

IMAQulate
Newton Fund Aquaculture GRP



Department of
BioTechnology,
Government
of India

सत्यमेव जयते



Department
for International
Development

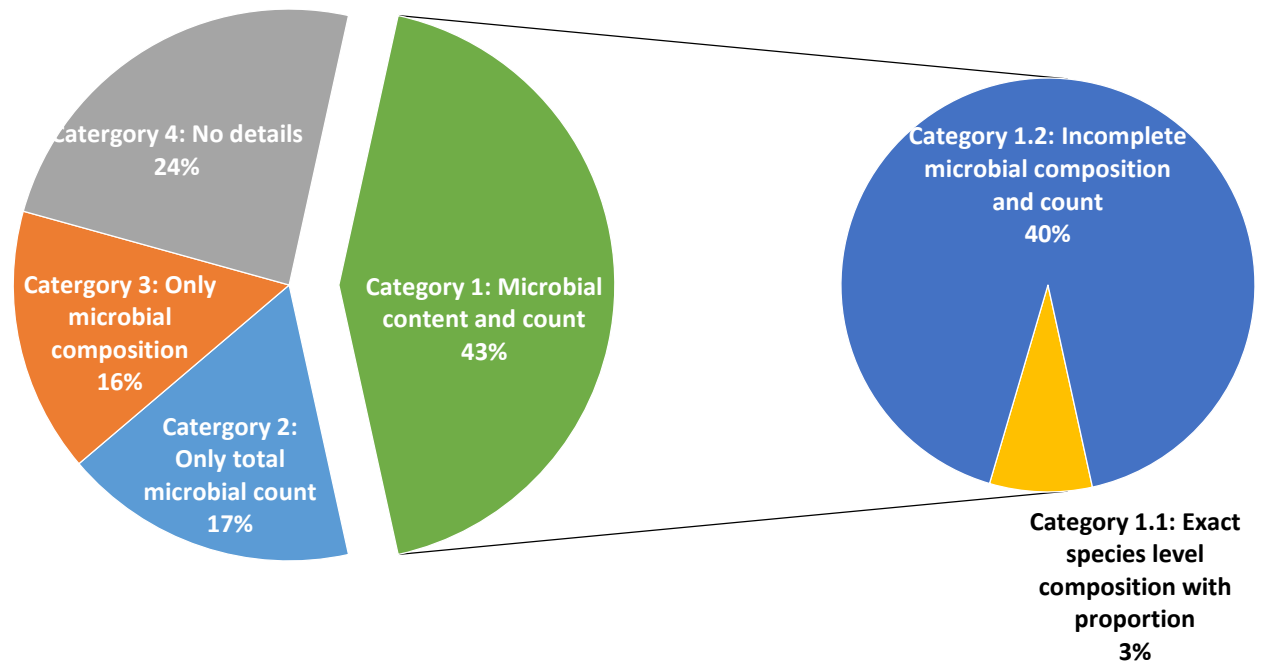
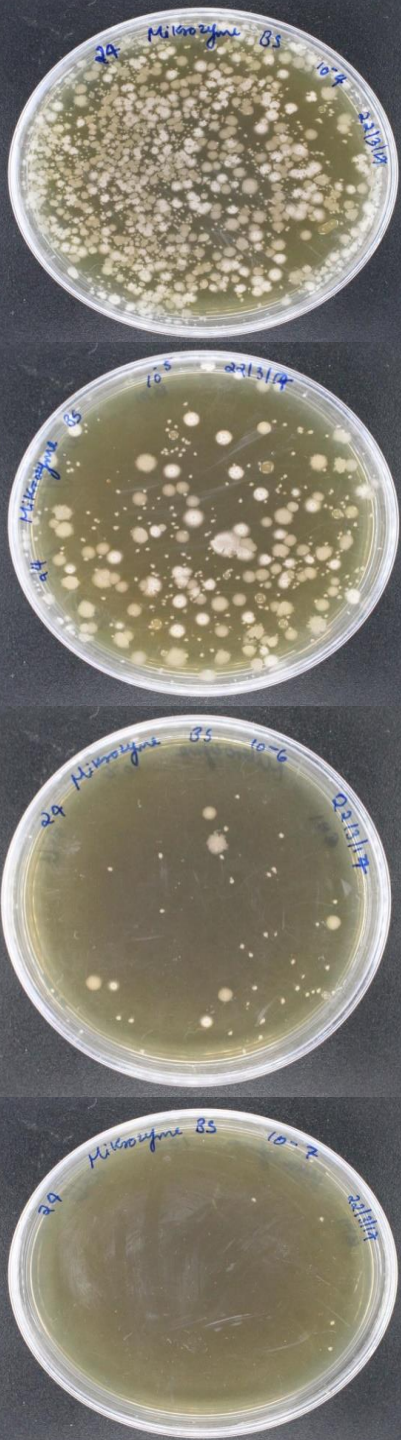
Evaluating Costs and Benefits of Prophylactic Health Products and Novel Alternatives on Smallholder Aquaculture Farmers in Asia and Africa (IMAQulate)

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Microbial Analysis of Commercial Aquaculture Probiotics Procured from India

Categorization of Probiotics Based on Label Information of Microbial Content



Composition :
 Strains : Safe and registered in National collections, Paris, France.
Bacillus subtilis Rosell-179 & *Pediacoccus 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
Bacillus Polymixa
Bacillus firmus
Bacillus mesentricus
Clostridium butyricum
Cellulomonas cartae
Pediacoccus
Aspergillus oryzae
Thiobacillus denitrificans
Perococcus denitrificans

Total concentration :
 NLT 12×10^8 cfu/gm

Objectives:
 Effectively controls NH_3 and H_2S
 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.

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 liter of water and spray in the pond.

Dosage :

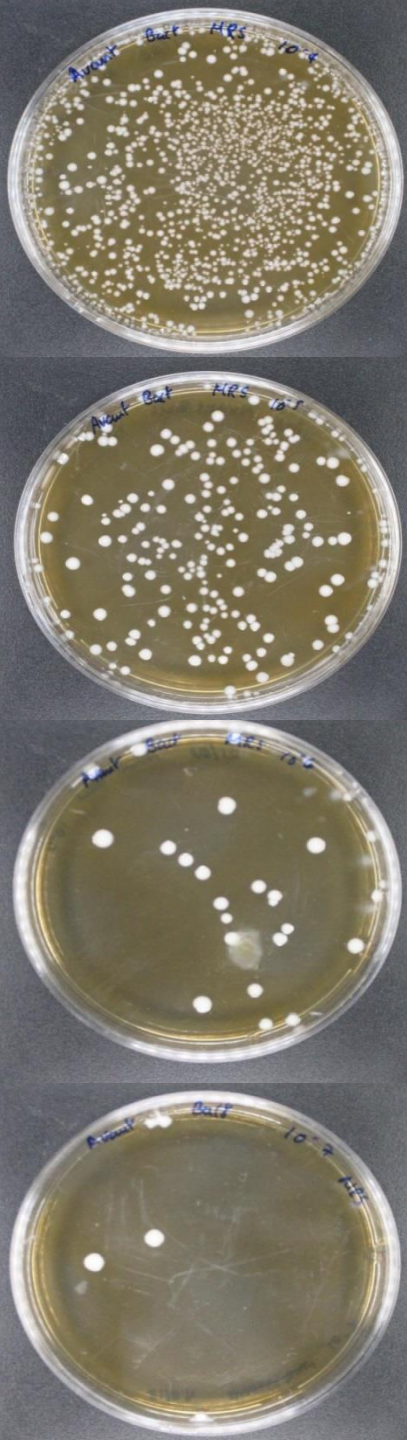
	Below 15 PCS/M ²	Above 15 PCS/M ²
0-60 days	500 gm/Hectare/week	750 gm/Hectare/week
60-120 days	750 gm/Hectare/week	1 kg/Hectare/week

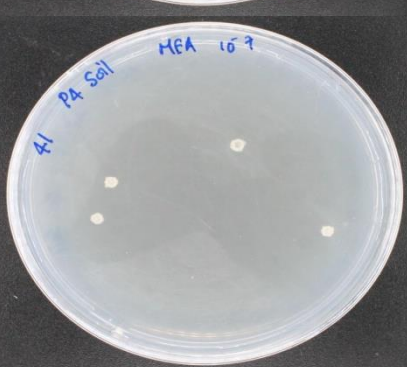
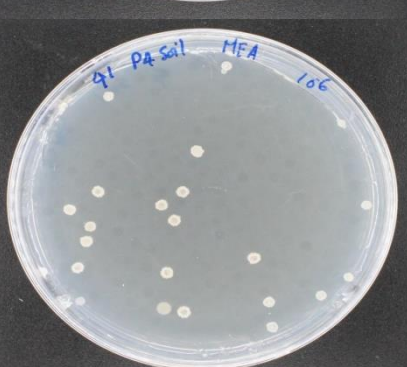
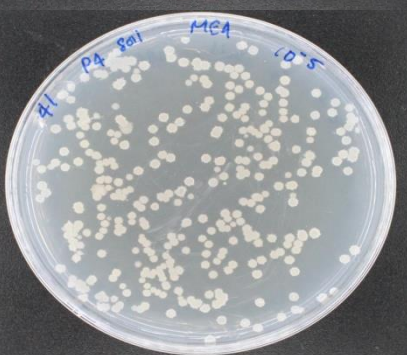
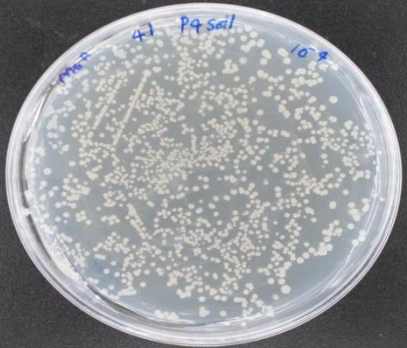
- Keep the pond clean by digesting organic matter and reduce H_2S , NH_3 , and other toxic materials.
- It compete in growth with vibrio and bring down vibrio population due to competitive exclusion.
- Decomposes organic sludge (including excess feed, dead plankton, excreta).
- Removes organic matter load through a continuous mineralisation process in a systemic way and release nutrients in to pond environment.
- 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.

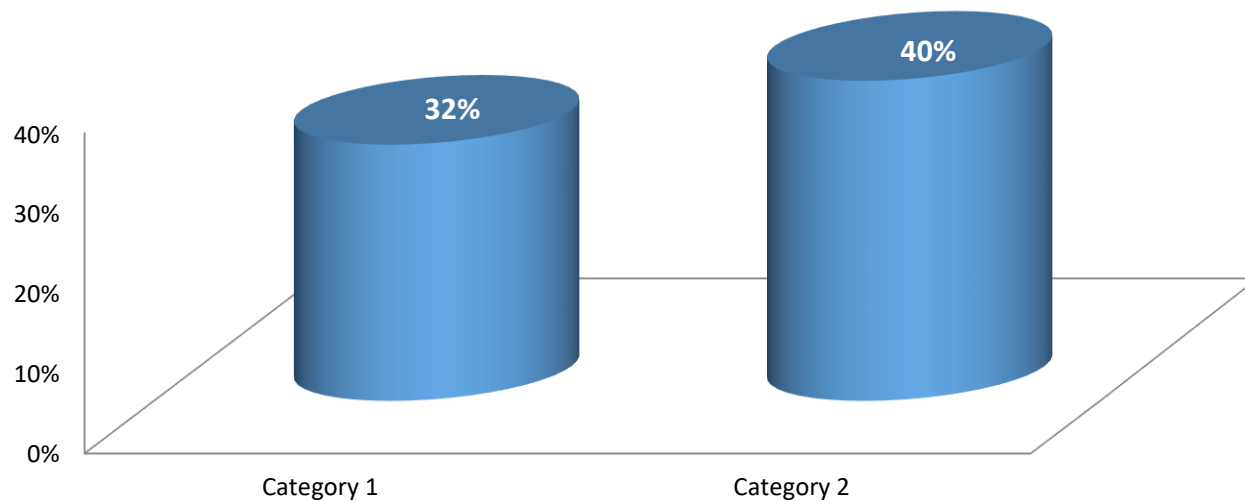
Mislabelling-Microbial Nomenclature

- ✘ Bacillus **Lichenformis**, B. **Pumilis**
- ✘ Lactobacillus Sporogenes
- ✘ *Saccharomyces cerevisiae* – 5g/500g
- ✘ P. Pantotrophus, P. Acidilactici
- ✘ Photo synthetic bacteria, Methanogenic bacteria, Sulphur utilising bacteria, Sulphur utilising yeast

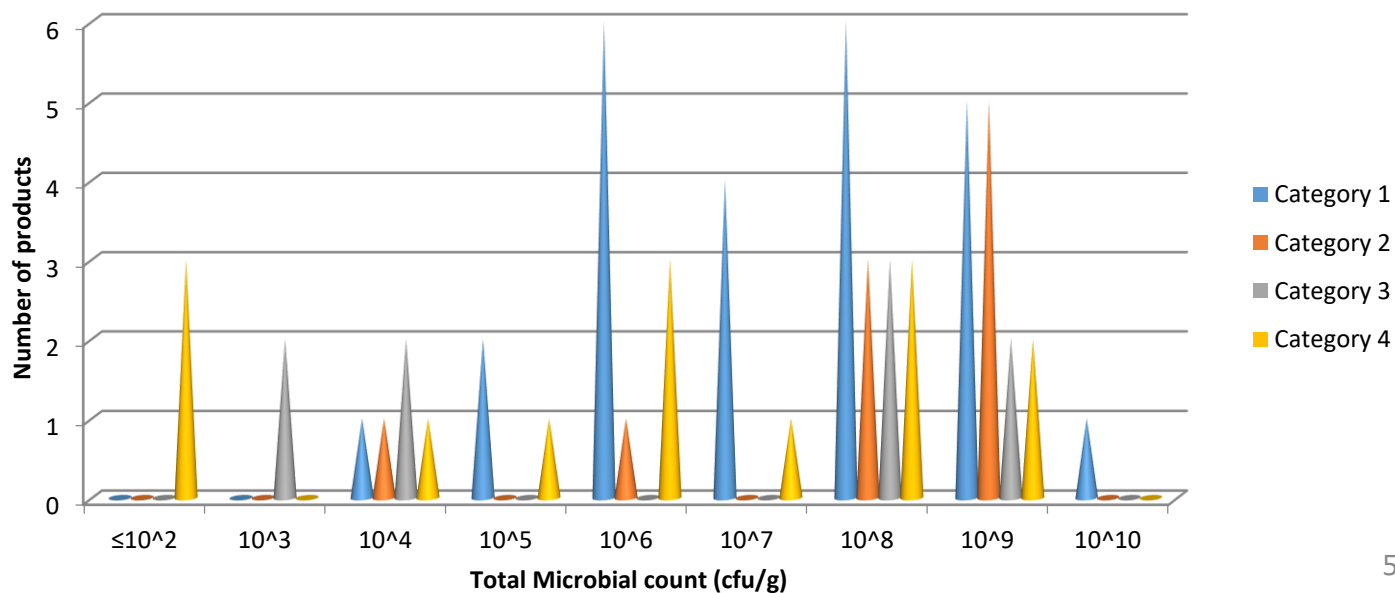




Percentage of probiotics that met claim on microbial load



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	<i>Bacillus subtilis</i> Rosell-179	Not Mentioned	1	25	<i>B. subtilis</i>	4.5×10^8
	<i>Pediococcus acidilactici</i> MA18/5M		1	35	Could not revive stored culture	
40	<i>B. subtilis</i>	1×10^5	1	43	<i>B. subtilis</i>	1.95×10^6
46	<i>B. subtilis</i>	7×10^8	1	34	<i>B. subtilis</i>	3.4×10^8
	<i>B.amyloliquefaciens</i>	2×10^8				
	<i>B.licheniformis</i>	5×10^8				
	<i>B.megaterium</i>	5×10^8				
	<i>B.pumilus</i>	5×10^8				
	<i>B.polymyxa</i>	5×10^8	2	30	<i>B. subtilis</i>	2.72×10^8
	<i>Alkaligenes faecalis</i>	1×10^9				
	<i>Saccharomyces cerevisiae</i>	1×10^8				
	<i>Nitrobacter</i>	2×10^4				
	<i>Nitrosomonas</i>	2×10^4				

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

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

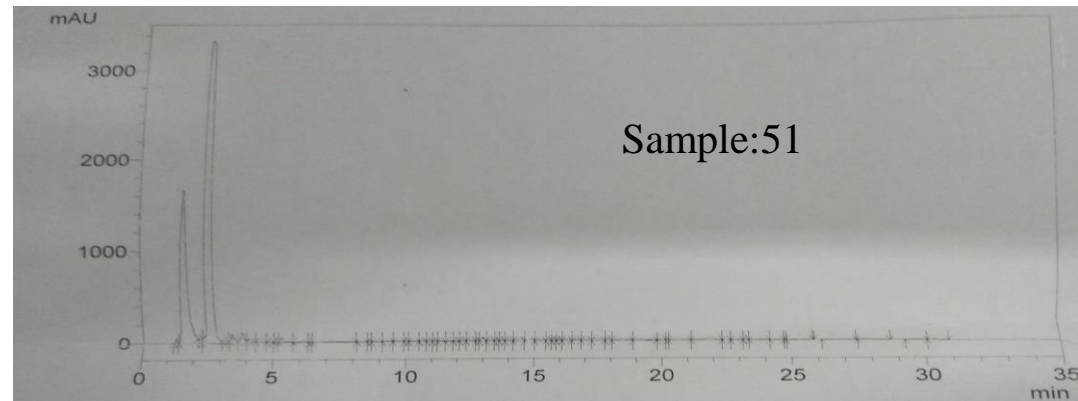
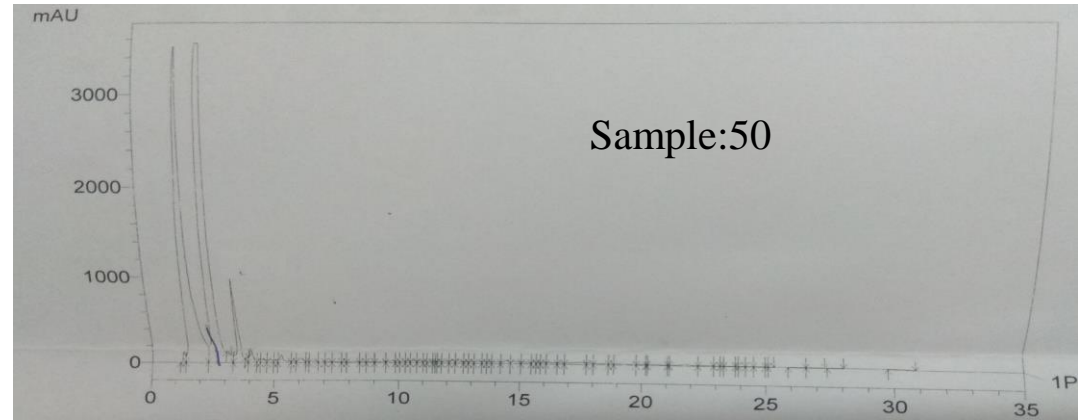
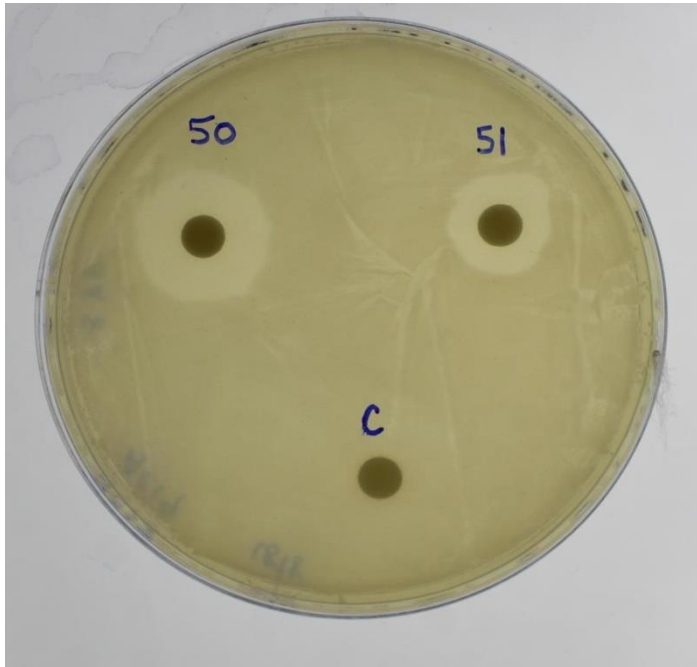
26% of the probiotic products had counts lower than 10^9 cfu/g

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

Major Findings

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

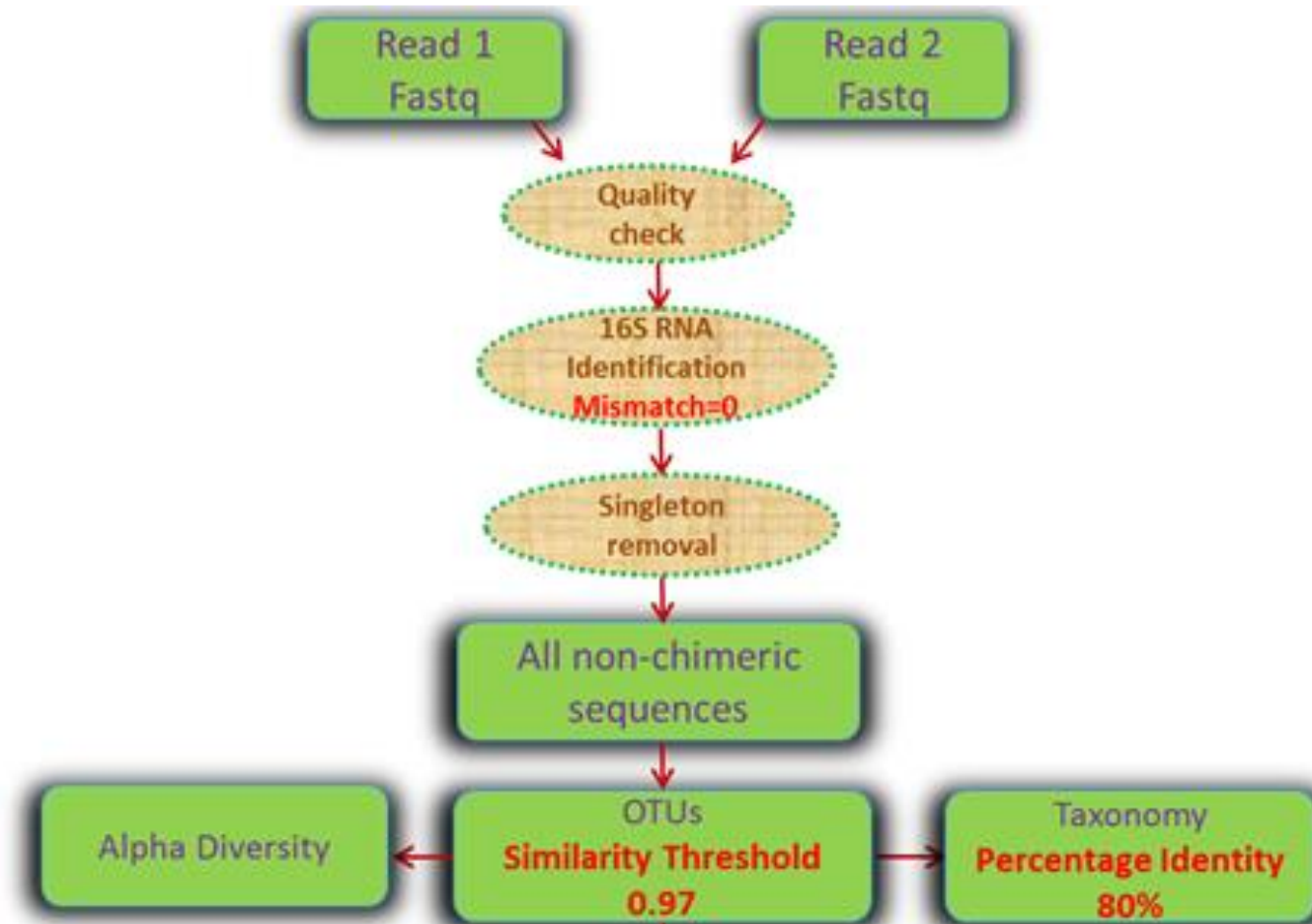


Antimicrobial activity of product extracts against *Bacillus subtilis* at pH 8

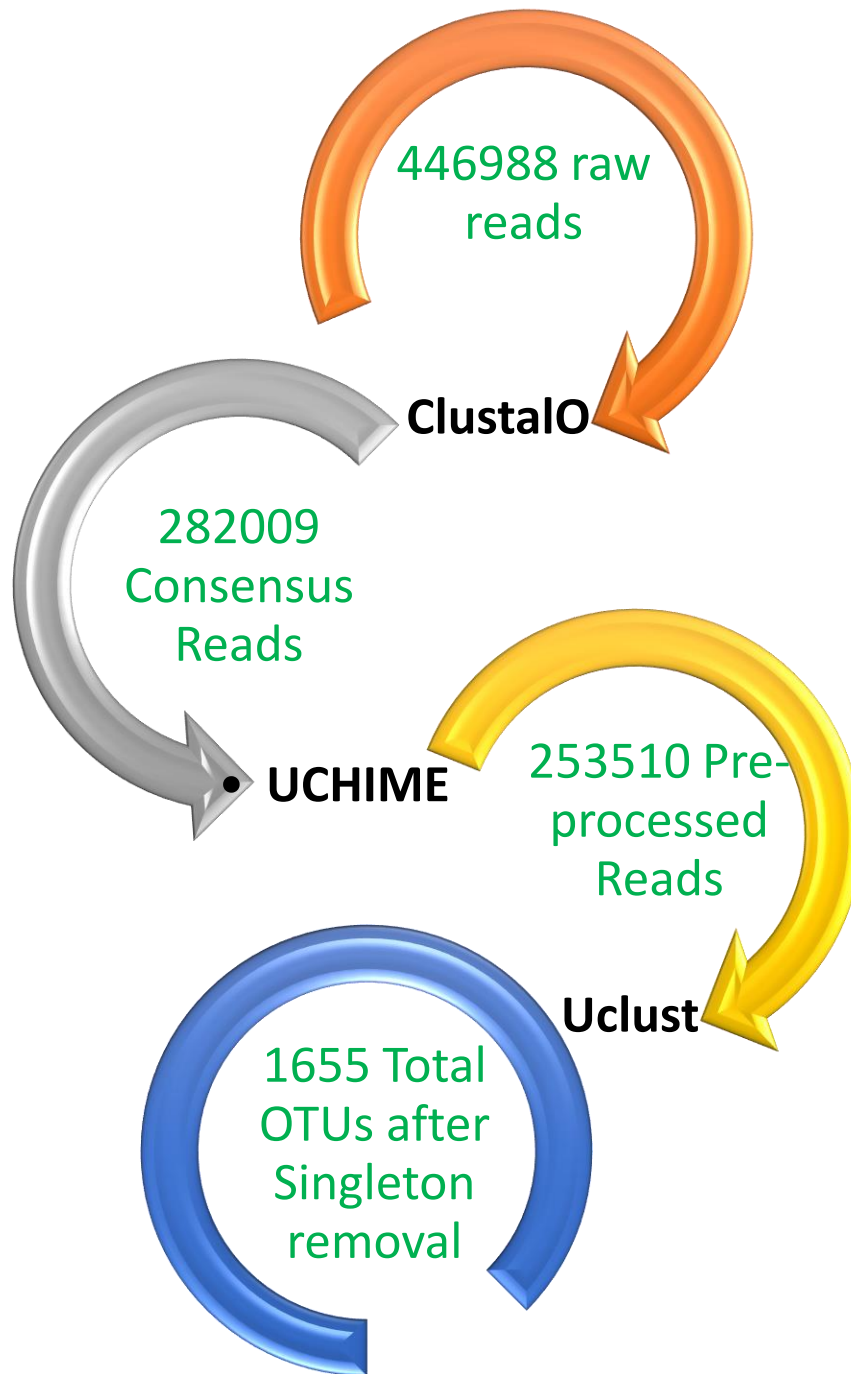
HPLC chromatograms of antimicrobial residue extracts from products 50 and 51

Microbiome Analysis of Commercial Probiotics

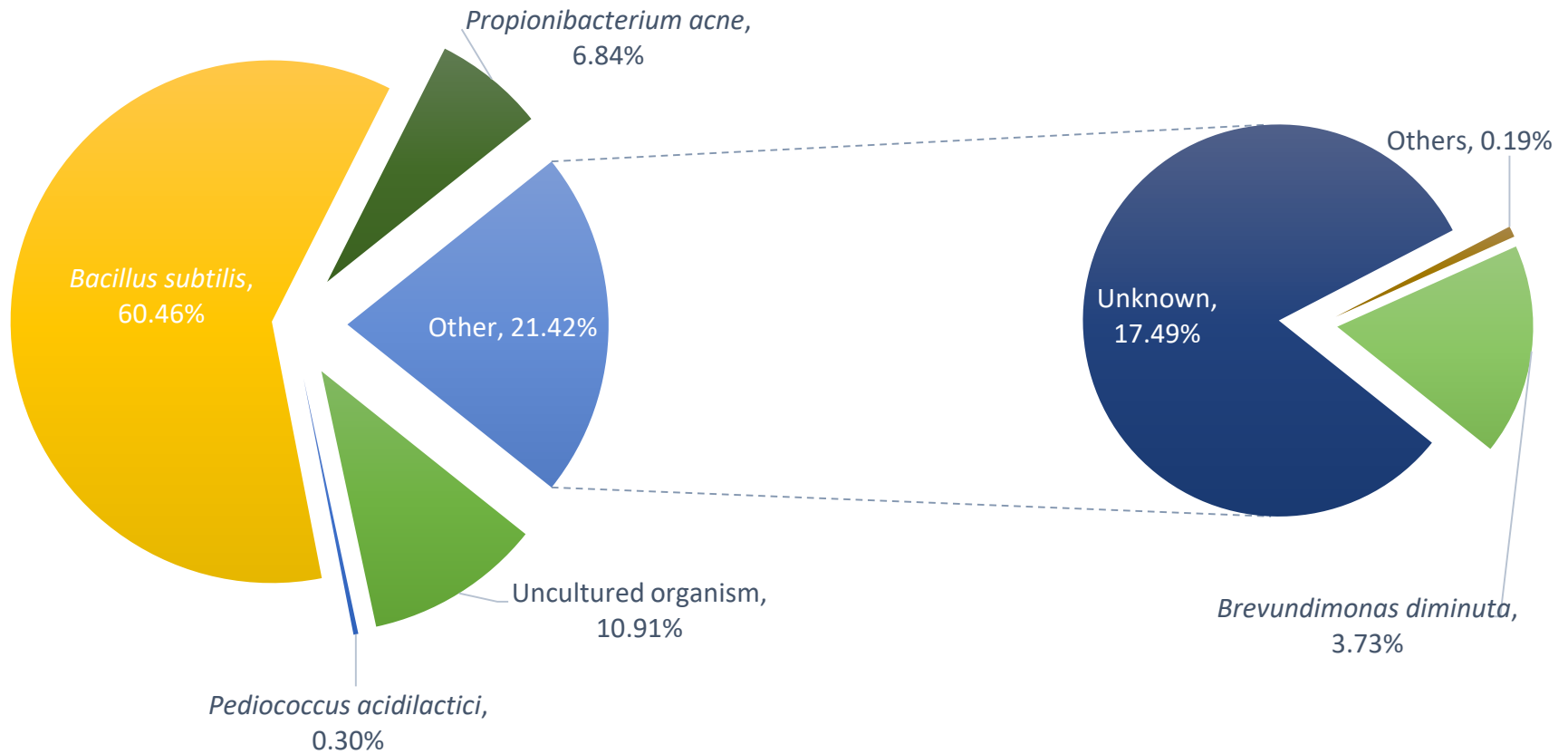
- **16S ribosomal RNA Amplicon Sequencing** performed using Illumina MiSeq platform for product with code number 13



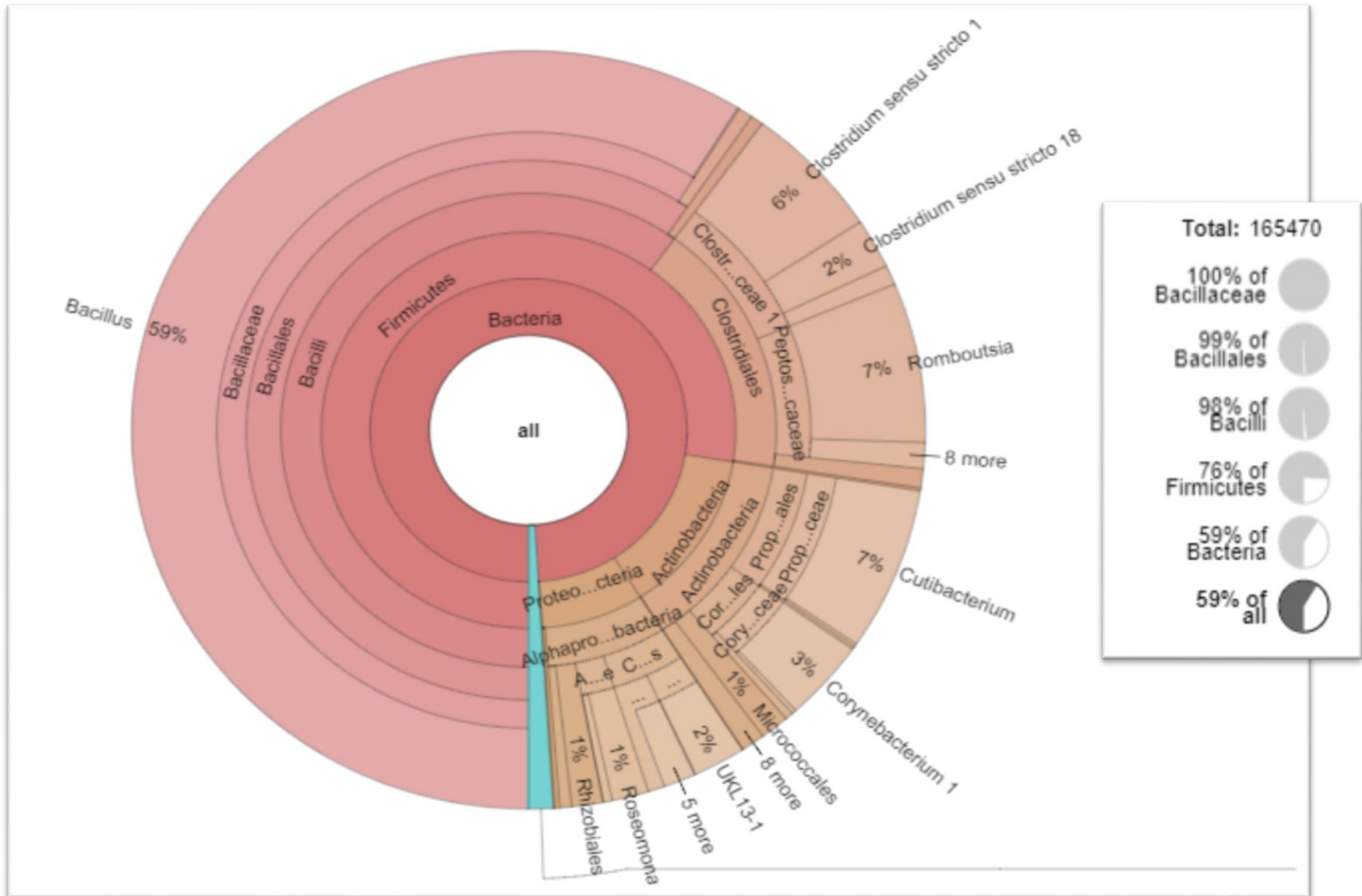
Metagenomics workflow

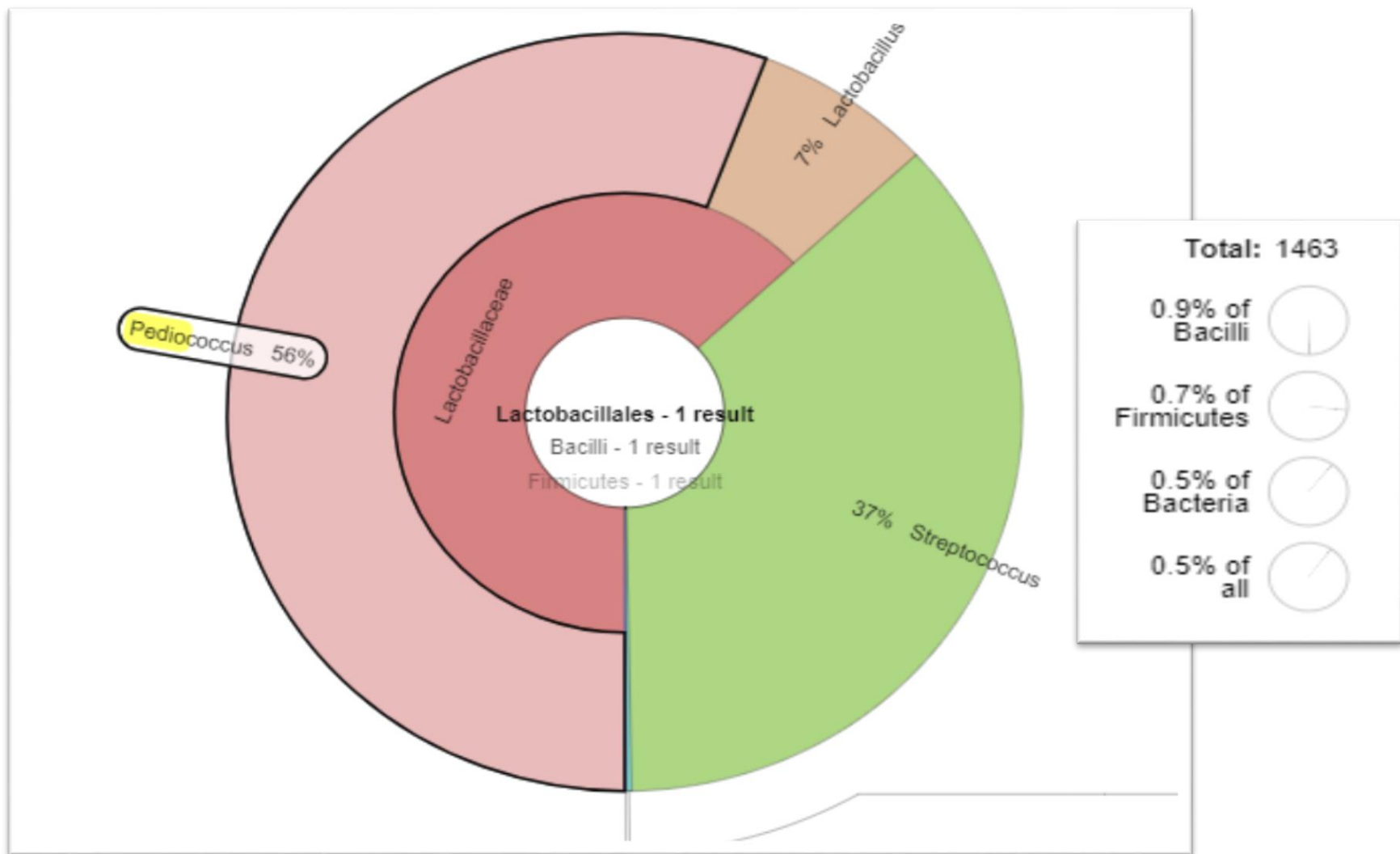


Species wise Distribution of OTUs



Genus level taxonomic classification





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*	11 th Indian Fisheries and Aquaculture Forum (IFAF)	21 st to 24 th November, 2017	Cochin, Kerala, India
2	Probiotic Use in <i>Litopenaeus vannamei</i> Farming in Andhra Pradesh	Chandra Rao P., Toms C. Joseph*, Madhusudana Rao B., Kenton Morgan, Francis Murray			

Thank you!!!



PROBIOTIC USE IN *LITOPENAEUS VANNAMEI* FARMING IN ANDHRA PRADESH

Chandra Rao, P., Toms C. Joseph, Madhusudana Rao, B., and Francis Murray*

*Madhusudana Rao, Toms C. Joseph, Madhusudana Rao, B., and Francis Murray are with the National Institute of Aquaculture Sciences, ICAR, Central Institute of Fisheries Technology (CICT), Pondicherry, Tamil Nadu, India.

Introduction:
Probiotics are formulations of beneficial microorganisms that are applied for shrimp farmers to improve pond water quality, enhance the shrimp health leading to better weight gain.

Material and Methods:
Questionnaire based survey was conducted in 182 *Litopenaeus vannamei* shrimp farms located in north coastal (n=20), central (n=80) and south coastal regions (s=82) of Andhra Pradesh, in order to understand the usage pattern of probiotics.

Results:

- 45 different probiotics brands supplied by 53 manufacturers were being used in *L. vannamei* farming.
- Three probiotics brands were used by 24% of the shrimp farmers. Probiotics ranged between Rs. 145/kg and Rs. 2000/kg.
- Price of probiotics (dose of probiotic per hectare pond farming) ranged between Rs. 363 and Rs. 2825.
- Cost per application dose of probiotic for applying ranged between Rs. 12 week shrimp rearing period range.
- Estimated cost incurred by the shrimp farmer per the probiotic, Rs. 12 week shrimp rearing period range between Rs. 4,200 to Rs. 94,500.
- Fresh technician collect about (44%) or with farmers, per the technician collect about (80%) decides the probiotic brand selection.

Number of probiotics applied

Probiotic Brand	Number of Farms
Brand 1	10
Brand 2	15
Brand 3	20
Brand 4	25
Brand 5	30
Brand 6	35
Brand 7	40
Brand 8	45
Brand 9	50
Brand 10	55
Brand 11	60
Brand 12	65
Brand 13	70
Brand 14	75
Brand 15	80
Brand 16	85
Brand 17	90
Brand 18	95
Brand 19	100
Brand 20	105
Brand 21	110
Brand 22	115
Brand 23	120
Brand 24	125
Brand 25	130
Brand 26	135
Brand 27	140
Brand 28	145
Brand 29	150
Brand 30	155
Brand 31	160
Brand 32	165
Brand 33	170
Brand 34	175
Brand 35	180
Brand 36	185
Brand 37	190
Brand 38	195
Brand 39	200
Brand 40	205
Brand 41	210
Brand 42	215
Brand 43	220
Brand 44	225
Brand 45	230
Brand 46	235
Brand 47	240
Brand 48	245
Brand 49	250
Brand 50	255
Brand 51	260
Brand 52	265
Brand 53	270

Types of probiotics used

Type	Percentage
Water probiotics (fluorocitrate)	50%
Soil probiotics	20%
Feed probiotics	10%
Other probiotics	20%

Probiotic advantages (based on product label claims)

- Help to maintain water quality
- Digest organic matter
- Reduce H₂S, NH₃, turbidity, hardness and shade
- Improve FCR
- Regulate pH
- Maintain stable and health phytoplankton bloom.
- Assist in rice seedling of shrimp.
- Improve feed conversion
- Probiotic microorganisms compete with harmful Vibrio sp. and bring down vibrio population in shrimp rearing environment

Conclusion:
Probiotics are being increasingly used by the shrimp farmers. Quality and efficacy of the probiotics available commercially needs to be monitored to ensure that the shrimp farmers use only good products for better shrimp production.

ICAR - Central Institute of Fisheries Technology

