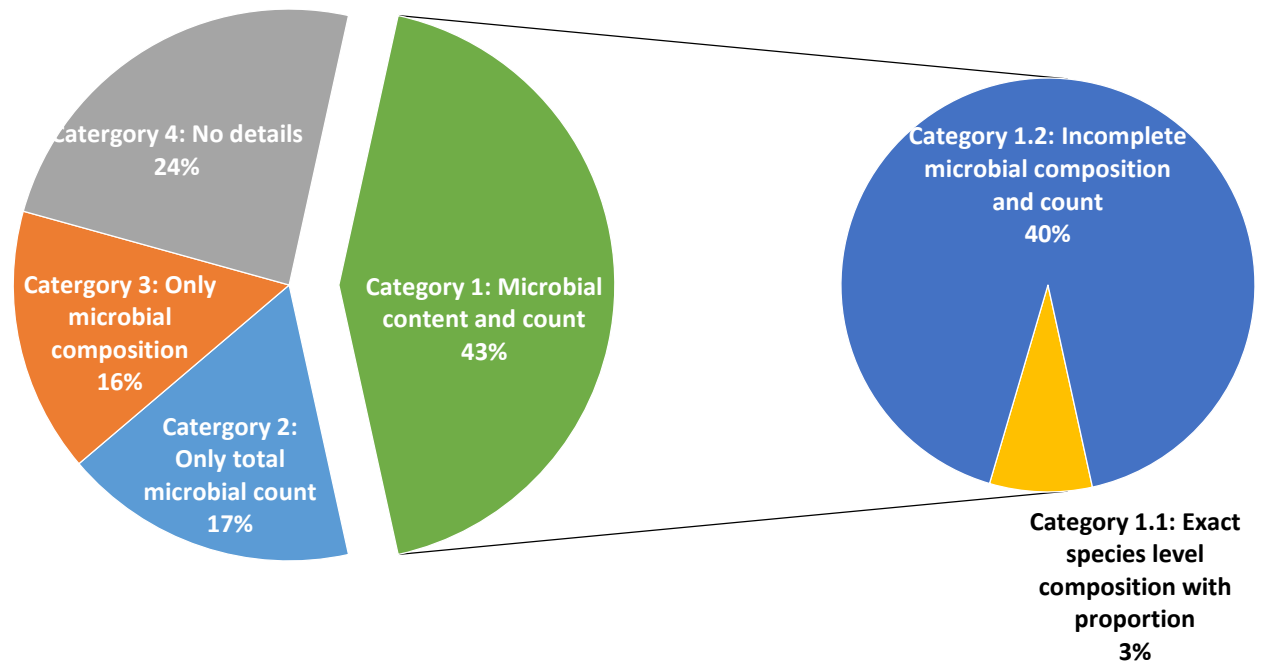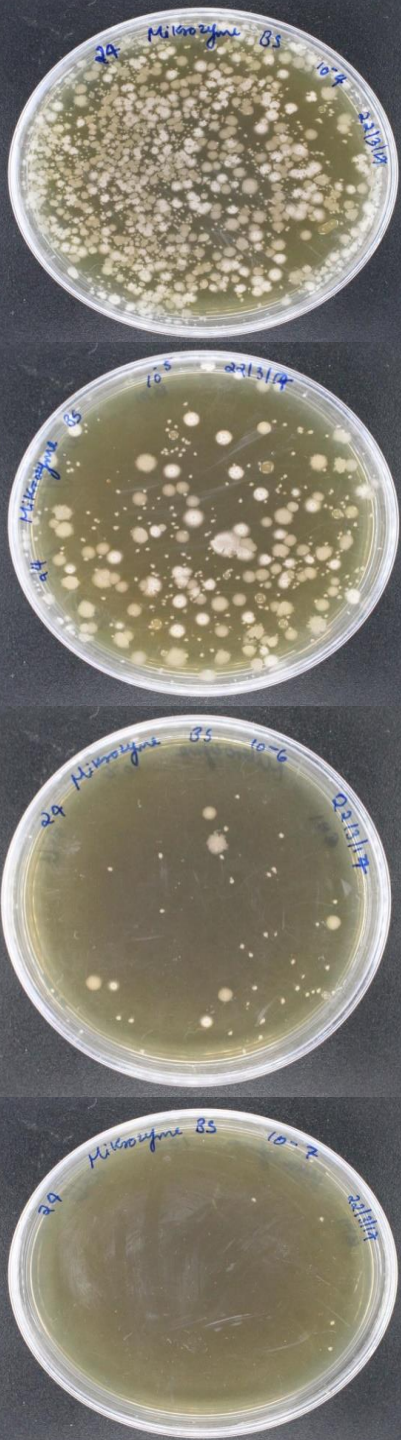# Evaluating Costs and Benefits of Prophylactic Health Products and Novel Alternatives on Smallholder Aquaculture Farmers in Asia and Africa (IMAQulate)

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ICAR-CIFT, Cochin

Microbial Analysis of Commercial Aquaculture Probiotics Procured from India

Categorization of Probiotics Based on Label Information of Microbial Content

Catergory 4: No details
24%

Catergory 3: Only microbial composition
16%

Catergory 2: Only total microbial count
17%

Category 1: Microbial content and count
43%

Category 1.2: Incomplete microbial composition and count
40%

Category 1.1: Exact species level composition with proportion
3%

Panel 1 (top left)

Composition :

Strains : Safe and registered in National collections, Paris, France.
Bacillus subtilis Rosell-179 & *Pediococcus acidilactici* MA18/5M

Content: Live Microbial Culture.

Product Specification

Contains minimum of 5×10^8 (Billion) cfu/g of aerobic and facultative anaerobic bacteria

In 1 kg. containing with

Bacillus subtilis 10^8 cfu

Composition :
Bacillus subtilis
Bacillus megatherium
Bacillus lecheniformis
BacilusPolymixa
Bacillus firmus
Bacillus mesentricus
Clostrridiumbutyricum
Cellulomonascartae
Pedicoccus
Aspergillusoryzae
Thiobacillusdenitrficans
Perococcusdenitrificans

Total concentration :
NLT 12×10^9 cfu/gm

Panel 2 (top right)

Uses :
- Strains are adaptable to different pH and Salinity conditions.
- Removes organic load of pond bottom and reduces the pollution of the pond.
- Reduces ammonia levels.
- Inhibits vibrio & other pathogenic bacteria and reduces bacterial toxins in the pond.
- Ensures healthy environment for the Prawn, Fish & Shrimp by reducing BOD and increasing DO levels.
- Improves water quality &reduces the frequency of water exchange.
- Reduces the mortality rate of Prawn, Fish & Shrimp.
- Improves growth and health performance.
- Enhances survival and immune system.
- Reduces pond bottom sludge

Dosage : 500 gms per acre.
**Administration : Mix required quantity of product in 100 liters of water and spray in the pond.**

Panel 3 (bottom left)

Objectives:
Effectively controls NH'3 and H2S
Digests cellulose, pectin and poly peptides.
Effectively controls COD.
Maintains stable Phyto and Zoo plankton
Reduces the use of Santizers.
Controls pathogenic bacteria.
Maintains overall health of the fish.

Dosage :
1 kg / acre once in every month 1 kg / ton of feed
Usage:
Soak the probiotic in fresh water overnight and apply evenly in the entire pond during sunny time.

Panel 4 (bottom right)

| Dosage :	Below 15 PCS/M2	Above 15 PCS/M2
0-60 days	500 gm/Hectare/week	750 gm/Hectare/week
60-120 days	750 gm/Hectare/week	1 kg/Hectare/week

- Keep the pond clean by digesting organic matter and reduce H_2S, NH3, and other toxic materials.
- It compete in growth with vibrio and brining down vibrio population due to competitive exclusion.
- Decomposes organic sludge (including excess feed, dead plankton, excreta).
- Removes organic matter load through a continuous mineralisation process in a systemic way and release nutrients in to pond enviornment.
- Prevents and treats large, benthic, filamentous algae.
- Promotes growth rate and production.
- Prevents and treats over blooming of algae.
- Prevents pond bottom from deterioration.
- Oxidize ammonia to nitrite and from nitrite to nitrate.
- Reduce BOD and COD.
- Reduces occurrence of disease.

**Consult your aquaculture specialist for specific dosage.**

Mislabelling-Microbial Nomenclature

✖ Bacillus Lichenformis, B. Pumilis

✖ Lactobacillus Sporogenes

✖ *Saccharomy cescerevisiae* – 5g/500g

✖ P. Pantotrophus, P. Acidilactici

✖ Photo synthetic bacteria, Methanogenic bacteria,
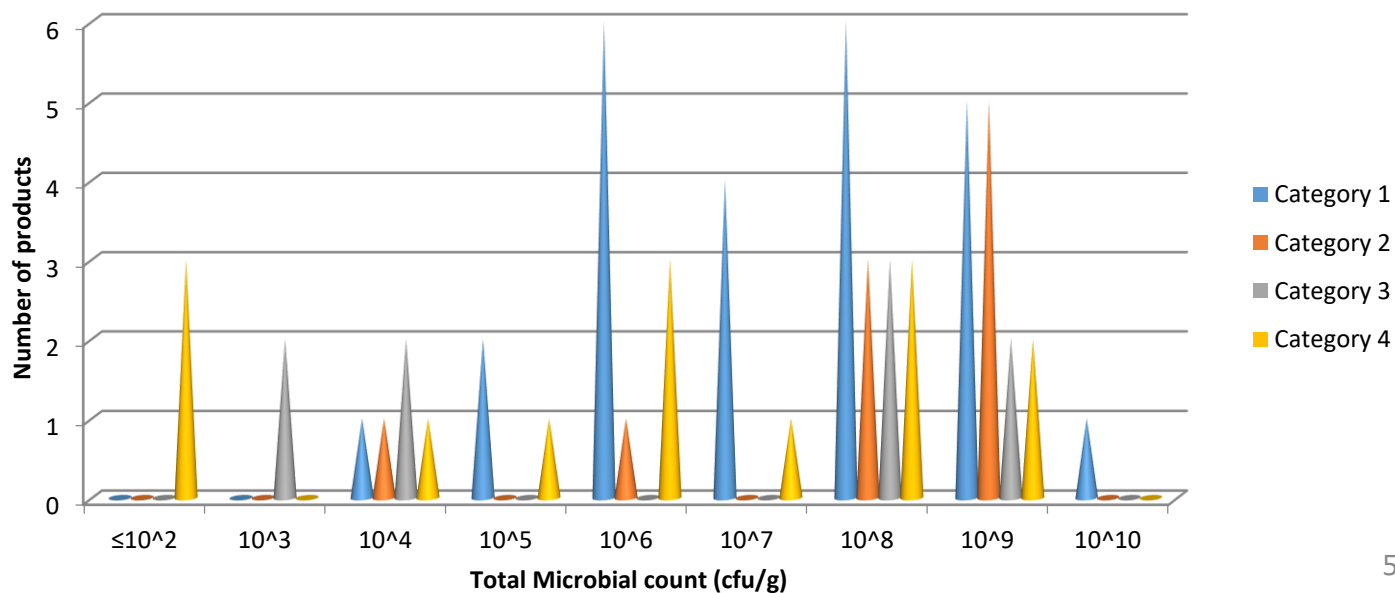
Sulphur utilising bacteria, Sulphur utilising yeast
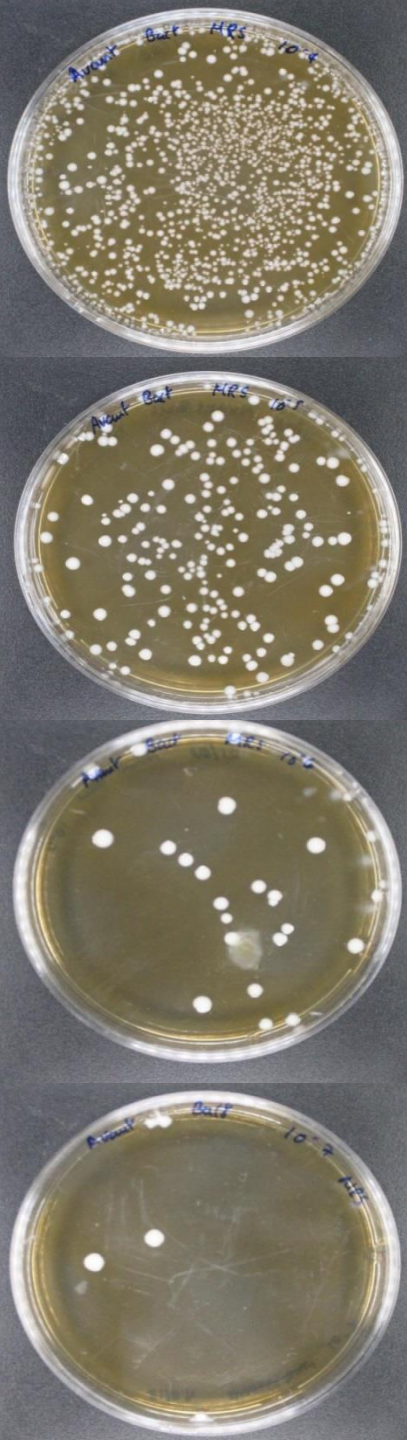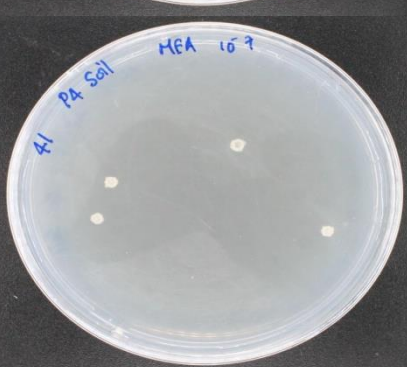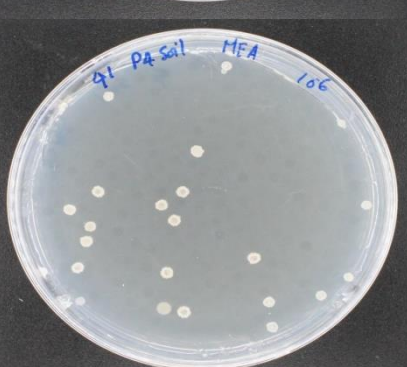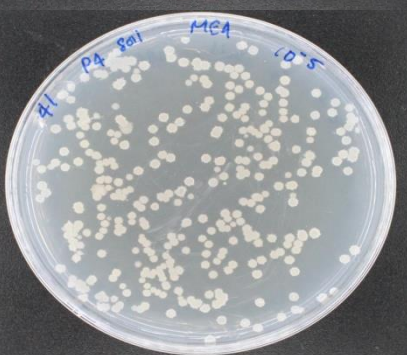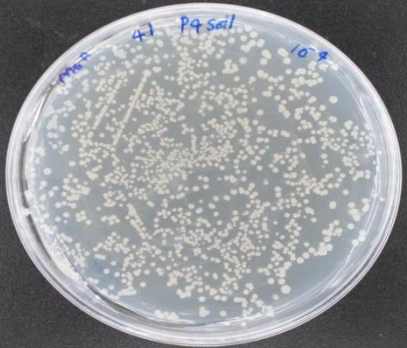
Percentage of probiotics that met claim on microbial load
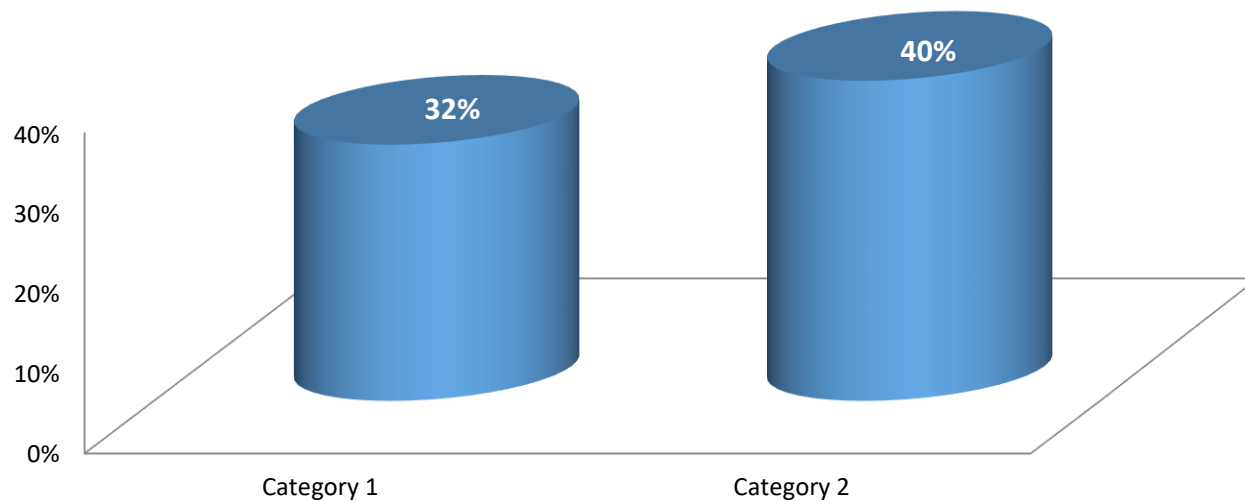
Total microbial load estimated

Microbial Qualitative Analysis of Selected Probiotics by Traditional Microbiology

Product code	Label Claim		Batch	Result of Microbiological Analysis		
	Microbial Composition	Concentration (CFU/g)		Number of isolates screened	Microorganisms present	Concentration (CFU/g)
13	*Bacillus subtilis* Rosell-179	Not Mentioned	1	25	*B. subtilis*	4.5×10^8
	Pediococuss acidilactici MA18/5M		1	35	Could not revive stored culture	
40	*B. subtilis*	1×10^5	1	43	*B. subtilis*	1.95×10^6
46	*B. subtilis*	7×10^8	1	34	*B. subtilis*	3.4×10^8
	B.amyloliquefaciens	2×10^8				
	B.licheniformis	5×10^8				
	B.megaterium	5×10^8				
	B.pumilus	5×10^8				
	B.polymyxa	5×10^8	2	30	*B. subtilis*	2.72×10^8
	Alkaligenes faecalis	1×10^9				
	Saccharomyces cerevisiae	1×10^8				
	Nitrobacter	2×10^4				
	Nitrosomonas	2×10^4				

Major Findings

Predominant microorganisms claimed on label are *Bacillus* spp., yeast, nitrifying bacteria, *Thiobacillus* spp., *Lactobacillus* spp., *Aspargillus* spp., *Pediococcus* spp.

Could not isolate nitrifying, denitrifying bacteria or *Thiobacillus* spp. from any of the 21% products having the claim for these organisms

26% of the probiotic products had counts lower than 10^9 cfu/g

Product code 46 claimed 10 different organisms, but only one strain was present

Estimation of Antimicrobial Residue in Probiotics

❑ Detected presence of antimicrobial compound in two products (code: 50 & 51)

❑ Both products are from same manufacturer and claimed to contain *Lactobacillus* and yeast; but found to have only yeast

❑ One of the product is a registered antibiotic-free aquaculture input under Coastal Aquaculture Authority
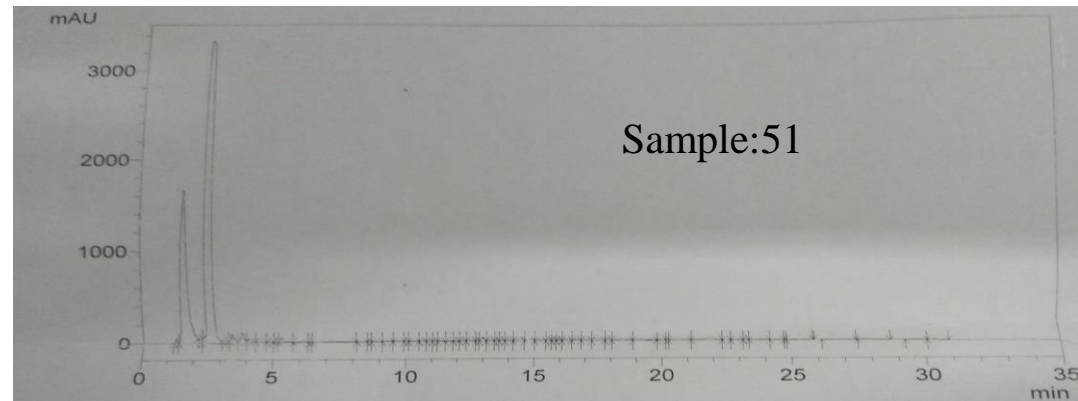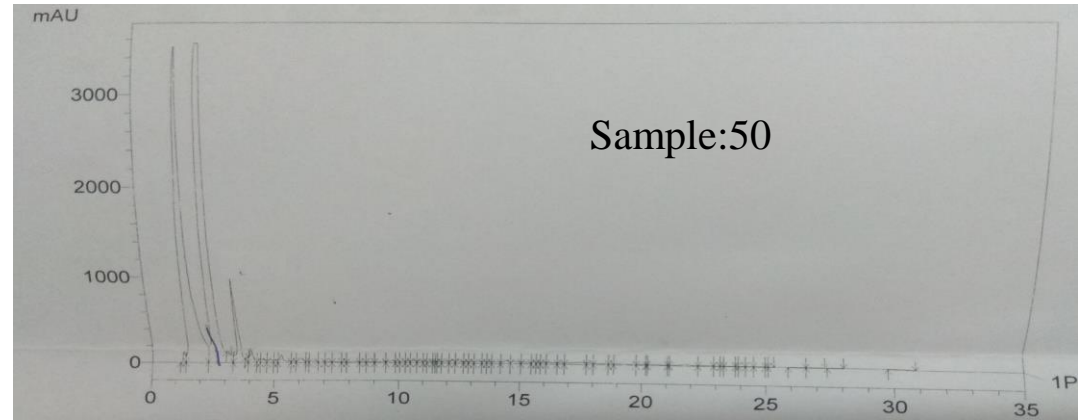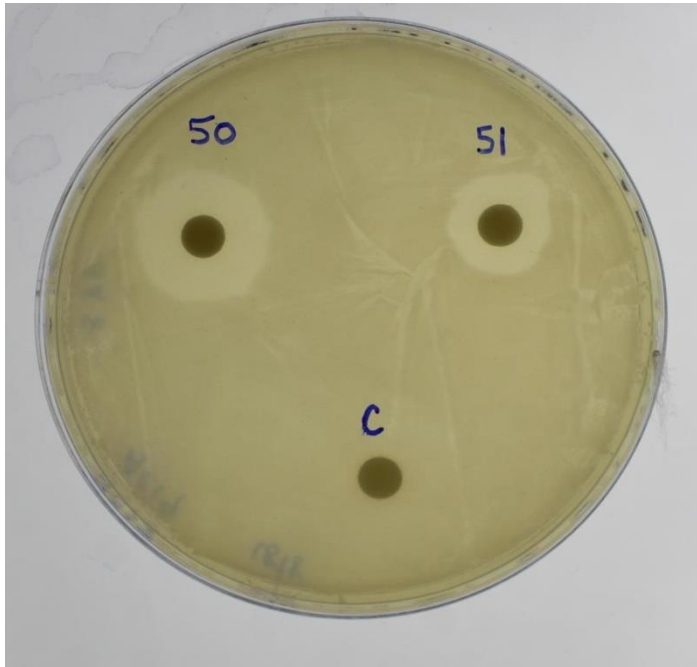
Antimicrobial activity of product extracts against *Bacillus subtilis* at pH 8

Sample:50

Sample:51

HPLC chromatograms of antimicrobial residue extracts from products 50 and 51

Microbiome Analysis of Commercial Probiotics

- **16S ribosomal RNA Amplicon Sequencing** performed using Illumina MiSeq platform for product with code number 13

Metagenomics workflow

446988 raw reads

ClustalO

282009 Consensus Reads

UCHIME

253510 Pre-processed Reads

Uclust

1655 Total OTUs after Singleton removal
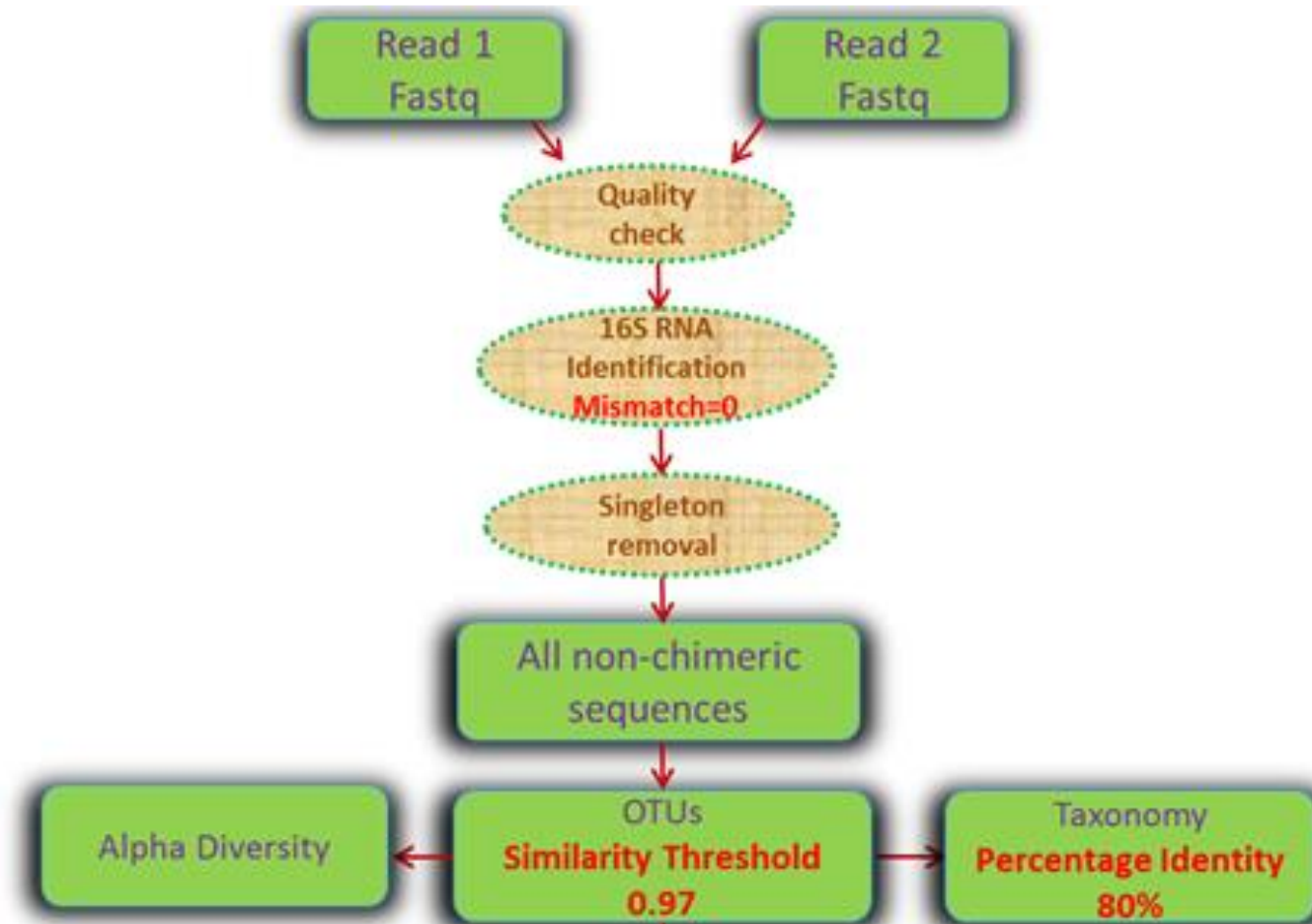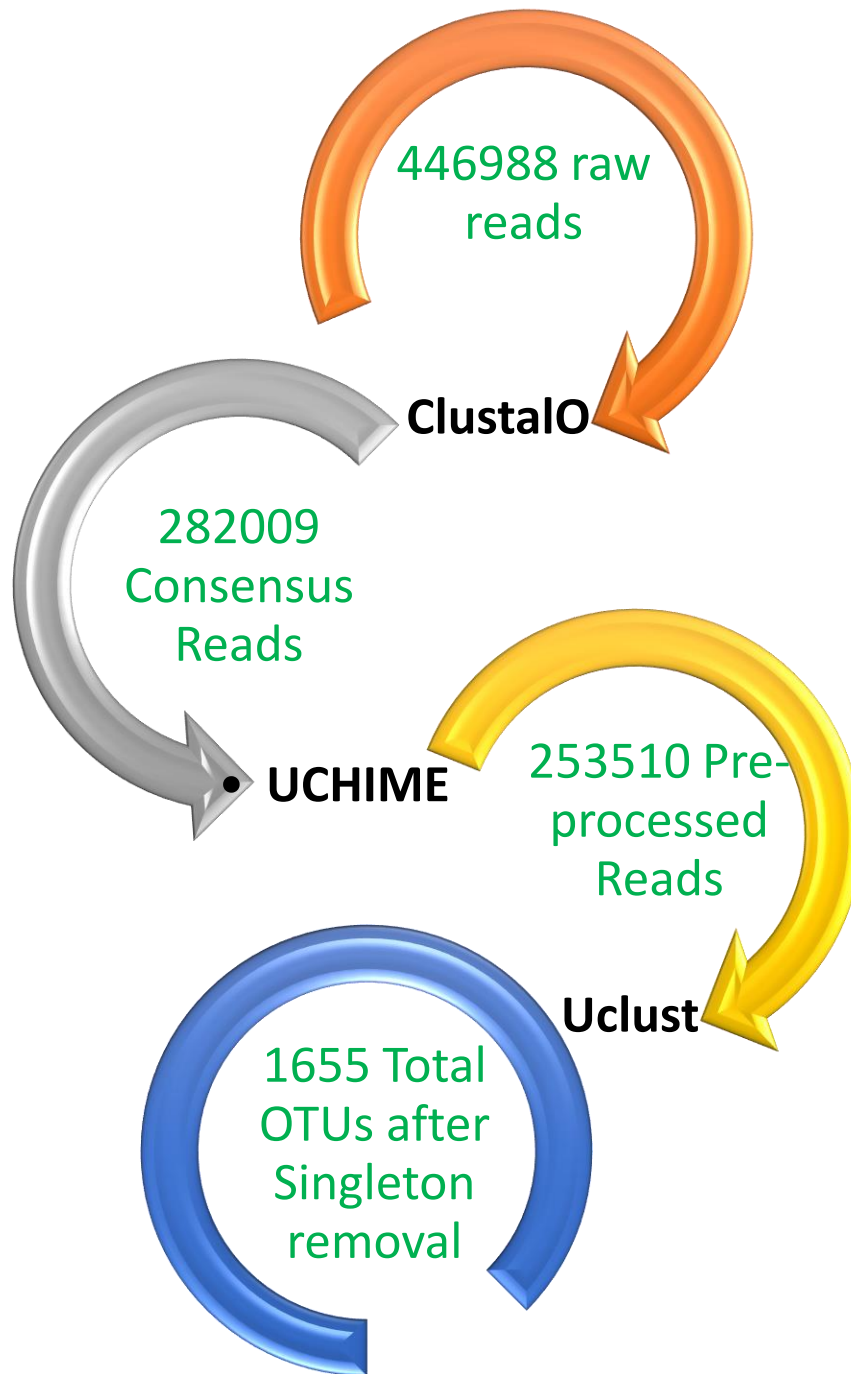
Species wise Distribution of OTUs
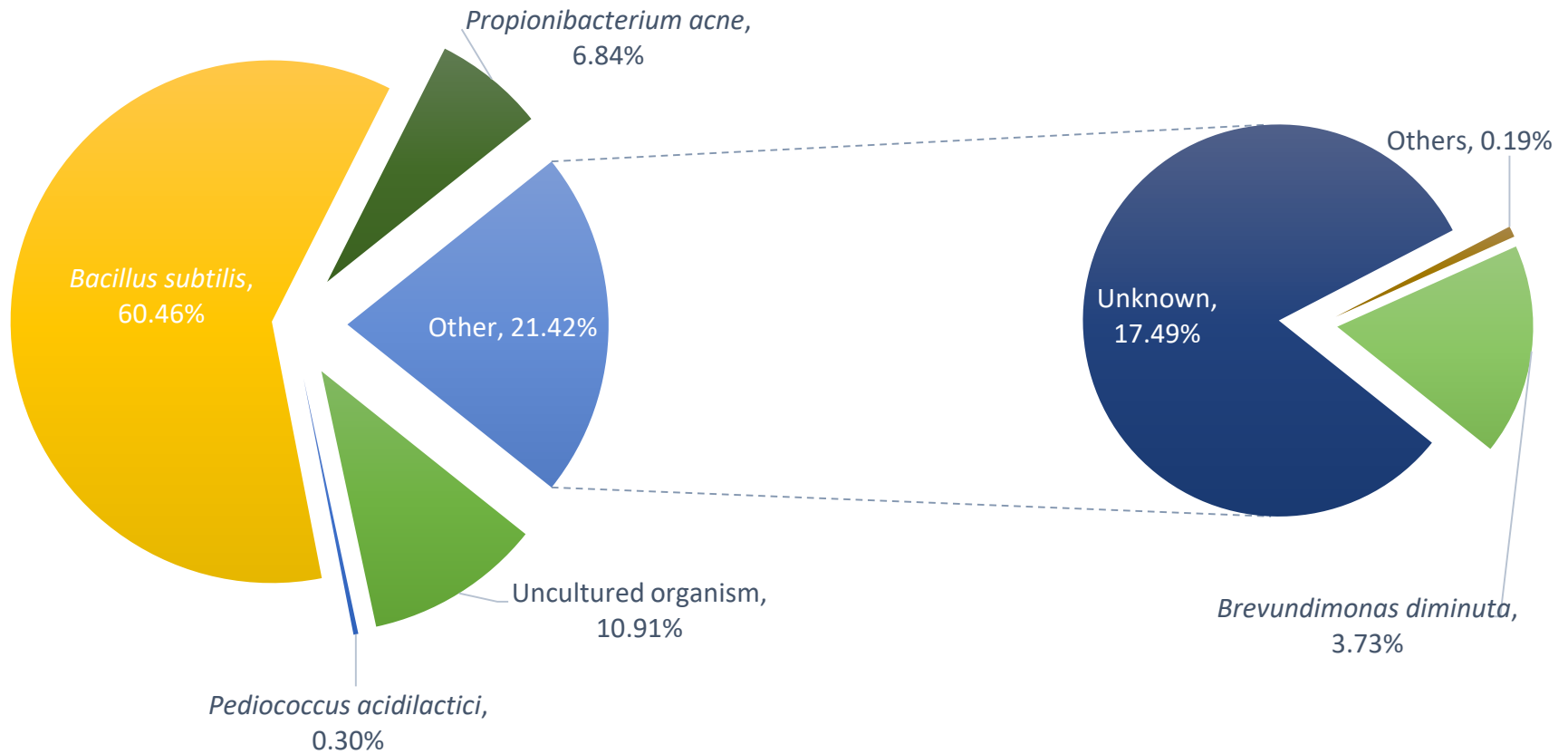
Genus level taxonomic classification

Pediococcus   56%

Lactobacillaceae

7% Lactobacillus

Lactobacillales - 1 result

Bacilli - 1 result

Firmicutes - 1 result

37% Streptococcus

Total: 1463

0.9% of
Bacilli

0.7% of
Firmicutes

0.5% of
Bacteria

0.5% of
all
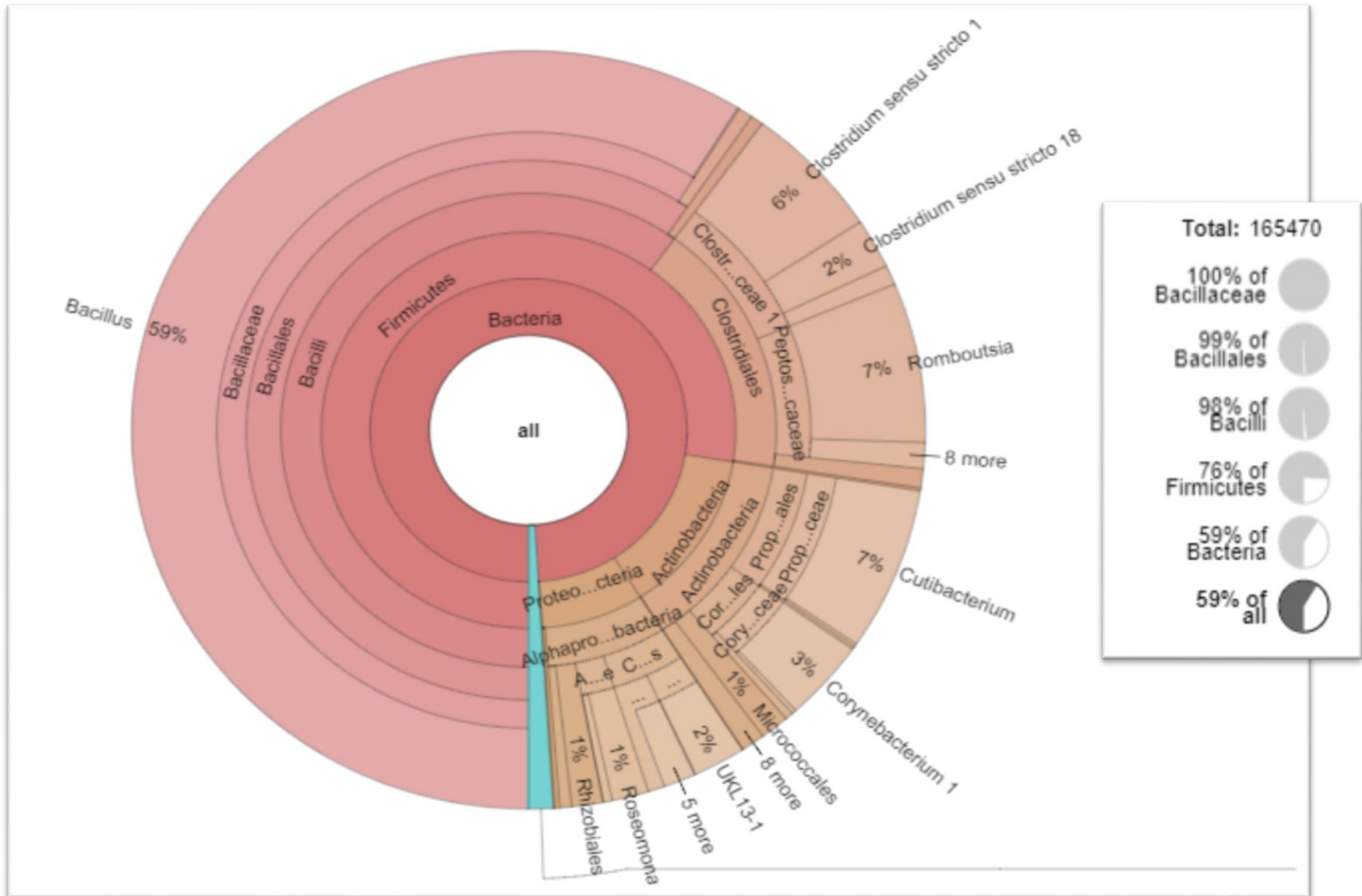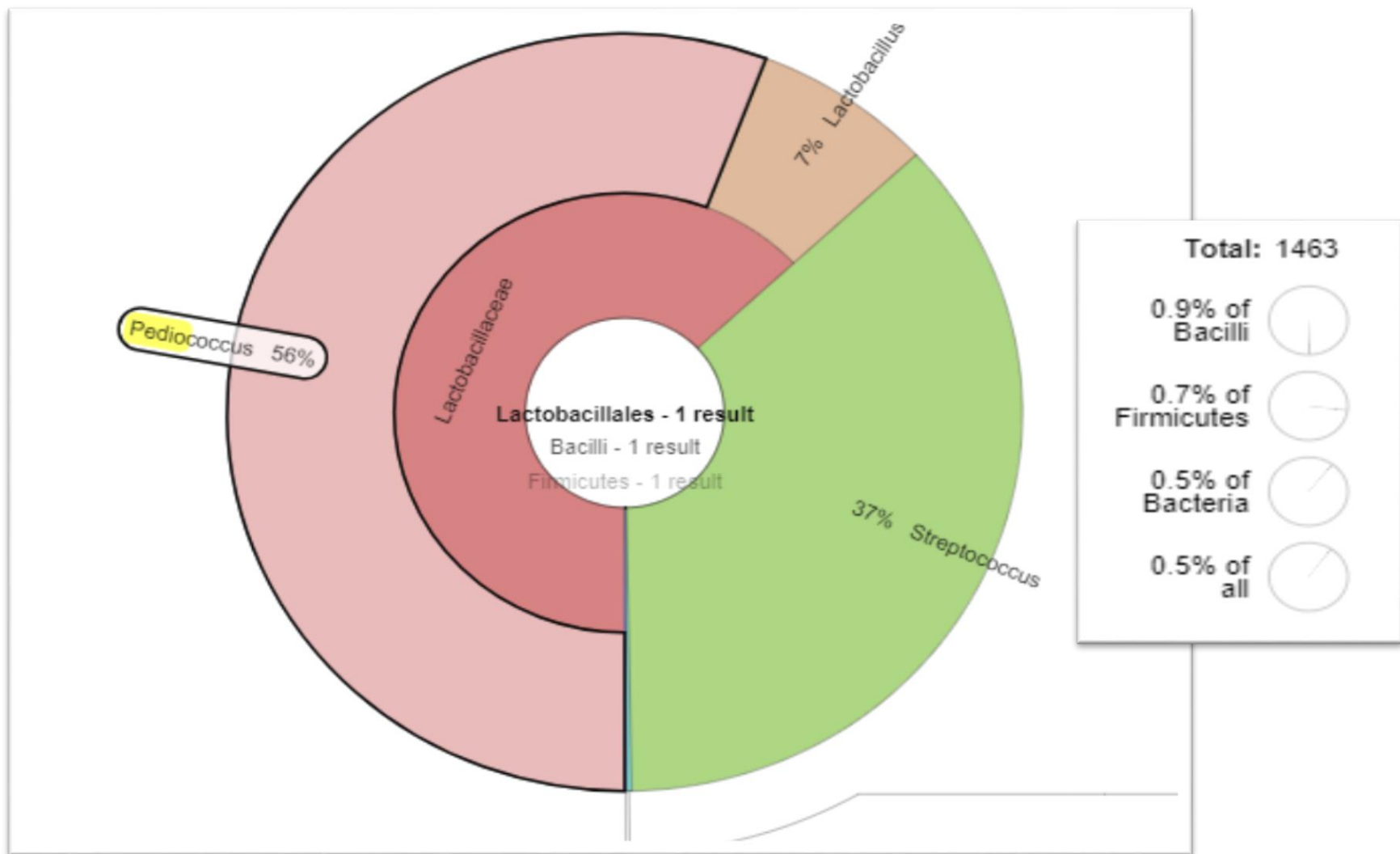
Expected Publications

1. Qualitative and quantitative evaluation of commercial probiotic products used for shrimp farming in Andhra Pradesh, India

2. Microbial evaluation of commercial probiotics by Next Generation Sequencing

3. AMR of bacteria from commercial aquaculture probiotics, as revealed by phenotypic and genotypic methods

4. Microbiome profiling of commercial probiotics treated shrimp aquaculture farms by Next Generation Sequencing

5. Immune parameters of shrimp fed with commercial probiotics

Sl. No.	Title	Authors	Dissemination activity & event	Dates	Location
1	Microbial Assessment of Aquaculture Probiotics Sold in the Indian Market	Lakshmi T. R, Bibindas K. S., B. Madhusudana Rao, Toms C. Joseph*	11th Indian Fisheries and Aquaculture Forum (IFAF)	21st to 24th November, 2017	Cochin, Kerala, India
2	Probiotic Use in *Litopenaeus vannamei* Farming in Andhra Pradesh	Chandra Rao P., Toms C. Joseph*, Madhusudana Rao B., Kenton Morgan, Francis Murray			

Thank you!!!