C: Details of Grant Utilization

Tezpur University part:**C1. Equipment Acquired or Placed Order with Actual Cost:**

Name of equipment	Quantity	Sanctioned Cost (Rs.)	Actual Purchased cost (Rs.)	Purchase details	Status
Bead Beater	1 no	3,00,000.00	2,99,100.00	Unigenetics Instruments, Make: Mini Bead Beater-16 (Biospec Products Inc. USA)	Installed
Ultra low temperature freezer (-86oC)	1 no	5,50,000.00	5,40,000.00	Eppendorf India Ltd.	Installed
Adjustable micropipette sets	6 nos				
pH meter	1 no	50,000.00	39,545.00	Hanna pH meter (Jyoti Pharmaceuticals Ltd)	Installed

C2. Manpower Staffing and Expenditure Details:

S. No.	Name & Designation of the Manpower engaged	Pay Scale provided	Date of Appointment	Salary disbursed
01	Ms. Preeti Sarkar JRF	Rs. 12,000.00 per month	03/11/2014	Rs. 3,59,200.00
02	Ms. Lopamudra Sarma JRF	Rs. 12,000.00 per month	01/08/2017	Rs. 84,000.00

C3. Details of Recurring Expenditure:

Heads	Sanctioned Cost (Rs.)	Utilized amount (Rs.)
Consumables	8,40,000.00	4,43,200.00
Out Sourcing for 16s RNA sequences	16,00,000.00	18,20,058.00
Travel	67,000.00	61,364.00
Contingency	59,000.00	59,008.00
Overhead charges	66,000.00	1,32,702.00

C4. Financial Requirements for the Next Year with Justifications: -NA-**NDRI part:****C1. Equipment Acquired or Placed Order with Actual Cost:**

Name of equipment	Quantity	Sanctioned Cost (Rs.)	Actual Purchased cost (Rs.)	Purchase details	Status
Bead Beater	1 no	3,00,000.00	2,90,990	Purchased Order placed with P.O. No. 46116400575 dated 05/08/205 to M/s MP Biomedicals India Pvt. Ltd. 7, Nagin Mahal, 2nd Floor 82, V N Road, Churchgate Mumbai- 400020	Installed

C2. Manpower Staffing and Expenditure Details:

S. No.	Name & Designation of the Manpower engaged	Pay Scale provided	Date of Appointment	Salary disbursed
01	Mr. Devendra Kumar Singh	Rs. 12,000.00 + 1200 (10%) HRA =Rs 13,200/- (Upto 23 rd May 2016) Rs. 28000+2800 (10%) HRA= 30,800	04/12/2014	6,82,483

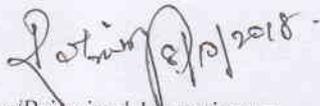
C3. Details of Recurring Expenditure:

Heads	Sanctioned Cost (Rs.)	Utilized amount (Rs.)
Consumables	8.59	8.4010
Contingency		
Travel	0.27	0.16581
Overhead charges	0.44	0.44
***The un-utilized amount of Rs. 35,845/- has been returned to DBT vide Cheque No. 984421 dated 05.06.2018		

C4. Financial Requirements for the Next Year (3rd year) with Justifications: NA

#Grant utilization details (UC&SE, Assets Certificate & manpower details) also required to be submitted separately as per the prescribed format

A) North-Eastern Institution



Signature of Project Coordinator/Principal Investigator

Assistant Professor

Deptt. of Food Engineering & Technology
Tezpur University, Napaam-784028

B) Collaborating Institutions Dist-Sonitpur (Assam)



Signature of Project Coordinator/Principal Investigator

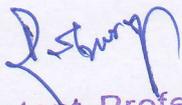
UTILISATION CERTIFICATE

(for the financial year ending 31st March 2018)

From 01/04/2017 to 31/03/2018

(Rs. in lakhs)

1. Title of the project/scheme : **“Comparative metagenome of human gut of North and North-eastern region of India”**
2. Name of the Organisation: **Tezpur University, Assam**
3. Principal Investigator : **Dr. Raj Kumar Duary**
4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project : **BT/536/NE/TBP/2013 dt 07 August, 2014**
5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given : **Rs. 4.76601 lakhs; BCIL/NER-BPMC/2017/365 dt 25/05/2017**
6. Amount received from DBT during the financial year (Please give No. and dates of sanction orders showing the amounts paid) : **Rs. 3.36 lakhs; BCIL/NER-BPMC/2017/365 dt 25/05/2017**
7. Other receipts/interest earned, if any, on the DBT grants : **Nil**
8. Total amount that was available for expenditure during the financial year (Sl. nos. 5, 6 and 7): **Rs. 8.12601 Lakhs**
9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed) : **Rs. 5.78961 Lakhs**
10. Unspent balance refunded, if any (Please give details of cheque No. etc.) **Rs. 2.33640 Lakhs**
11. Balance amount available at the end of the financial year : **Rs. 2.33640 Lakhs**
12. Amount allowed to be carried forward to the next financial year vide letter No. & date : **Not required**

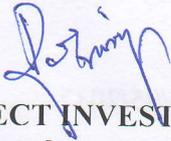

Assistant Professor
Deptt. of Food Engineering & Technology
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Dist-Sonitpur (Assam)


Finance Officer
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1. Certified that the amount of **Rs. 5.78961 Lakhs** mentioned against col. 9 has been utilised on the project / scheme for the purpose for which it was sanctioned and that the balance of + **Rs. 2.33640 Lakhs** remaining unutilized at the end of the year has been surrendered to Govt. (Vide No.dated) / will be adjusted towards the grants-in-aid payable during the next year.
2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled / are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statements



(PROJECT INVESTIGATOR)
(Signed and stamped)
 Assistant Professor
 Deptt. of Food Engineering & Technology
 Tezpur University, Napaam-784028
 Dist-Sonitpur (Assam)



(FINANCE OFFICER)
(Signed and stamped)
 Finance Officer
 Tezpur University



(HEAD OF THE INSTITUTE)
(Signed and stamped)
 Registrar
 Tezpur University

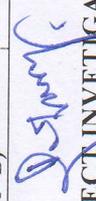
(To be countersigned by the DBT Officer-in-charge)

**FINAL CONSOLIDATED STATEMENT OF EXPENDITURE
(FOR FINAL SETTLEMENT OF ACCOUNTS)**

1. Title of the Project : Comparative metagenome of human gut of North and North-eastern region of India
2. Sanctioned Project Cost : Rs. 45.05 Lakhs
3. Revised cost, if any : Nil
4. Duration of the project : 3 years (extended up to March, 2018)
5. Sanction Order No. & Date : BT/536/NE/TBP/2013 dt 07 August, 2014
6. Date of commencement of Project : August, 2014
7. Extension, if any : till March, 2018
8. Date of completion of project : 31/03/2018

Details of grant, expenditure and balance

S. No.	Heads	Sanctioned Cost (Rs. in Lakhs)	page no.-	Year-wise Releases made (Rs. In Lakhs)				Total	Year-wise Expenditure incurred (Rs. In Lakhs)				Rs. In Lakhs Balance	
				1 st yr	2 nd yr	3 rd Yr	4 th Yr		1 st yr	2 nd yr	3 rd yr	4 th yr		Total
A.	Non-recurring	9.00		9.00	0.00	0.00	-	9.00	2.9910	5.79545	0.00000	-	8.78645	0.21355
	Equipments	9.00		9.00	0.00	0.00	-	9.00	2.9910	5.79545	0.00000	-	8.78645	0.21355
B.	Recurring													
1.	Manpower	6.60		2.11	1.19000	1.95000	-	5.25000	0.47200	1.44000	1.44000	1.08000	4.43200	0.81800
2.	Consumables	9.00		4.00	2.99000	1.41000	-	8.40000	0.88722	3.10032	0.00000	0.96331	4.95085	3.44915
3.	Outsourcing	17.00		11.00	5.00000	0.00000	-	16.00000	0.00000	10.0000	6.00000	2.20058	18.20058	-2.20058
4.	Travel	1.50		0.50	0.17000	0.00000	-	0.67000	0.16677	0.00000	0.05669	0.39018	0.61364	0.05636
5.	Contingency	0.70		0.30	0.29000	0.00000	-	0.59000	0.28800	0.00000	0.00000	0.30208	0.59008	-0.00008
6.	Overhead	1.25		0.50	0.16000	0.00000	-	0.660000	0.31250	0.16106	0.00000	0.85346	1.32702	-0.66702
7.	Interest	-		0.00	0.50585	0.16117	-	0.66702	-	-	-	-	-	0.66702
	Total	36.05		18.41	10.30585	3.52117	0.00	32.23702	2.12649	14.70138	7.49669	5.78961	30.11417	2.12285
	Grand Total (A+B)	45.05		27.41	10.30585	3.52117	0.00	41.23702	5.11749	20.49683	7.49669	5.78961	38.90062	2.3364


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Manpower Staffing Details (In the financial year wise manner)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR (Rs.)	TOTAL SALARY PAID DURING PROJECT PERIOD
Ms. Preeti Sarkar	JRF (2014-15)	3/11/2014	-	Rs. 12,000.00	47,200.00	3,59,200.00
	JRF (2015-16)	-	-	Rs. 12,000.00	1,44,000.00	
	JRF (2016-17)	-	-	Rs. 12,000.00	1,44,000.00	
	JRF (2017-18)	-	31/05/2018	Rs. 12,000.00	24,000.00	
Ms. Lopamudra Sarma	JRF (2017-18)	01/08/2018		Rs. 12,000.00	84,000.00	84,000.00



(Signature of Principal Investigator)

Assistant Professor

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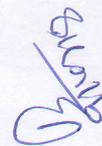
Dist-Sonitpur (Assam)



(Signature of Accounts Officer)

Finance Officer

Tezpur University



(SIGNATURE OF HEAD OF THE INSTITUTE)

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Tezpur University

Manpower Expenditure Details (In financial year wise manner)*:

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELEASES BY DBT	ACTUAL EXPENDITURE	BALANCE
JRF	01	JRF without NET: 12000/-+HRA for 1st & 2 nd Yr and 14000/-+ HRA For 3 rd Yr	Rs. 1.44 (In Lakhs)	Rs. 6.60 (In Lakhs)	-	-	-	Rs. 5.25 (In Lakhs)	Rs. 4.432 (In Lakhs)	Rs. 0.8180 (In Lakhs)



(Signature of Principal Investigator)
Assistant Professor

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(Signature of Accounts Officer)

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* Details of manpower salary/ fellowship revision along with the statement and arrears requested should be given separately, if applicable.

Due-Drawn Statement

Name of the Project Staff	Month and Year	Due	Drawn	Difference
Ms. Preeti Sarkar	3/11/14 to 31/3/15	47,200.00	47,200.00	0.00
	1/4/15 to 31/3/16	1,44,000.00	1,44,000.00	0.00
	1/4/16 to 31/3/17	1,44,000.00	1,44,000.00	0.00
	1/4/17 to 31/5/17	24,000.00	24,000.00	0.00
Ms. Lopamudra Sarma	1/8/17 to 31/3/18	84,000.00	84,000.00	0.00



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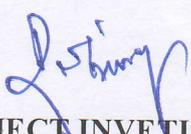
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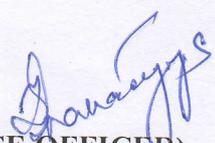


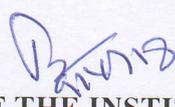
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List of Assets

S. No.	Name of equipment	Quantity	Sanctioned Cost	Actual Purchased cost	Purchase details	Status
01	Bead Beater	01	Rs. 3,00,000.00	Rs. 2,99,100.00	Unigenetics Instruments, Make: Mini Bead Beater-16 (Biospec Products Inc. USA)	Installed
02	Ultra low temperature freezer (-86°C)	01	Rs. 5,00,000.00	Rs. 5,40,000.00	Model: U410-Upright, Eppendorf India Limited	Installed
03	Adjustable volume of pipettes (sets)	01 Set	Rs. 50,000.00		Make: Eppendorf Research Plus, Eppendorf India Limited	Procured
04	pH meter	01	Rs. 50,000.00	Rs. 39,545.00	Model: HI2211, Make: HANNA Instrument, Jyoti Pharmaceuticals,	Installed
	Total		Rs. 9,00,000.00	Rs. 8,78,645.00		


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