ANNEXURE I

LIST OF PUBLISHED PAPER

1. Narzary, Y., Brahma, J., Brahma, C., & Das, S. (2016). A study on indigenous fermented foods and beverages of Kokrajhar, Assam, India. *Journal of Ethnic foods*, *3*(4), 284-291.

2. Narzary, Y., Brahma, C., & Das, S. (2019). Annalysis of Minerals, Amino acid and Fatty acids in Fermented Fish Napham traditionally prepared by Bodo ethnic group of Kokrajhar, Assam. *International Journal emerging Tecnologies and Innovative Research*, *6*(4), 90-97.

3. Narzary, Y. & Das., S. (2021). Study on biochemical constituent, microbial dynamics and 16SrRNA of traditionally prepared fermented fish *napham*. RTBSRNEI-2021. ISBBN: 9788136939029.

4. Narzary, Y., Das, S., Goyal, A. K., Lam, S. S., Sarma, H., & Sharma, D. (2021). Fermented fish products in South and Southeast Asian cuisine: indigenous technology processes, nutrient composition, and cultural significance. *Journal of Ethnic Foods*, *8*(1), 1-19.

LIST OF CONFERENCE ATTENDED

1. Narzary, Y. (2018). Role of women in preserving indigenous knowledge of food processing and preservation for achieving household food security in Bodo society. *"The changing status of women: Myth and Reality with special reference to Northeast India".* UGC sponsored International seminar, Kokrajhar, Assam.

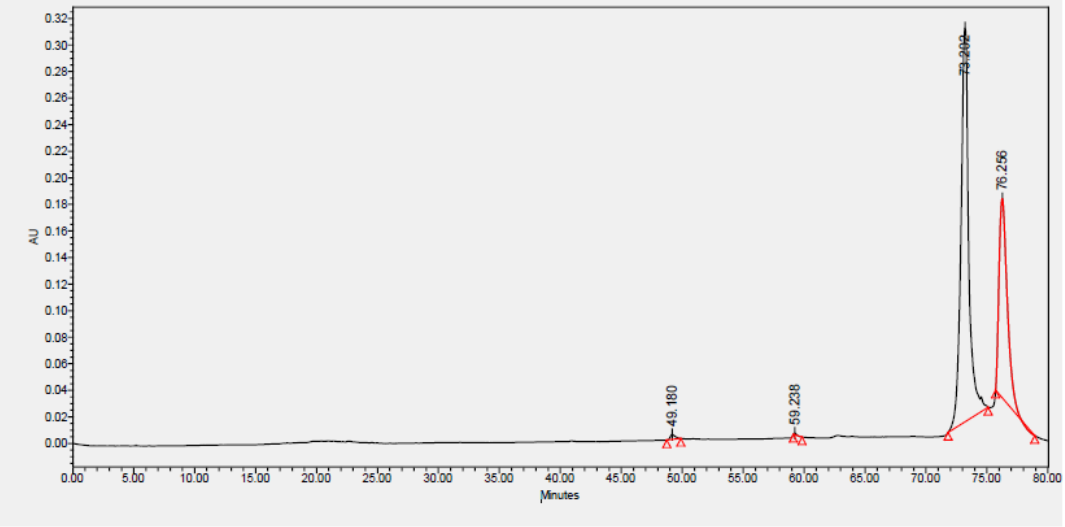
2. Narzary, Y. & Das., S. (2021). Study on biochemical constituent, microbial dynamics and 16SrRNA of traditionally prepared fermented fish *napham*. RTBSRNEI-2021 National Seminar. Hilakandi, Assam.

ANNEXURE II

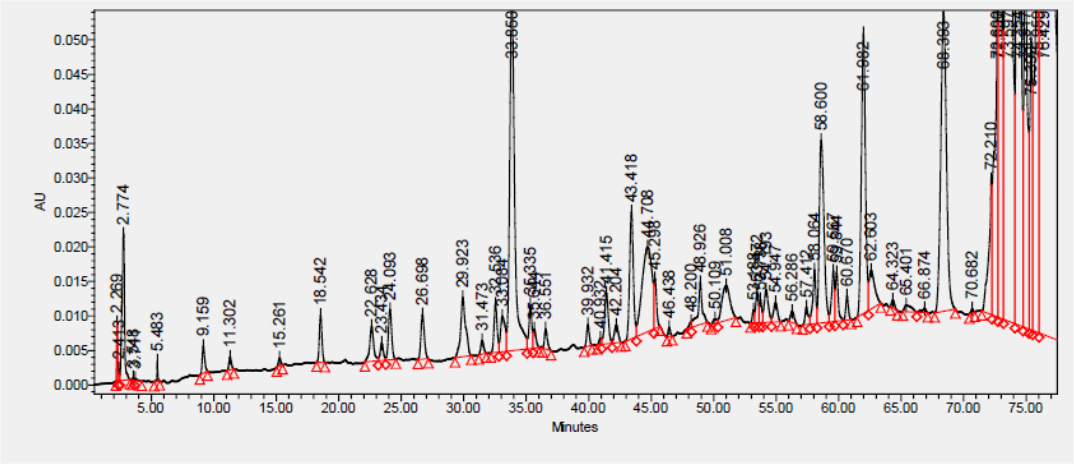
Chromatogram report

1. HPLC Blank and Standard Chromatogram and retention time

Blank Chromatogram:



Standard Chromatogram:

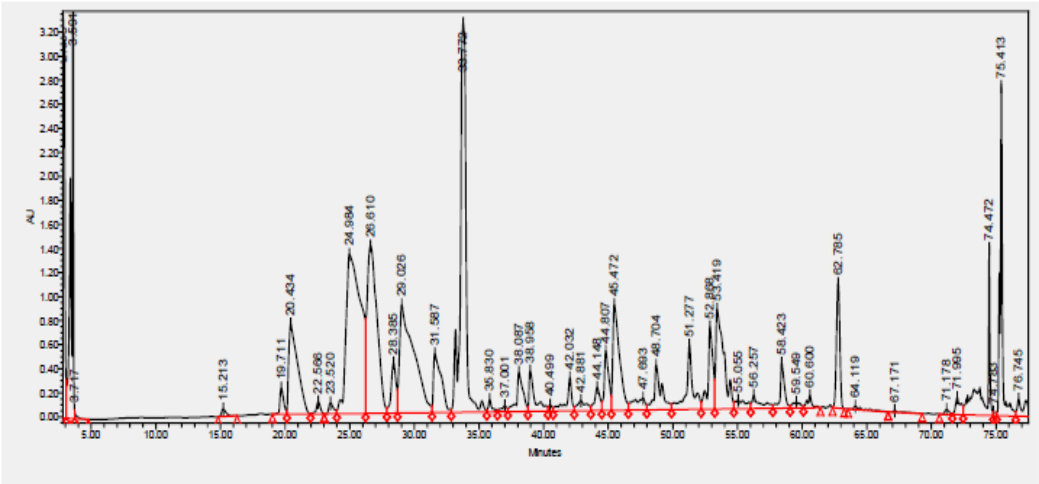


Name	Retention Time	Area
Aspartic acid	18.542	98311
Threonine	22.628	113238
Serine	24.093	133463
Asparagine	26.698	122475
Glutamic acid	29.923	239314
Glutamine	33.85	1419116
Glycine	43.418	383891
Alanine	44.708	676585
Valine	48.926	141401
Cysteine	58.6	696440
Methionine	59.567	118894
Iso leucine	59.844	108666
Leucine	60.67	49622
Tyrosine	61.982	913044
Phenylalanine	62.603	173845
Tryptophan	72.21	335252
Histidine	72.69	793896
Lysine	74.917	1423933
Arginine	75.959	1656903

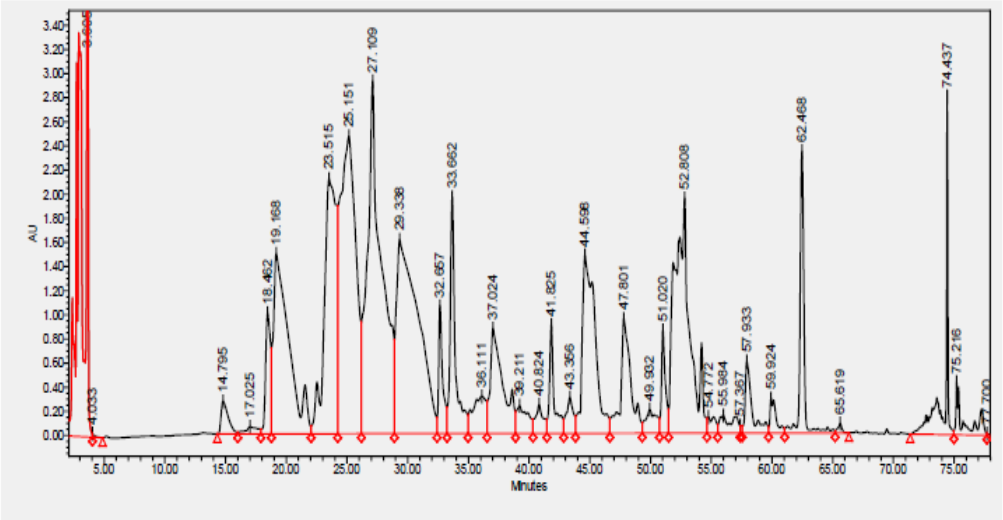
Retention time of Standard

2. HPLC Sample Chromatogram
Raw sample

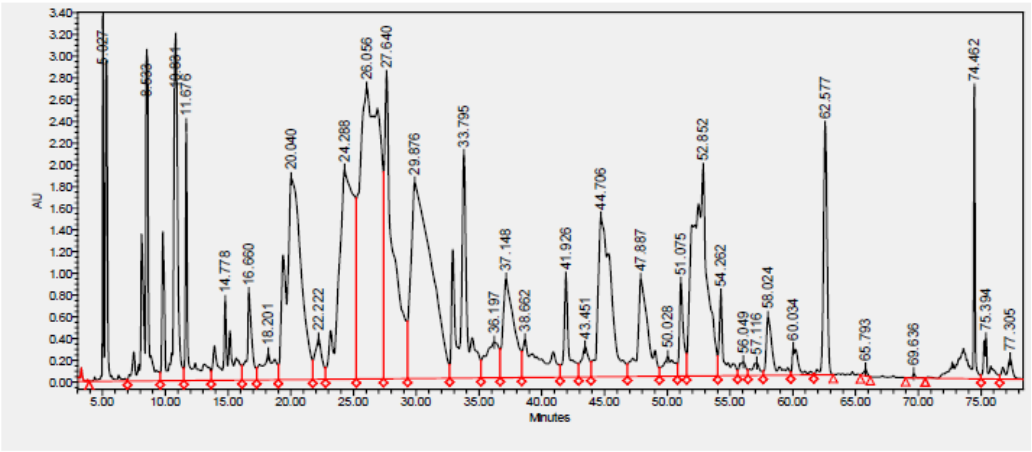
Sample 1 NB(RAW)_R1



Fermented fish *napham* F1



Fermented fish *napham* F2



a)

Name	Retention Time
Aspartic acid	18.117
Threonine	22.566
Serine	24.281
Asparagine	28.385
Glutamic acid	31.587
Glutamine	33.772
Glycine	42.032
Alanine	44.148
Valine	49.157
Cysteine	58.423
Methionine	59.314
Iso leucine	60.355
Leucine	60.6
Tyrosine	62.785
Phenylalanine	64.119
Tryptophan	72.696
Histidine	72.81
Lysine	74.472
Arginine	75.251

b)

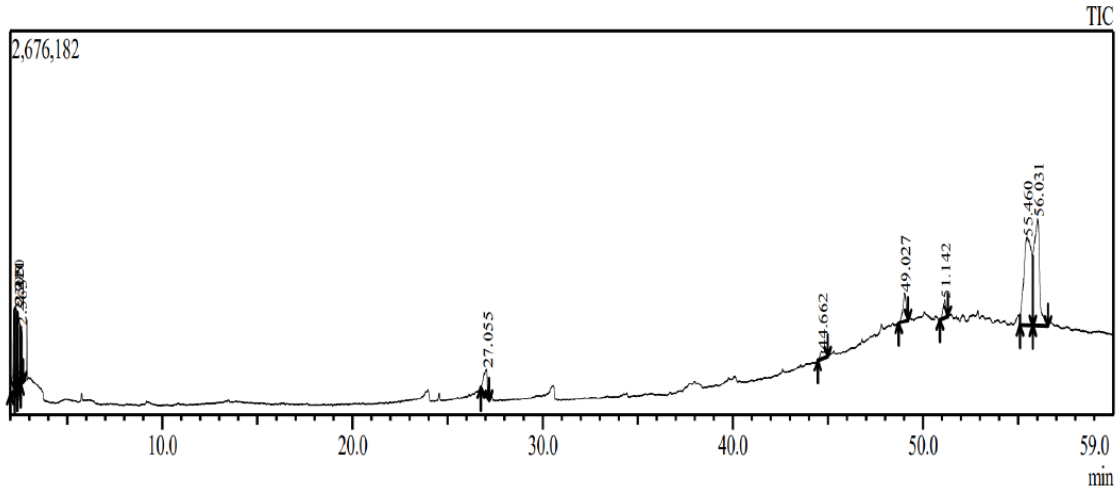
Name	Retention Time
Aspartic acid	18.201
Threonine	22.222
Serine	23.18
Asparagine	29.235
Glutamic acid	32.9
Glutamine	33.795
Glycine	42.429
Alanine	44.149
Valine	49.015
Cysteine	58.024
Methionine	58.936
Iso leucine	60.233
Leucine	60.944
Tyrosine	63.608
Phenylalanine	64.352
Tryptophan	72.698
Histidine	73.032
Lysine	74.462
Arginine	75.241

c)

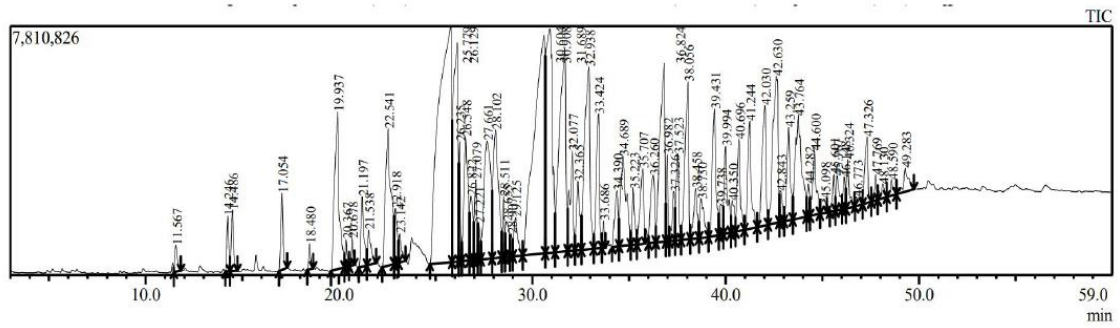
Name	Retention Time
Aspartic acid	18.254
Threonine	22.111
Serine	23.217
Asparagine	28.05
Glutamic acid	32.744
Glutamine	34.063
Glycine	40.51
Alanine	42.548
Valine	48.99
Cysteine	56.154
Methionine	57.317
Iso leucine	60.274
Leucine	61.276
Tyrosine	62.033
Phenylalanine	63.962
Tryptophan	71.452
Histidine	72.748
Lysine	73.671
Arginine	75.352

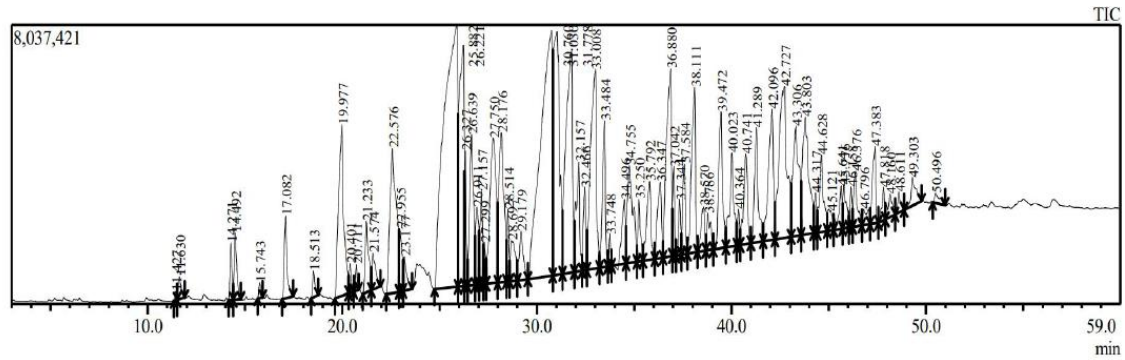
Retention time a) Raw sample and b) fermented sample F1 c) Fermented sample F3

3. GCMS Blank Chromatogram

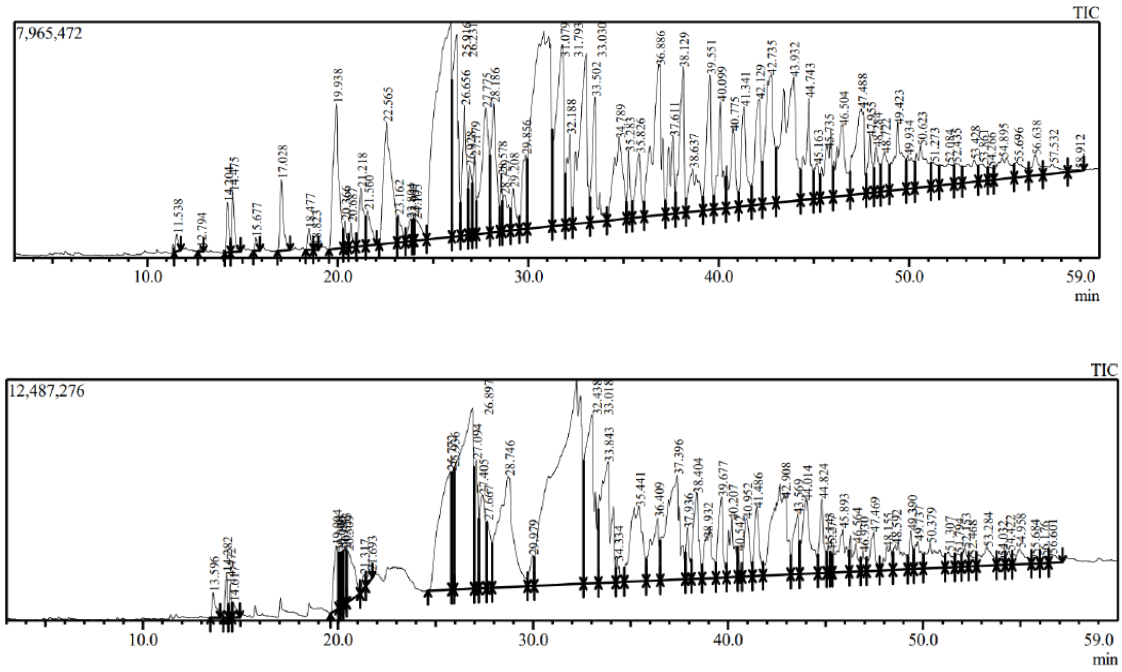


GCMS Raw sample Chromatogram





GCMS Chromatogram of fermented fish sample F1 and F2



1. **Retention time detected in Raw sample**

IUPAC name of fatty acids	ret time	IUPAC name	ret time
4,7,10,13,16,19-Docosahexaenoic acid	42.63	Arachidonic acid	41.244
5,8,11,14,17-Eicosapentaenoic acid	46.148	Heptadecanoic acid	26.822
5,8,11,14-Eicosatetraenoic acid	40.696	Heptadecanoic acid	27.661
6-Hexadecenoic acid	27.221	Heptadecanoic acid	40.35
9,12,15-Octadecatrienoic acid	36.982	Octadecanoic acid	30.604
9,12-Octadecadienoic acid	33.686	Octadecanoic acid	43.259
9,12-Octadecadienoic acid	44.6	PENTANEDIOIC ACD	28.907
9-Hexadecenoic acid	28.102	Tetradecanoic acid	18.48
9-Octadecenoic acid	30.908	Tetradecanoic acid	21.538
9-Octadecenoic acid	34.689	Tridecanoic acid	17.054
9-Octadecenoic acid	43.764	9-Hexadecenoic acid	26.235
9-Octadecenoic acid (Z)-	23.142	9-Octadecenoic acid (Z)	20.367
Arachidonic acid	49.283	7,10,13-Eicosatrienoic acid	36.26
Dodecanoic acid(Lauric acid)	14.246	9,12,15-Octadecatrienoic acid	32.938
Eicosanoic acid	46.773	9,12-Hexadecadienoic acid	27.079
Heptadecanoic acid	26.548	9,12-Octadecadienoic acid	35.707
Heptadecanoic acid	34.39	7,10,13-Eicosatrienoic acid	36.26
Hexadecanoic acid	25.779		
Hexanoic acid	44.282		
n-Hexadecanoic acid	39.431		
Nonadecanoic acid	32.365		
Nonadecanoic acid	38.458		
Pentadecanoic acid	22.541		
Pentadecylic acid	37.523		
Tetradecanoic acid	35.223		
Z-7-Hexadecenoic acid	39.994		
11,14,17-Eicosatrienoic acid	45.767		
11-Hexadecenoic acid	26.129		
6,9,12-Octadecatrienoic acid	32.077		

2. Retention time in F1

IUPAC name of fatty acids	ret time	IUPAC name	ret time
4,7,10,13,16,19-Docosahexaenoic acid	42.568	Eicosanoic acid	34.479
5,8,11,14,17-Eicosapentaenoic acid	41.271	Heptadecanoic acid	26.599
5,8,11,14-Eicosatetraenoic acid	40.741	Heptadecanoic acid	27.649
6,9,12-Octadecatrienoic acid	32.231	Heptadecanoic acid	40.397
6-Hexadecenoic acid	27.288	Hexadecanoic acid	25.4
7,10,13-Eicosatrienoic acid	36.343	n-Hexadecanoic acid	39.457
9,12,15-Octadecatrienoic acid	45.779	Nonadecanoic acid	32.463
9,12-Octadecadienoic acid	33.764	Octadecanoic acid	43.28
9,12-Octadecadienoic acid	31.865	Pentadecanoic acid	21.228
9,12-Octadecadienoic acid (Z,Z)-	44.625	Pentadecanoic acid	22.463
9-Hexadecenoic acid	34.764	Pentadecanoic acid	37.36
9-Octadecenoic acid	42.921	Tetradecanoic acid	18.508
Arachidonic acid	49.306	Tetradecanoic acid	19.868
18-Nonadecenoic acid	40.031		
5,8,11,14-Eicosatetraenoic acid	36.881		
9,12,15-Octadecatrienoic acid	33.051		
9,12,15-Octadecatrienoic acid	37.038		
9,12-Hexadecadienoic acid	27.118		
9,12-Octadecadienoic acid	35.788		
9-Hexadecenoic acid	26.28		
9-Octadecenoic acid	35.301		
Docosanoic acid	38.519		
Dodecanoic acid	14.238		
Heptadecanoic acid	26.883		
Hexadecanoic acid	25.831		
Octadec-9-enoic acid	43.781		
Tridecanoic acid	17.039		

1. Retention time in F2

IUPAC name of fatty acids	ret time	IUPAC name	ret time
11-Eicosenoic acid	35.441	Docosanoic acid	38.932
11-Hexadecenoic acid	27.094	Dodecanoic acid	14.282
4,7,10,13,16,19-Docosahexaenoic acid	41.486	Heptadecanoic acid	27.405
4,7,10,13,16,19-Docosahexaenoic acid	42.908	Hexadecanoic acid	25.772
9,12-Octadecadienoic acid (Z,Z)	33.018	Hexadecanoic acid	25.936
9,12-Octadecadienoic acid (Z,Z)	34.334	Hexadecanoic acid	39.677
9-Octadecenoic acid (Z)-	56.176	Octadecanoic acid	29.979
Z-7-Hexadecenoic acid	40.207	Pentadecanoic acid	21.317
10-Undecenoic acid	14.617	Tetradecanoic acid	20.353
11,13-Eicosadienoic acid	36.409	Tetradecanoic acid	19.904
5,8,11,14,17-Eicosapentaenoic acid	56.601	Tetradecanoic acid	20.197
9,12,15-Octadecatrienoic acid	33.843	Tetradecanoic acid	20.509
9,12,15-Octadecatrienoic acid	45.893	Tetradecanoic acid	21.693
9,12-Hexadecadienoic acid	27.667		
9,12-Octadecadienoic acid (Z,Z)-	44.824		
9-Octadecenoic acid (Z)	20.096		
Arachidonic acid	49.39		
Eicosanoic acid	46.93		
Hexadecanoic acid	26.897		
Nonadecanoic acid	45.143		
Octadecanoic acid	43.569		
Oxalic acid	50.379		

ANNEXURE III

CULTURE MEDIA USED

1. Plate count Agar (PCA) (Himedia)

Ingredients	gm/l
Tryptone	5
Yeast extract	2.5
Dextrose (Glucose)	1
Agar	15
Distilled water	1000
Final pH (at 25°C)	7±0.2

2. PCA with 10% NaCl (Himedia)

Ingredients	gm/l
Tryptone	5
Yeast extract	2.5
Dextrose (Glucose)	1
Agar	15
NaCl	100
Final pH	7
Distilled water	1000

3. Nutrient Agar media supplemented with 10% and 1% Casein Hydrolysate (Tanasupawat et al., 1992)

Ingredients	gm/l
Peptone	5
Beef Extract	3
NaCl	105
Agar	15
Casien hydrolysate	10
Final pH	
Distilled water	1000

4. De Megan, Rogosa and Sharpe (MRS) Agar (Himedia)

Ingredients	gm/l
Proteose peptone	10
HM Peptone B	10
Yeast extract	5
Dextrose (Glucose)	20
Tween 80 (Polysorbate 80)	1
Ammonium citrate	2
Sodium acetate	5
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium hydrogen phosphate	2
Calcium carbonate	20
Agar	12
Final pH (at 25°C)	6.5±0.2
Distilled water	1000

5**. PDA (Himedia)**

Ingredients	gm/l
Potatoes	200
Dextrose	200
Agar	15
Final pH (at 25°C)	5.6±0.2
Distilled water	1000

6. M17 (Himedia)

Ingredients	gm/l
Peptone	5
Soya peptone	5
Yeast extract	2.5
HM peptone B #	5
Ascorbic acid	0.5
Magnesium sulphate	0.25
Lactose	5
Agar	10
Final pH (at 25°C)	7.1±0.2
Distilled water	1000

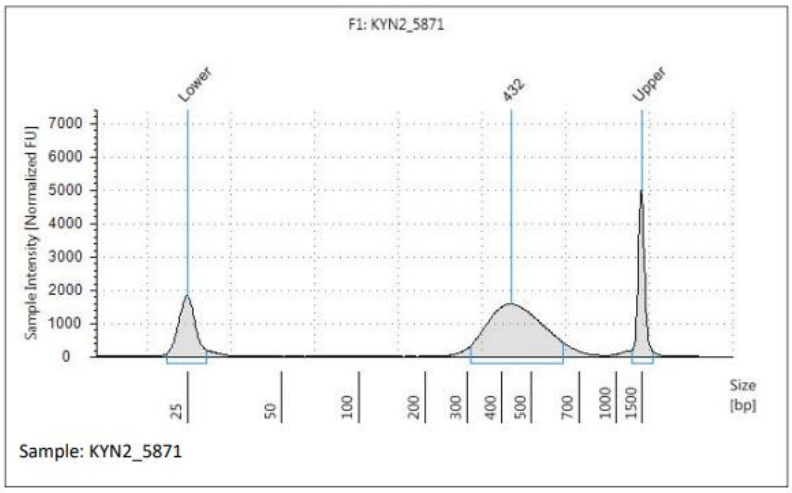
7. EMB Agar (Himedia)

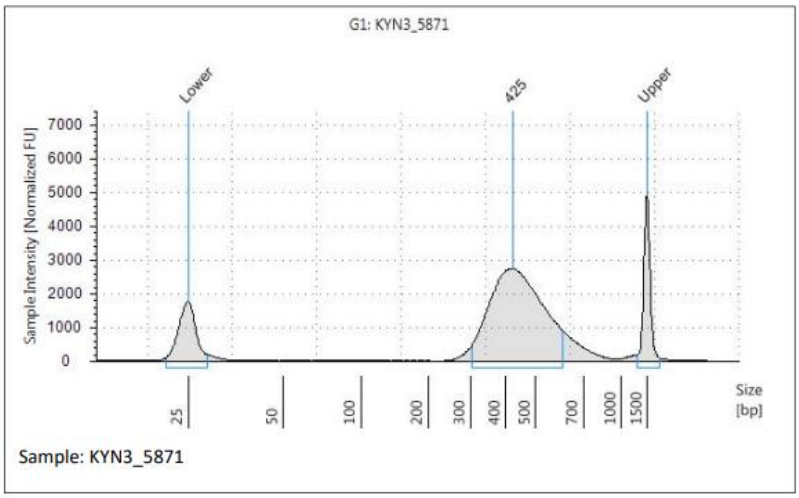
Ingredients	gm/l
Ingredients Gms / Litre	10.000
Peptone	
Dipotassium hydrogen phosphate	2.000
Lactose	5.000
Saccharose (Sucrose)	5.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	7.2±0.2
Distilled water	1000

ANNEXURE IV

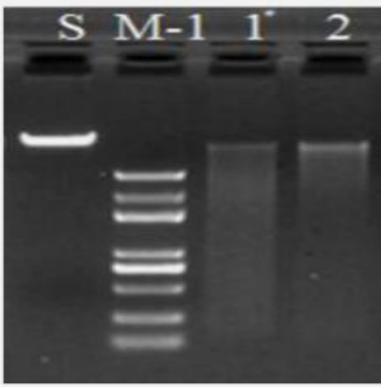
1. Whole genome metagenomics: QC report

TAPESTATION PROFILE OF LIBRARY

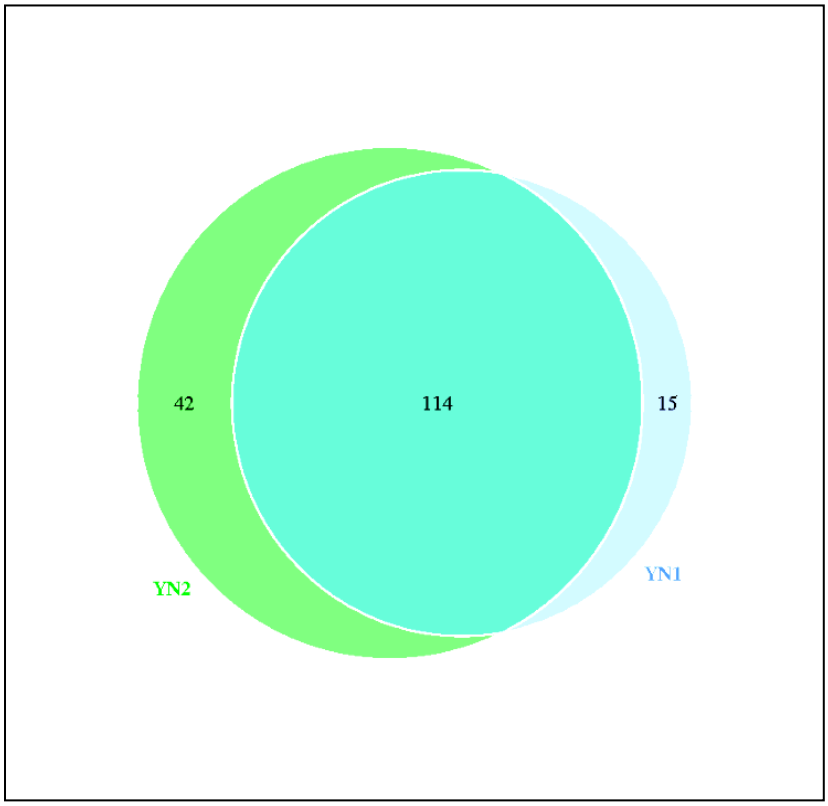




1. Gel electrophoresis of samples S3 and S4

A. QC - Gel Electrophoresis (for DNA/RNA) / RIN Profile (for RNA)
 **DNA QC(1 – S3, 2 – S4, S-Standard, M1 – Trans 15K plus)**
Figure: 0.8 % Agarose gel image

2. **Venn diagram generated from OTU of two samples S3 and S4**



- **Values in overlapping parts represent common OTUs. The others are specific OTUs in each sample.**

3. **NCBI Accession No. of WGS metagenomic sequence**

   PRJNA689966

   SAMN17224118 YN3 (Tax ID: 496924)

   SAMN17224117 YN2 (Tax ID: 496924)

4. **NCBI Accession No. of 16SrRNA metagenomic sequence**

   PRJNA715595

   SAMN20088403:16SrRNA metagenome of Napham (TaxID: 870726)

ANNEXURE VI

1. Plant identification report from BSI, Shillong, Meghalaya

भारत सरकार/GOVERNMENT OF INDIA
पर्यावरण वन एवंजलवायु परिवर्तन मंत्रालय/MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण/BOTANICAL SURVEY OF INDIA
प्रभारी वैज्ञानिक का कार्यालय/OFFICE OF THE SCIENTIST IN-CHARGE
पूर्वी क्षेत्रीय केंद्र/EASTERN REGIONAL CENTRE
शिलोंग-793003/SHILLONG - 793003

दूरभाष/Telephone: 0364- 2223971, 2223618 ई-मेल/e-mail- bsibsishll@yahoo.co.in Telefax: 0364- 2224119

संख्या/No.: BSI/ERC/Tech/2020-21/ 148 दिनाक/Dated: 16.03.2021

सेवा मे/To,
Ms Yutika Narzary
Dept. of Botany,
Bodoland University
Kokrajhar-783370\
Assam(BTC)
Mobile: 9435296442
Email: yutika.2lu@gmail.com

विषय/Sub.: Accession of plant specimens and incorporation at ASSAM herbarium. reg.

Dear Ms Narzary,

With reference to your letter No. Nil, dated, 12th March, 2021 regarding the subject cited above, I am to inform you that your plant specimens has been incorporated in ASSAM with accession numbers as below:-

1. *Benincasa hispida* (Thunb.) Cogn. (Cucurbitaceae) (Coll.no. - 01, Accession no. 96691)
2. *Colocasia esculenta* (L.) Schott (Araceae) (Coll.no. 02, Accession no. 96690)
3. *Hibiscus sabdariffa* L.(Malvaceae) (Coll.no. 03, Accession no. 96692)

Thanking you / सधन्यवाद

भवदीय/Yours sincerely

16/3/2021

(Dr. N. Odyuo)
वैज्ञानिक- E एवं कार्यालय प्रमुख/ Scientist-E & HoO

2. Fish identification report from ZSI, Shillong, Meghalaya

भारत सरकार
GOVERNMENT OF INDIA

MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE

उत्तर पूर्वी क्षेत्रीय केन्द्र
भारतीय प्राणी सर्वेक्षण
विसा कालोनी, शिलांग
दूरभाष: 0364-2223638
फैक्स: 0364-2226495

zoological Survey of India
north Eastern Regional Centre
ilsa Colony, Shillong-793003
Phone:0364-2223638,
Fax:0364-2226495
E-mail:

No.F. 2-4/2020-21/Tech./ Dated: March, 2021.

To
Smt. Yutika Narzary
Assisistant Professor,
Department of Botany
Bodoland University, Kokrajhar – 783370, BTC, Assam

Subject: Identification Report

Madam,

This has reference to your letter dated 12.03.2021 regarding identification of fishes for research purpose. The fish specimens submitted by you were identified as follows:

Phylum: Chordata
Class: Actinopterygii
Order: Cypriniformes

Sample No.	**Family: Cyprinidae**	*No. of examples*
13	*Amblypharyngodon mola* (Hamilton, 1822)	5
14	*Esomus danrica* (Hamilton, 1822)	4
15	*Danio rerio* (Hamilton, 1822)	Damaged
1	*Puntius chola* (Hamilton, 1822)	1
1	*Puntius sophore* (Hamilton, 1822)	2
1	*Puntius terio* (Hamilton, 1822)	2
	Family: Botiidae	
16	*Botia Dario* (Hamilton, 1822)	1
	Family: Cobitidae	
5	*Lepidocephalichthys guntea* (Hamilton, 1822)	2
	Order: Siluriformes	
	Family: Bagridae	
9	*Mystus bleekeri* (Day, 1877)	2
9	*Mystus cavcio* (Hamilton, 1822)	2

	Order: Synbranchiformes	
	Family: Mastacembelidae	
6	*Macrognathus pancalus* (Hamilton, 1822)	3
	Order: Perciformes	
	Family: Ambassidae	
11	*Parambassis ranga* (Hamilton, 1822)	4
11	*Parambassis lala* (Hamilton, 1822)	1
12	*Parambassis lala* (Hamilton, 1822)	6
	Family: Nandidae	
7	*Nandus nandus* (Hamilton, 1822)	2
	Family: Badidae	
3	*Badis badis* (Hamilton, 1822)	7
	Family: Gobiidae	
2	*Glossogobius giuris* (Hamilton, 1822)	4
	Family: Osphronemidae	
4	*Trichogaster fasciata* Bloch & Schneider, 1801	2
4	*Trichogaster lalius* (Hamilton, 1822)	1
	Family: Channidae	
8	*Channa gachua* (Hamilton, 1822)	3
	Order: Tetraodontiformes	
	Family: Tetraodontidae	
17	*Leiodon cutcutia* (Hamilton, 1822)	3

Thanking you,

Yours faithfully

(Dr. D. Khyriam)

Forwarded:

Officer-in-Charge

Note: The fish specimens submitted could not be registered due to lack of location details.

ANNEXURE VII

PHOTO PLATE I
Method of preparation on *napham*

Fish cleaned and sun dried for *napham*

Dried and smoked fish and *Colocassia esculanta*

Pounding of raw material of *napham*

Inserted inside the bottle

Woman preparing *napham* in bamboo stem

Small catchment area for fish used by *napham* vendors

PHOTO PLATE II

Documentation of *napham* with villagers and in market place

Vendors whose livelihood is based on fish and fish related products like *napham*

Napham sold in market, each pouch costs Rs.10/-and bottles Rs100/- to Rs.150/bottle

Dry fish and fermented fish products sold in Kokrajhar Market

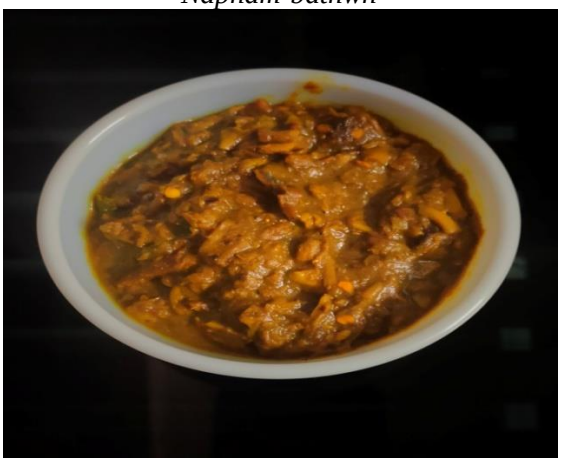
Napham sold in bottles (cost: Rs.100/--Rs.150/- bottle)

PHOTO PLATE II

Traditional cuisines prepared from *napham*

Napham-bathwn

Napham thaso-athing

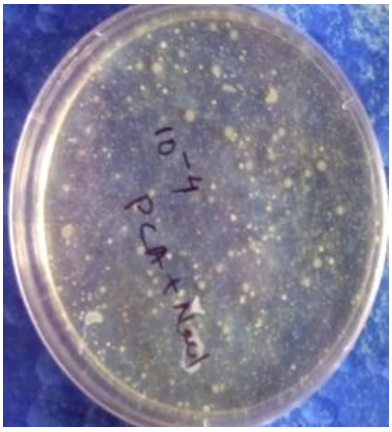


Napham-thasobibar

Napham-bidwi

PHOTO PLATE III

Plate Count Agar

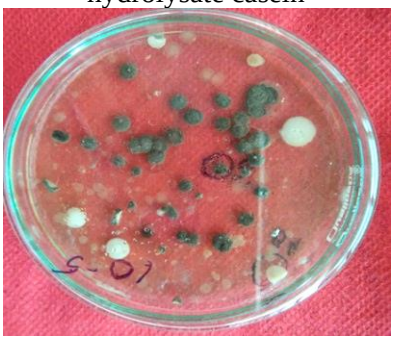


PCA with 10% NaCl

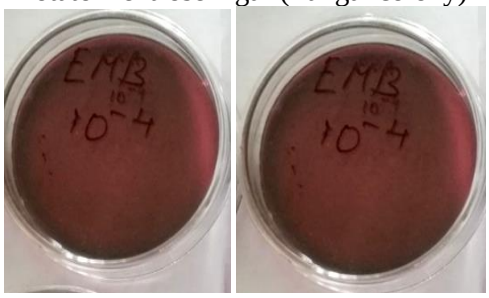
M17 Agar

Nutrient Agar supplemented with hydrolysate casein

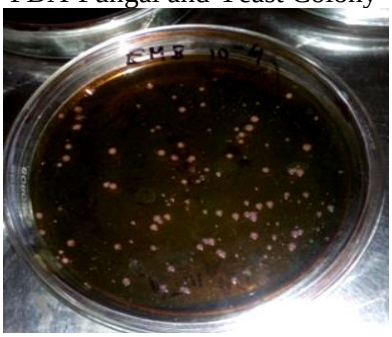
Potato Dextrose Agar (Fungal colony)



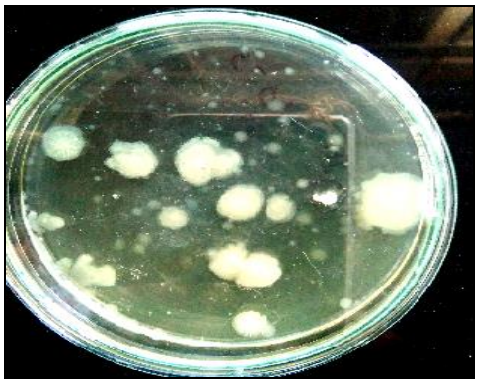
PDA-Fungal and Yeast Colony



No bacterial growth in EMB Agar on 6th and 12th month

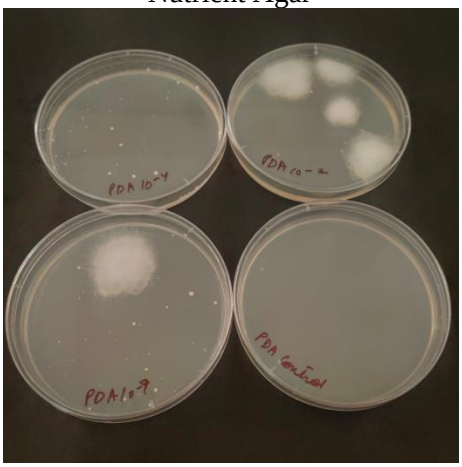


EMB-Agar

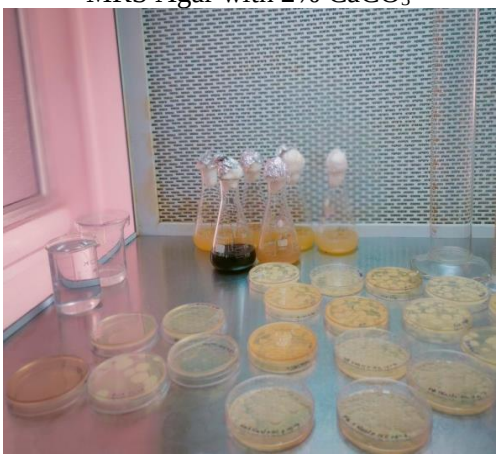


Nutrient Agar

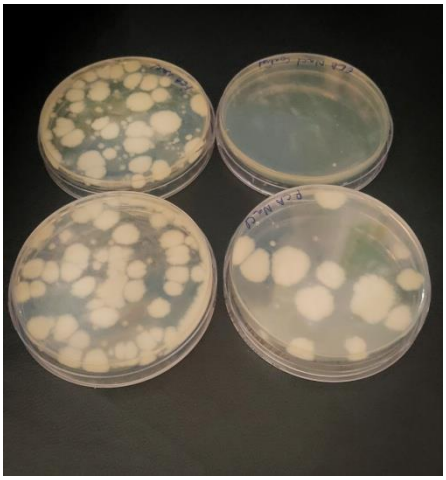
MRS Agar with 2% $CaCO_3$



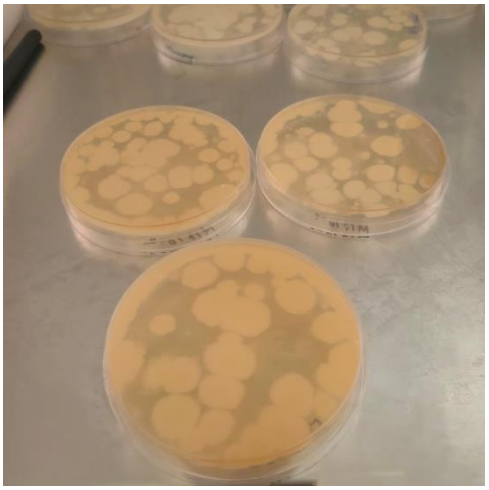
Fungi and yeast in PDA at different dilution for cfu count



Isolation of bacteria and fungi in different media for cfu count



Bacterial colonies in plate count agar supplemented with NaCl



Bacterial colonies in M17 agar

PHOTO PLATE IV

Channa gachua (Hamilton, 1822), local name-Na gwri

Mystus carcio (Hamilton, 1822), Local name-na thengwna

Lepidocephalichthys guntea (Hamilton, 1822), Local name-na balabathia

Glossogobius giuris (Hamilton, 1822), Local name-na mutura

Amblypharyngodon mola (Hamilton, 1822), Local name-na maowa

Trichogaster spp. Local name-Na bingsi

Trichogaster spp. Local name-Na bingsi

Parambassis spp.
Local name-Na chandanga

Macrognathus pancalus (Hamilton, 1822), local name- Na thuri

Puntius spp. Local name-Na phitikri

Nandus nandus (Hamilton, 1822), Local name-Na thotha

Badis badis (Hamilton, 1822), Local name-Na duthumwi

Esomus danrica (Hamilton, 1822), Local name-borali

Glossogobius giuris (Hamilton, 1822), Local name-na mutura

ABREBIATION

ABI	Application Binary Interface
ACE	Angiotensin-converting enzyme
ACE	Abundance-based coverage esGmators
AOAC	Association of Official Analytical Collaboration
BA	Biogenic Amine
BLAST	Basic Local Alignment Search Tool
BLAST	Basic Local Alignment Search Tool
BSI	Botanical Survey of India
CFU	Colony forming Unit
CTAB	Cetyltrimethylammonium Bromide
DHA	Docosahexaenoic Acid
DNA	Deoxynucleic Acid
DNA QC	Deoxyribonucleic acid *quality control*
DPP-IV	Dipeptidyl peptidase- IV
EMB	*Eosin methylene blue*
EPA	Eicosapentaenoic acid
EPS	Exopolysaccharide
ERC	Eastern Regional Center
FDA	Food and Drug Administration
FLASH	Fast Length Adjustment of SHort reads
GCMS	Gas chromatography mass spectrometry
GF ASS	Graphite Furnace Atomic Absorption Spectroscopy
GRAS	Generally considered as safe
HPLC	High performance liquid Chromatography
IPP	Isoleucyl-prolyl-proline
ITS	Iterspecific region
LAB	Lactic Acid Bacteria
MEGA6	Molecular Evolutionary Genetics Analysis
MGA	Meta gene Annotation
MRS	De Man, Rogosa and Sharpe agar

MUSCLE	MUltiple Sequence Comparison by Log-Expectation
NCBI	National Center for Biotechnology Information
NEHU	North-Eastern Hill University
NERC	Nort-Eastern Regional Center
NGS	Next Generation Sequencing
ORF	Open Reading Frame
OTU	Operational Taxonomic Unit
PCA	Plate Count Agar
PCR	Polymerase Chain Reactio
PDA	Potato Dextrose Agar
PD_whole tree	Phylogenetic diversity_whole tree
PE	Paired End
PERL	Practical Extraction And Report Language
PITC	Phenylisothiocyanate
QUIME	Quantitative Insights Into Microbial Ecology
RDP	Remote Desktop Protocol
rRNA	Ribosomal Ribonucleic Acid
SAIF	Sophisticated Analytical Instrument Facility
SDS	Sodium Dodecyl Sulfate
SSU	Small Ribosomal Subunit
T2D	Type 2 Diabetis
TEM	Transmission Electron Microscope
VPP	Valyl Polyproline
WGS	Whole genome sequencing