WHERE SCIENCE SERVES NATURE

Focus on VALAGRO'S INNOVATION in the field of BIOLOGICALS

DR. ANITHA CHOWDARY
Valagro Biosciences, India

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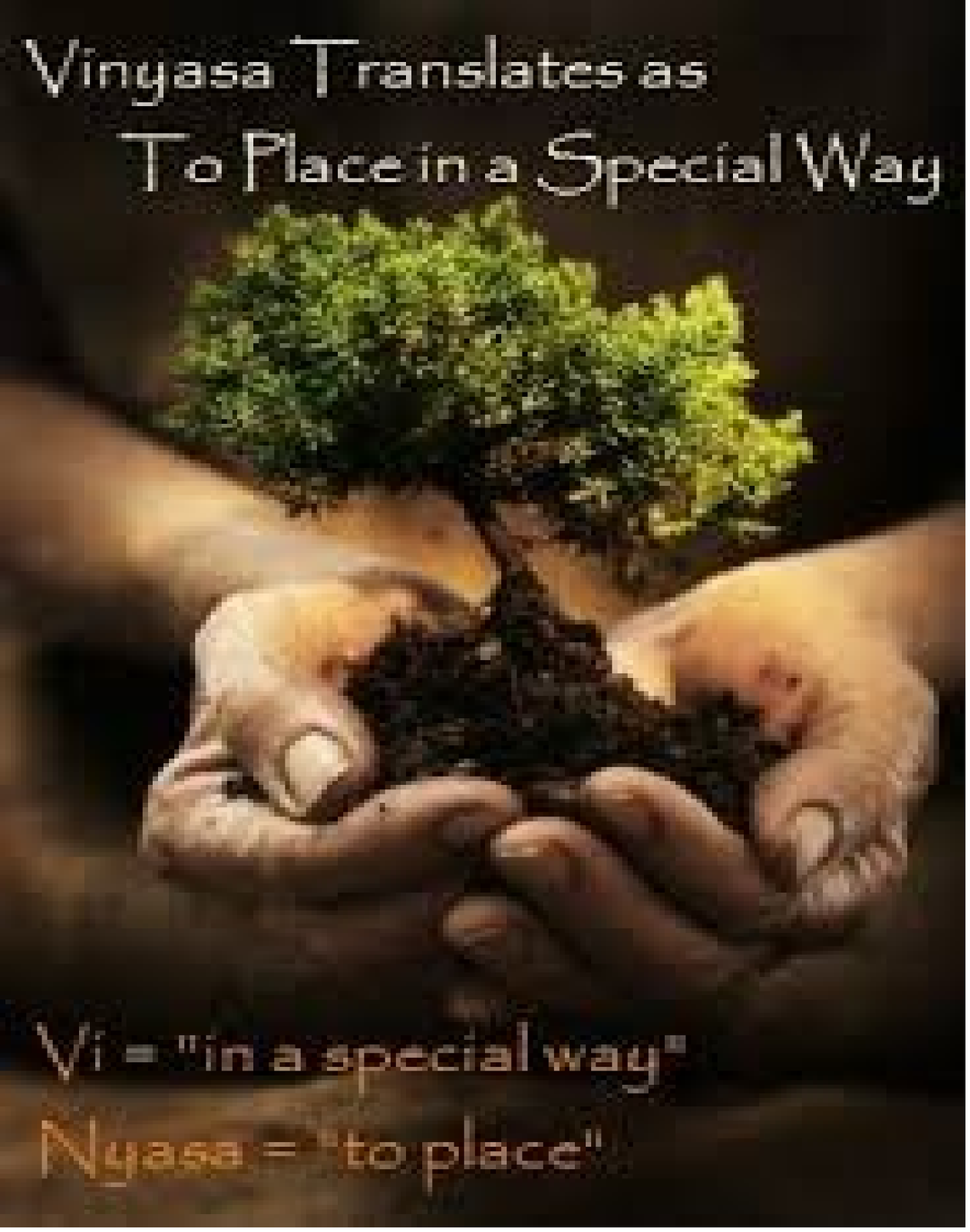

INNOVATION

Change that Unlocks New Value

Jamie Notter

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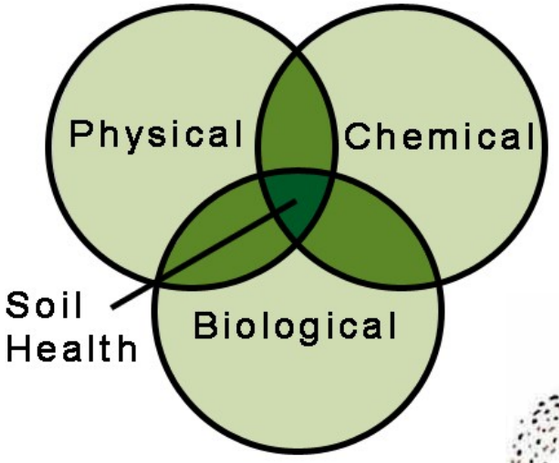
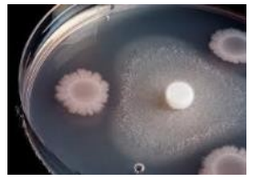
AGRICULTURAL BIOLOGICALS



Naturally Occurring Solutions

1. PLANT EXTRACTS
2. MICROBIALS
3. ORGANIC MATERIALS
4. BENEFICIAL INSECTS



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GEAPOWER,
THE EXCLUSIVE
VALAGRO TECHNOLOGY PLATFORM

Focus on the
MICROBIAL PLATFORM

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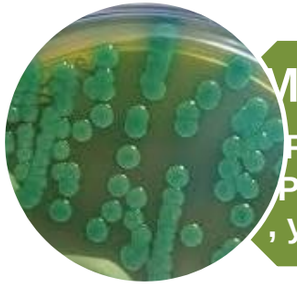
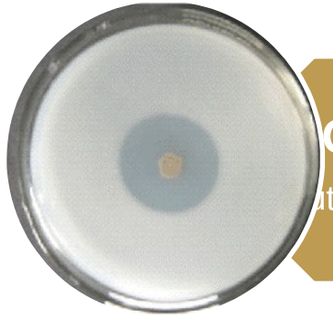
MAIN RESEARCH AREAS



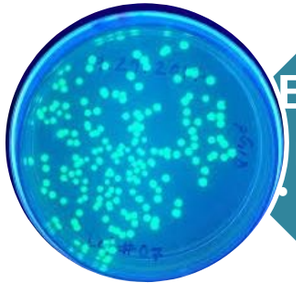
MICROBIAL BIOSTIMULANTS

- Foliar and root application
- Plant growth hormones, abiotic stress tolerance
- , yield &quality *etc.*



O FERTILIZERS

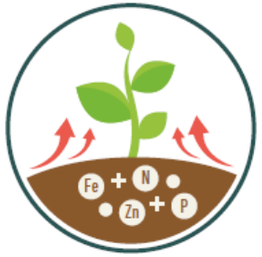
- trient availability-PGPRs



BIOCONTROL

- New actives for disease & insest control
- Induced host defence mechanism

1 IMPROVE TOLLERANCE TO ABIOTIC STRESS



2 IMPROVE NUTRIENT USE EFFICENCY

3 IMPROVE CROP QUALITY

IPR INTRODUCTION PROCESS

01 RESEARCH TRIALS & TESTING

02 INNOVATION

03 RESEARCH & DEVELOPMENT

04 INVESTMENT

05 PROTECTION

06 IPR

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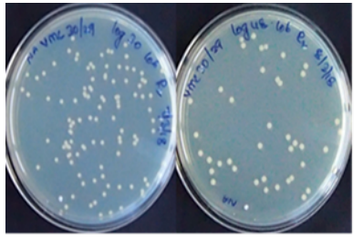
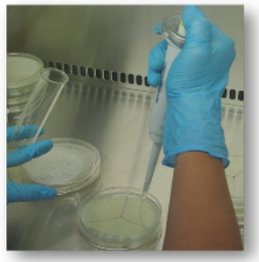
VALAGRO APPROACH TOWARDS INNOVATIVE SOLUTIONS

- Compliance with Biodiversity Act, 2002, India
- Identification of Novel Microbes
- Development of high through put screening technologies
- Innovative Process development to demonstrate MOA through OMICS
- Unique Formulation development using novel raw materials-New Actives.
- IP Protection-Patented Technologies

QUALITY CONTROL ⟷ **MANUFACTURING**





DISCOVERY | IN VITRO SCREENING | PROTOTYPING | PRIMARY SCREENING | OPEN FIELD TESTING

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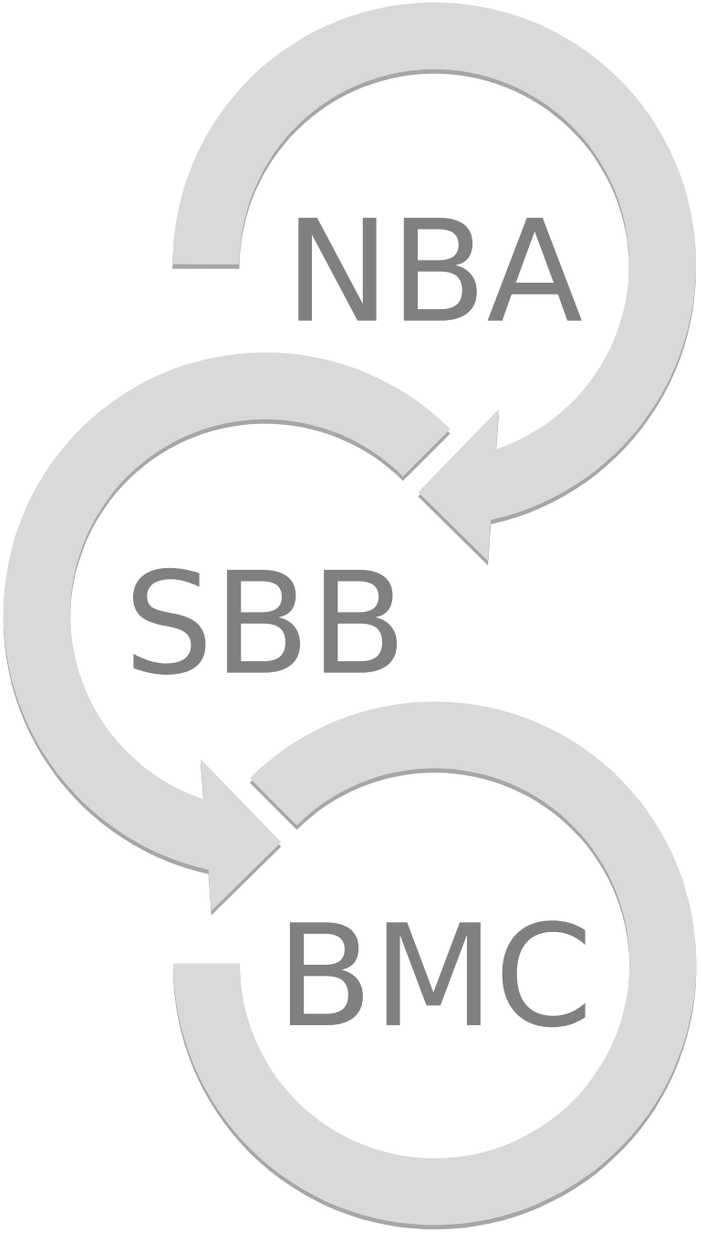
REGULATORY COMPLIANCE- BIO DIVERSITY ACT, INDIA

National level

State Level

Local Bodies



राष्ट्रीय जैव विविधता प्राधिकरण
National Biodiversity Authority, India
2003

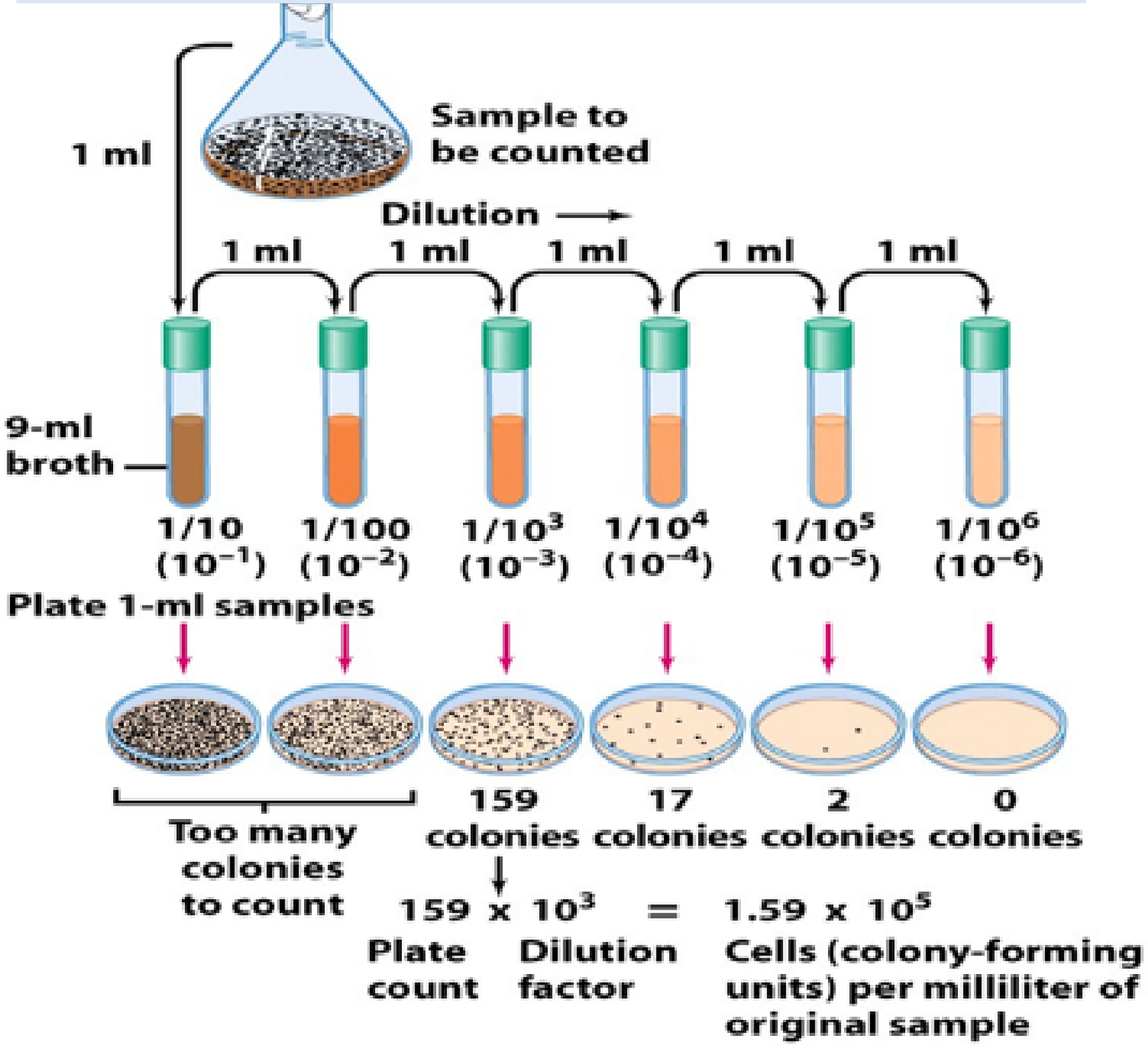
Form I- Access &Benefit Sharing
Form II- Transfer of research
Form III- Prior approval -IPRs
Form IV- Third party transfer of accessed Bio resources

COLLECTION OF RHIZOSPHERIC SOIL SAMPLES FROM DIFFERENT CROPS

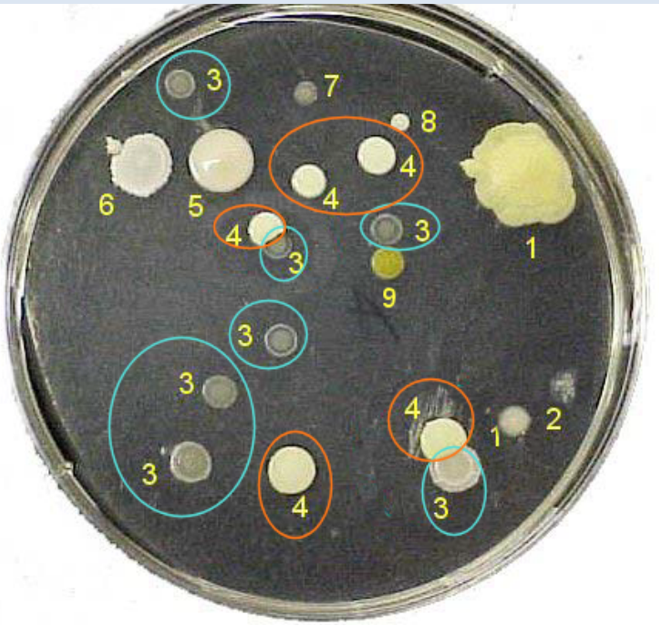
Strain Code	No.of strains	Date of Isolation	Crop	Village
VIMC-01 to VIMC-49	49	03.01.2018	Paddy	Indrakaran
VIMC-50 to VIMC-89	40	08.01.2018	Turmeric	Dharmaram
VIMC-90 to VIMC-127	38	06.02.2018	Chilli	Dharmaram
VIMC-128 to VIMC-142	15	15.02.2018	Coriander	Dharmaram
VIMC-143 to VIMC-161	19	15.02.2018	cowpea	Dharmaram
VIMC-162 to VIMC-183	22	15.02.2018	Cotton	Komaramvelly
VIMC-184 to VIMC-216	33	18.04.2018	Tomato	Dharmaram
VIMC-217 to VIMC-224	8	16.05.2018	Bamboo	Mizoram
VIMC-225 to VIMC-238	14	16.05.2018	Rock soil	Mizoram
VIMC-239 to VIMC-289	51	26.06.2018	Cotton	Kasipur
VIMC-290 to VIMC-312	23	09.07.2018	Fodder Jowar	Julkal
VIMC-313 to VIMC-503	29	09.07.2018	Fenugreek	Shamirpet

Figure: **A**-Rhizosphere soil samples of all the crops; **B** and **E**- Chilli, **C**-Turmeric, **D**- Onion, **F**- Cowpea, **G**- Tomato and **I**-Garlic from Dharmaram village, Karimnagar; **H**-Fenugreek from Shamirpet village, Warangal; **J**-Paddy from Kadaverugu village, Warangal and **K**- diseased chilli leaves from Dharmaram, Karimnagar.

Isolation of Soil Microbes by Serial dilution Technique



Purification of Soil Microbes



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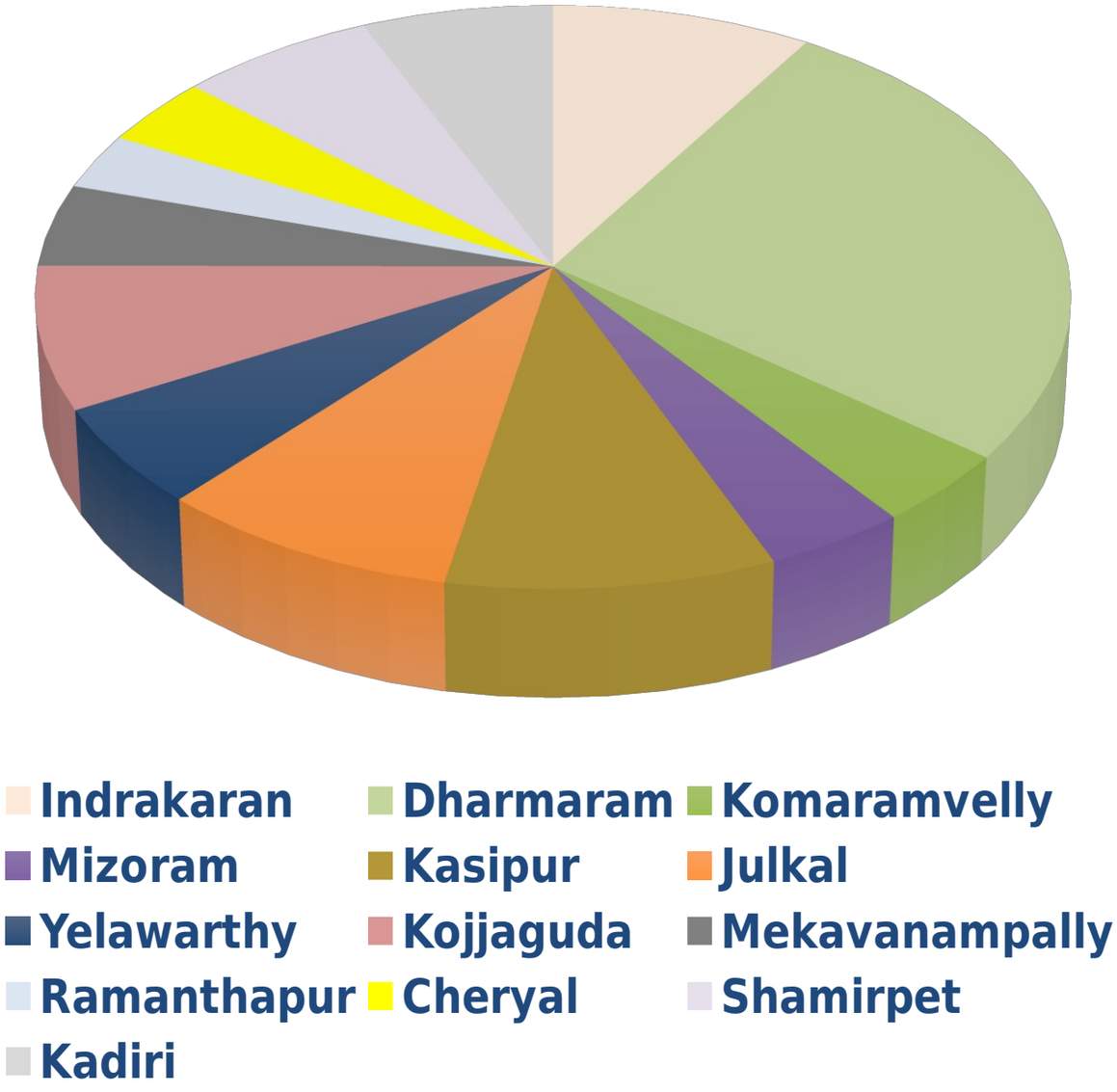
Strain Code	Isolates	Crop	Village
VIMC-01 to VIMC-49	49	Paddy	Indrakaran
VIMC-50 to VIMC-89		Turmeric	
VIMC-90 to VIMC-127		Chilli	
VIMC-128 to VIMC-142	145	Coriander	Dharmaram
VIMC-143 to VIMC-161		cowpea	
VIMC-184 to VIMC-216		Tomato	
VIMC-162 to VIMC-183	22	Cotton	Komaramvelly
VIMC-217 to VIMC-224	22	Bamboo	Mizoram
VIMC-225 to VIMC-238		Rock soil	
VIMC-239 to VIMC-289	51	Cotton	Kasipur
VIMC-290 to VIMC-312	47	Fodder Jowar	Julkal
VIMC-313 to VIMC-336		Onion	
VIMC-337 to VIMC-366	30	Mango	Yelawarthy
VIMC-367 to VIMC-410	43	Spinach	Kojjaguda
VIMC-411 to VIMC-435	25	Turmeric	Mekavanampally
VIMC-436 to VIMC-	545	300 strains shortlisted	

Number of isolates from different villages



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THE NEED TO EXPLORE…

The knowledge on the benefits of Microbials is constantly improving (consistent increase of research papers)…

Less is known about their “mode of action”…

Natural but Complex matricec… what makes them so «special»?

Generally, about **2–5%** of **Rhizosphere bacteria** are **PGPR**

Plant Growth Promoting Microbes

Direct plant growth promotion

Nutrient Solubilization

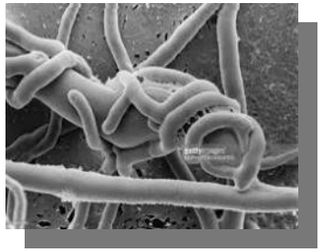
1.Nitrogen fixation2.Phosphate solubilization

3.Potassium Mobilization 4.Zinc Solubilization

Phytohormone production

1.Auxins 2.Gibberellins 3. Ethylene 4. Abscisic acid

Indirect plant growth promotion

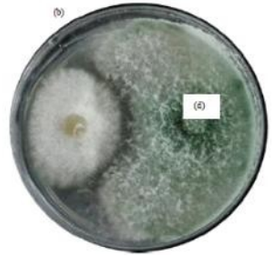


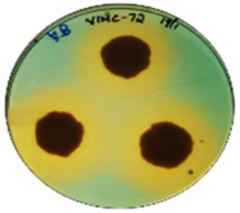
Plant protection

1.Antibiotic production 2.Hydrolytic enzymes production

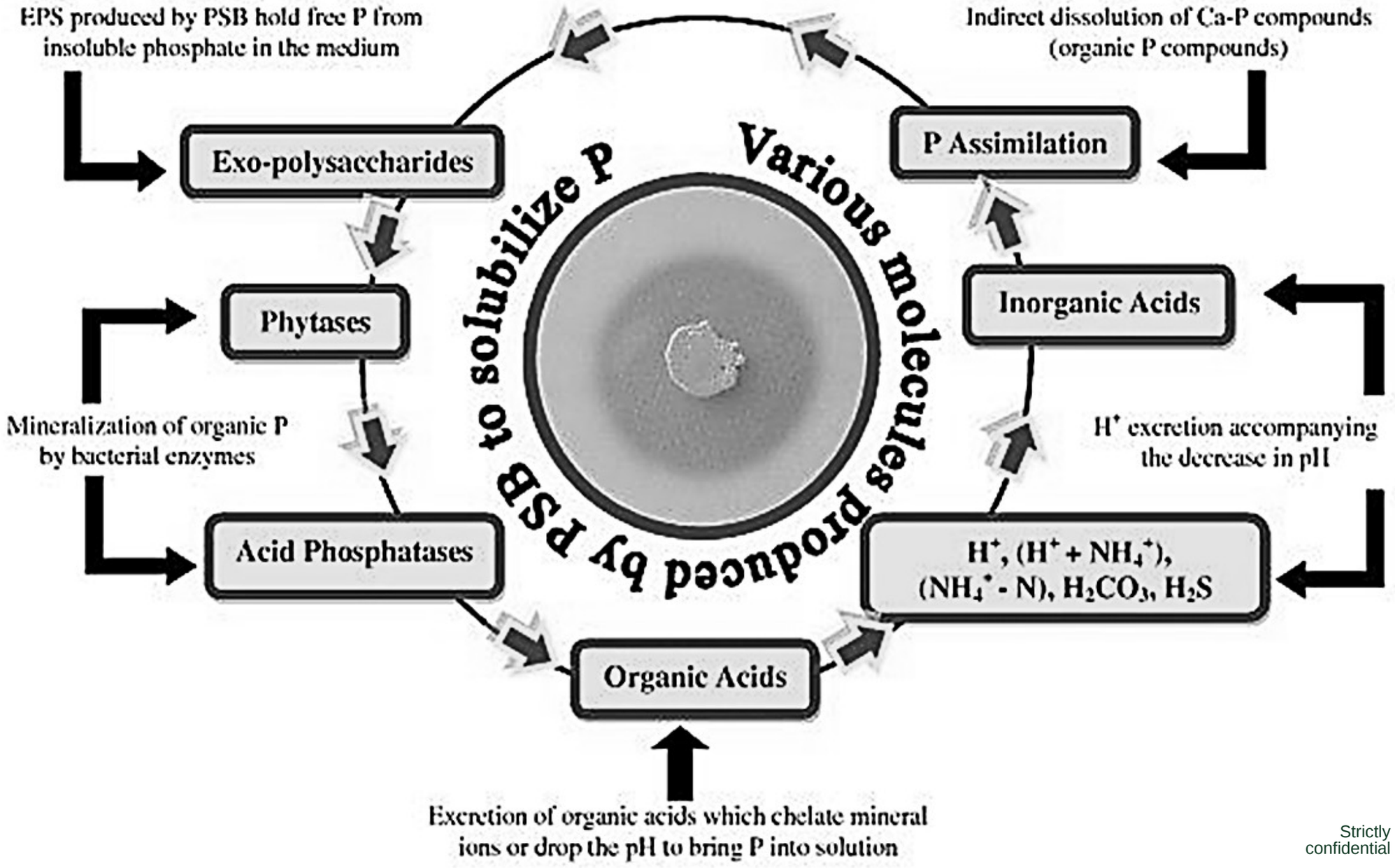
3.Siderophore production 4.Induced Systemic Resistance

5.Exopolysaccharide production





MECHANISM OF P SOLUBILIZATION BY SOIL MICROBES



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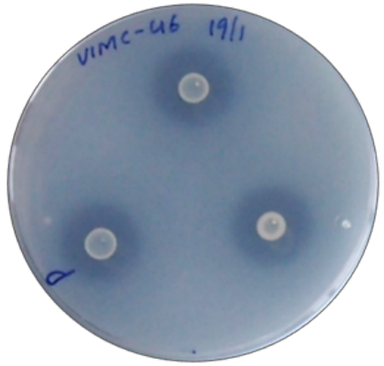
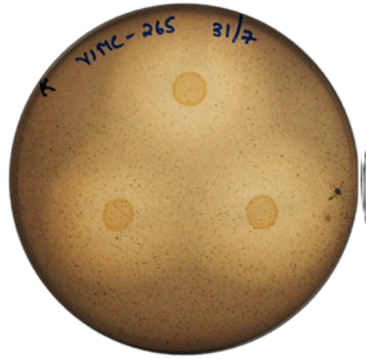
Nitrogen Fixation | P Solubilization | K Mobilizatio | Zn Solubilization

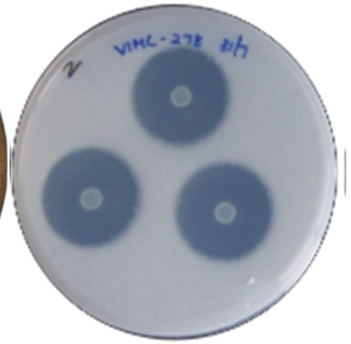
Strain	N-fixation (mm)	Strain	PSB (mm)	Strain	KMB (mm)	Strain	ZSB (mm)
VMC30/269	26.67	VMC30/232	12.67	VMC30/310	17.5	VMC30/307	20
VMC30/238	26	VMC30/273	10	VMC30/271	16.33	VMC30/303	17
VMC30/344	25	VMC30/307	10	VMC30/303	15.5	VMC30/310	16
VMC-30/318	23.33	VMC30/216	9.33	VMC30/270	15	VMC30/198	17.67
VMC30/232	22.67	VMC30/304	8	VMC30/307	15	VMC30/304	15.67
VMC30/216	22.67	VMC30/303	5.5	VMC30/232	14.67	VMC30/366	16
VMC30/333	20.67	VMC30/333	5	VMC30/289	14.33	VMC30/371	20
VMC30/322	19.5	VMC-30/391	7	VMC30/340	10	VMC30/378	10.33
VMC30/310	19	VMC-30/403	7.67	VMC30/198	19.33	VMC30/439	11
VMC30/335	18	VMC-30/404	11	VMC30/269	12		
VMC30/307	16.5	VMC-30/374	8	VMC30/272	16.33		
VMC30/240	16.33	VMC-30/392	8.33	VMC30/273	12		
VMC30/303	15	VMC-30/460	8.67	VMC30/304	16		
VMC-30/311	20	VMC-30/462	9	VMC30/359	14		
		VMC-30/475	9	VMC30/366	17		
		VMC-30/476	9	VMC30/369	12.67		



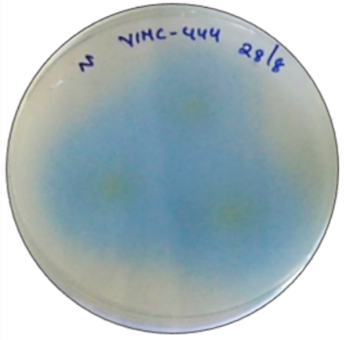
P solubilization



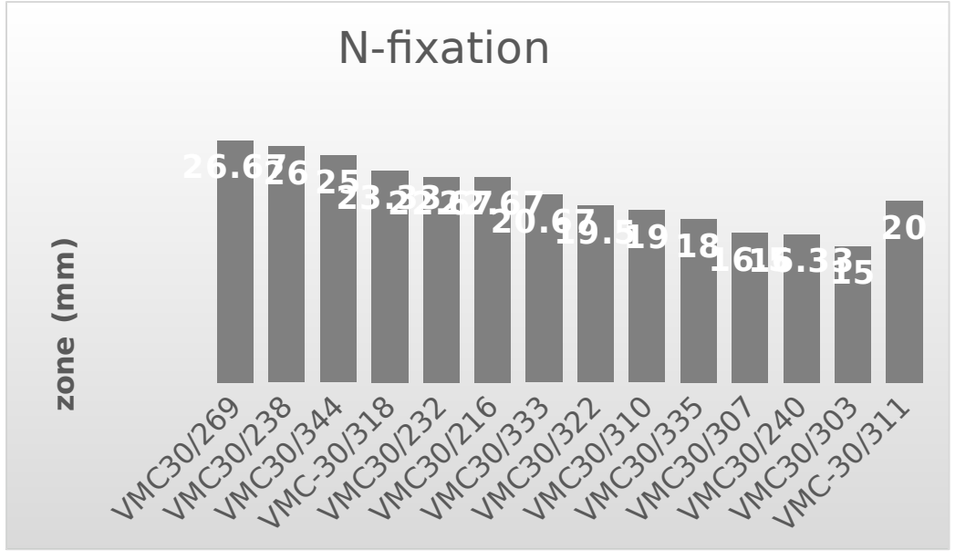
K solubilization



Zn solubilization



N2 fixation



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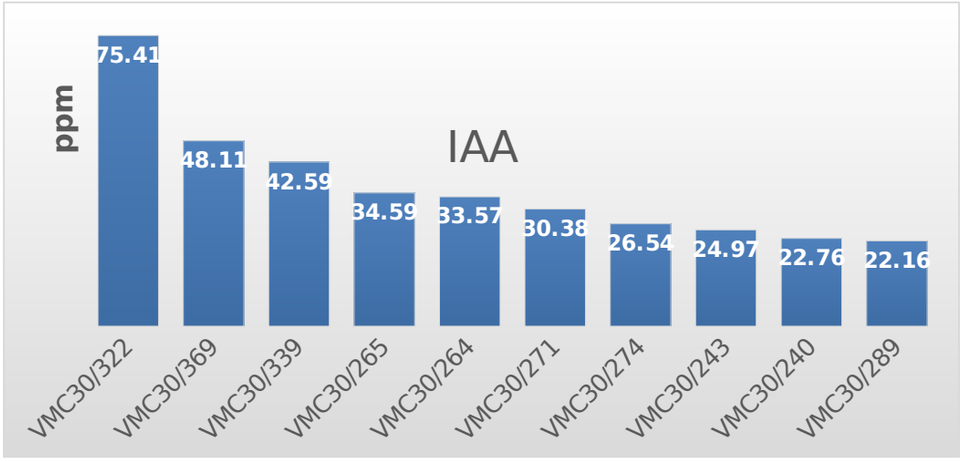
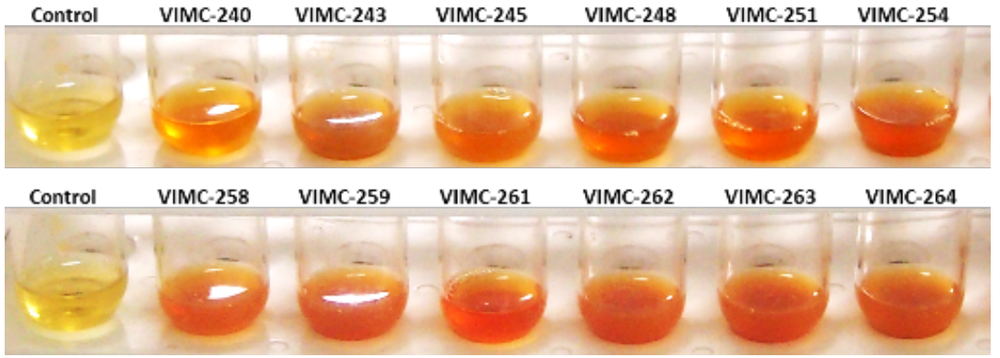
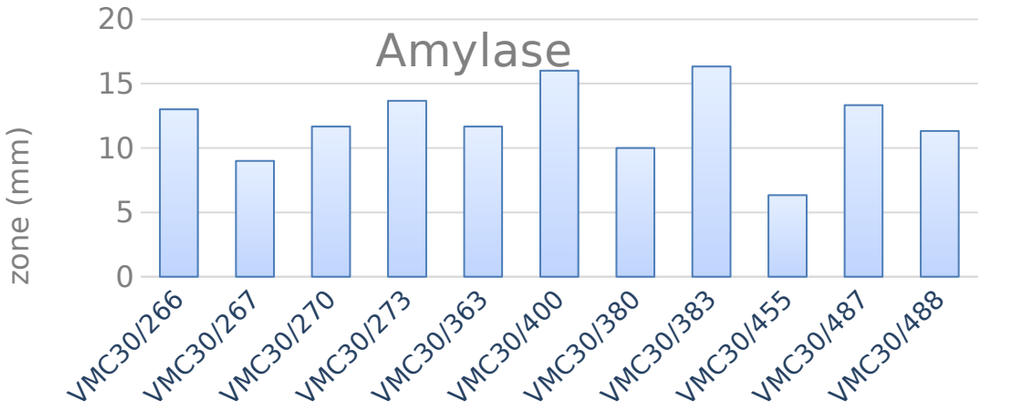
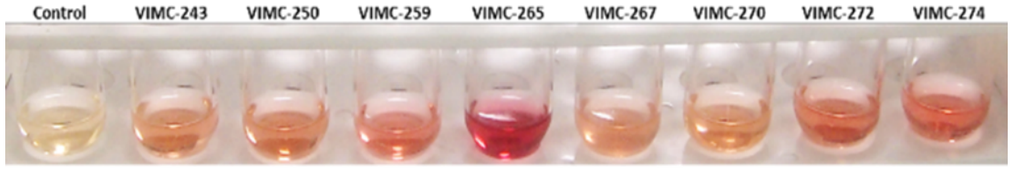
Fe Chelation | Ammonia production | IAA Productio | CMC Degradation | Amylase Activity

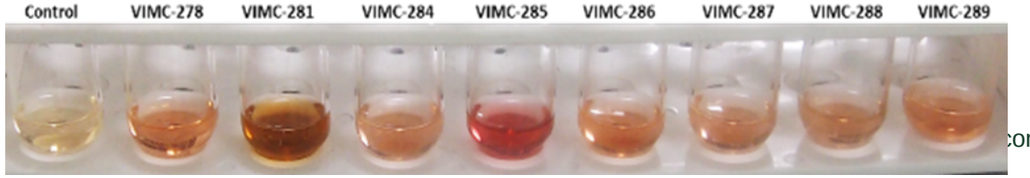


Strain	Ammonia (ppm)	Strain	Siderophore (mm)	Strain	CMC (mm)
VMC30/311	150	VMC30/303	21	VMC30/339	12.33
VMC30/318	152.41	VMC30/216	14.67	VMC30/316	9
VMC30/376	432.41	VMC30/218	13.33	VMC30/269	8.33
VMC30/403	142.41	VMC30/209	19.33	VMC30/319	8.33
VMC30/411	222.78	VMC30/217	14.33	VMC30/266	7.33
VMC30/378	320.56	VMC30/210	18	VMC30/267	10
VMC30/380	300	VMC30/215	15.33	VMC30/270	8.33
VMC30/439	298.7	VMC30/212	16.67	VMC30/271	5.33
VMC30/455	466.48	VMC30/207	20.67	VMC30/330	6.67



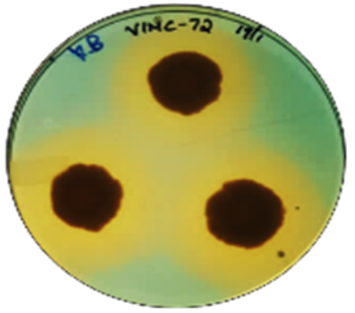






Starch hydrolysis

CMC degradation



Siderophores-Fe

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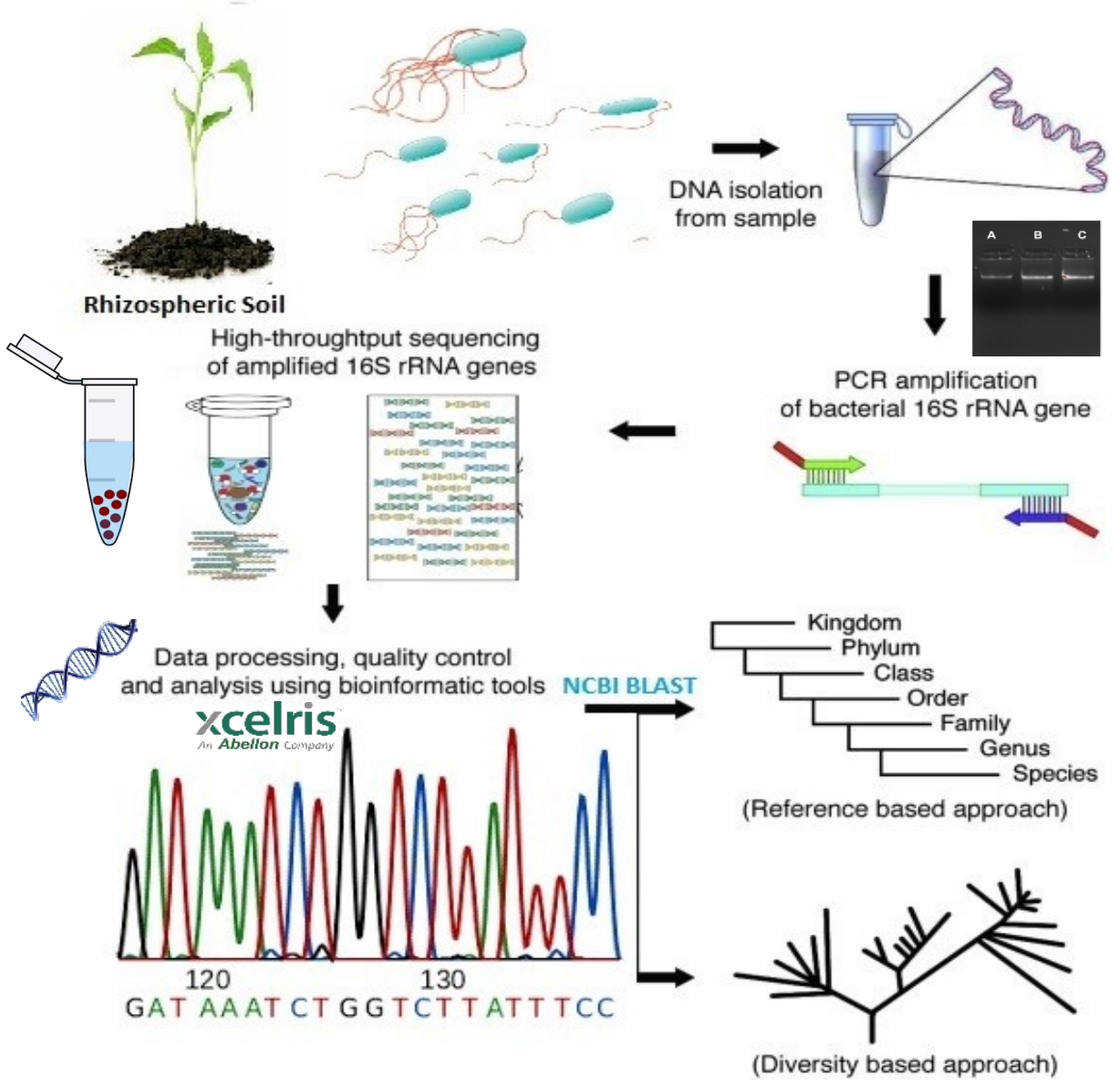
SCREENING FOR MULTI PGP TRAITS

Strain code	N-fixation		P-solubilization		K-solubilization		Zn-solubilization		Siderophore production		IAA production
	zone (mm)	Index	zone (mm)	SI	zone (mm)	SI	zone (mm)	SE	zone (mm)	SI	conc. (ppm)
VMC30/29	10.00	2.11	-	-	-	-			2.67	1.20	32.38
VMC30/46	24.33	4.04	-	-	3.00	1.64	6	138.57			2.05
VMC30/55	15.33	2.92	-	-	5.67	1.81	18.00	450.00	13.00	1.71	
VMC30/61	**11.00**	**2.38**	**2.00**	**1.18**	**6.00**	**2.20**	**18.67**	**400.00**	**11.00**	**1.85**	
VMC30/166	13.67	2.86	-	-	-	-			6.67	3.30	
VMC30/195	24.67	3.64	3.00	1.33	-	-	10.00	157.89			
VMC30/196	10.33	2.35			9.67	2.38					11.84
VMC30/229	**6.67**	**1.80**	**14.67**	**3.60**	**12.00**	**3.43**	**8.33**	**2.44**	**18.67**	**1.88**	**19.73**
VMC30/232	**22.67**	**4.09**	**12.00**	**3.11**	**31.00**	**4.14**	**4.67**	**1.83**	**-**	**-**	**10.97**
VMC30/216	22.67	3.83	4.00	1.90	3.33	1.81	-	-	14.67	1.72	**10.65**

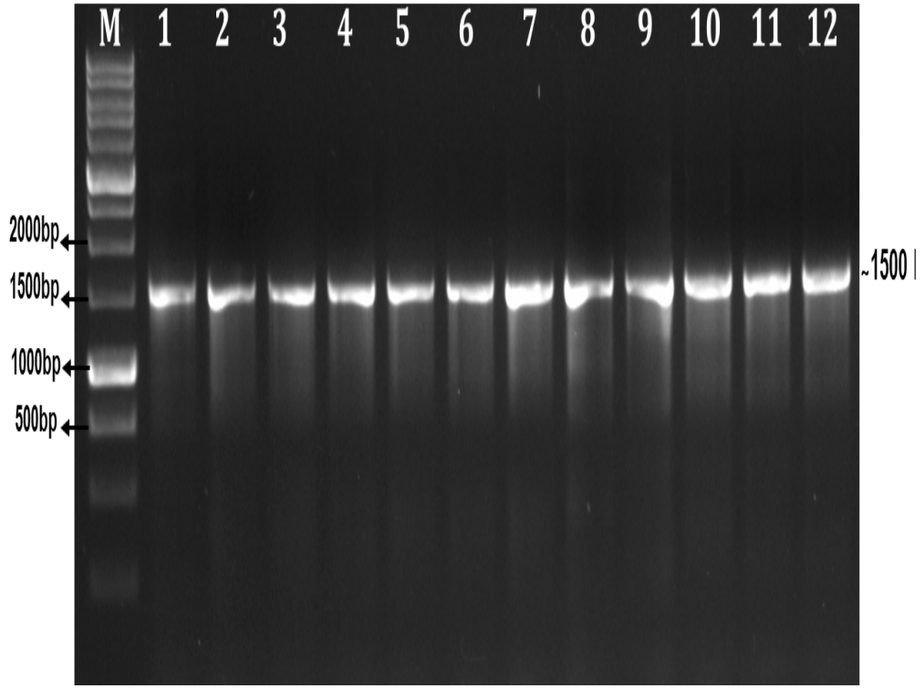
Internal criteria fixed for Selection

- ✓ N-fixation-10mm halo zone,
- ✓ P-solubilization -5mm,
- ✓ K-solubilization-10mm,
- ✓ Zinc-10mm,
- ✓ siderophore-10mm,
- ✓ IAA-10ppm,
- ✓ Ammonia-'+++'.

MOLECULAR CHARACTERIZATION STUDIES

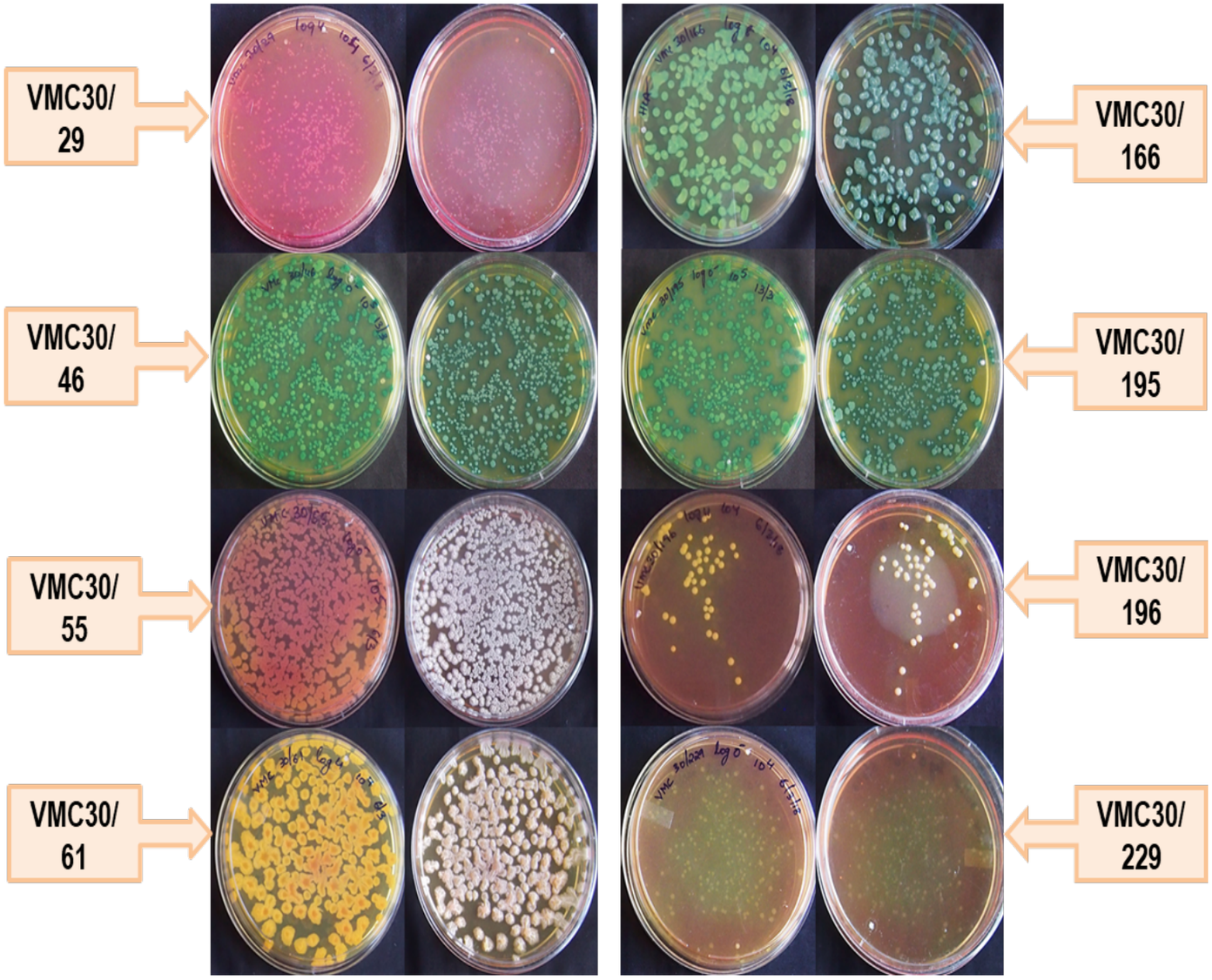


16SrRNA gene amplification

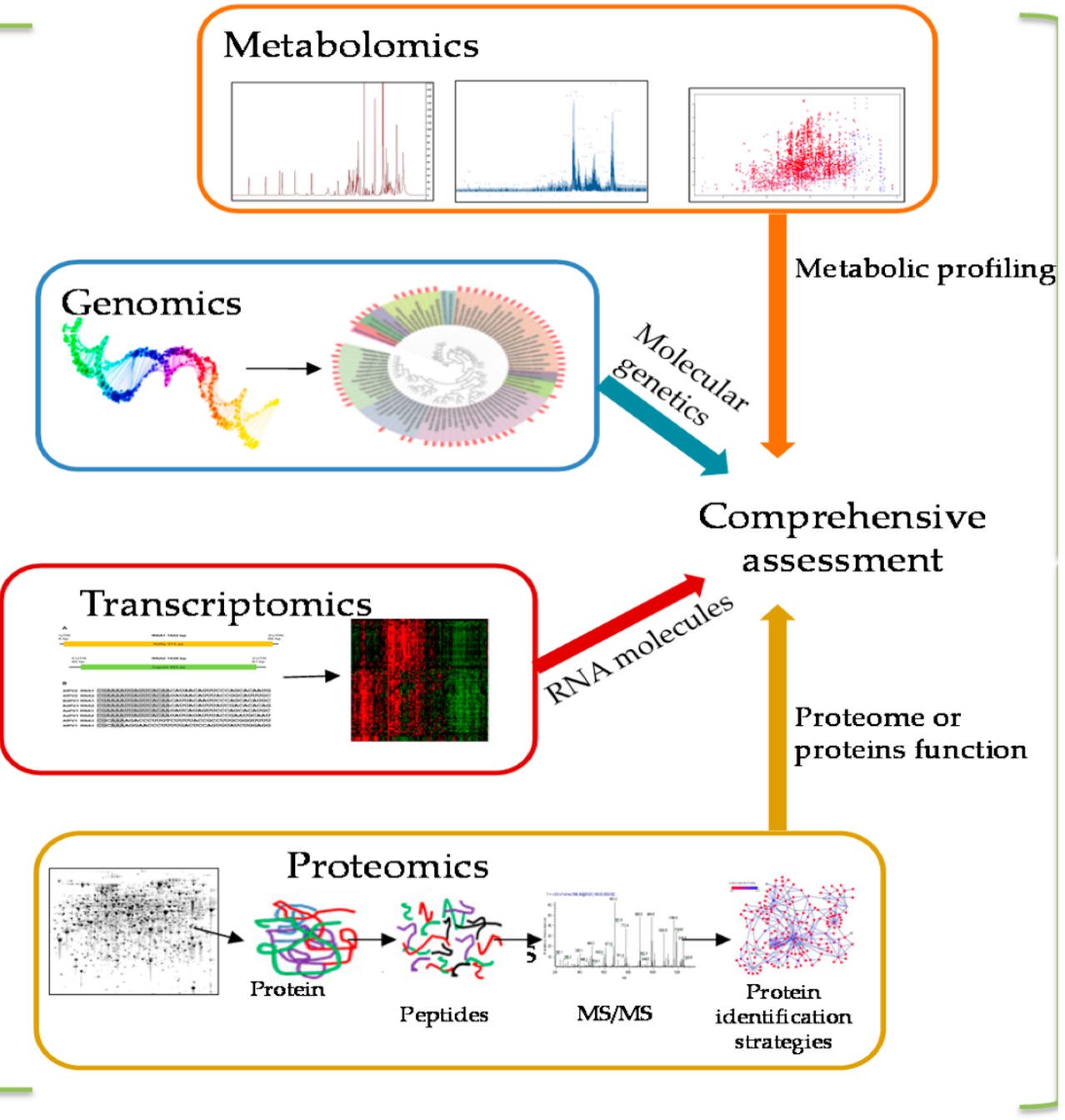


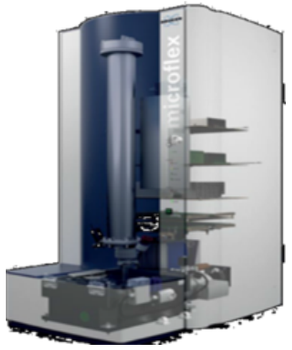
M-1 kb DNA ladder
1-VMC30/240
2-VMC30/234
3-VMC30/217
4-VMC30/210
5-VMC30/235
6-VMC30/215
7-VMC30/212
8-VMC30/207
9-VMC30/198
10-VMC30/262
11-VMC30/235
12-VMC30/263

NBA APPROVED STRAINS



Strain Code	Taxonomic designation	NCBI Accession Nos.	NCMR Accession Nos
VMC30/196	*Bacillus megaterium*	MG952575	MCC 0524
VMC30/29	*Bacillus simplex*	MG952569	MCC 0525
VMC30/46	*B. pumilus*	MG952570	MCC 0526
VMC30/55	*Bacillus subtilis subsp. subtilis*	MG952572	MCC 0527
VMC30/61	*Bacillus amyloliquefaciens*	MG952571	MCC 0528
VMC30/166	*Bacillus licheniformis*	MG952573	MCC 0529
VMC30/195	*Bacillus pumilus*	MG952574	MCC 0530
VMC30/229	*Bacillus oceanisediminis*	MG996509	MCC 0531
VMC30/216	*Pseudomonas putida*	MG996511	MCC 0532
VMC30/232	*Enterobacter hormaechei*	MG996510	MCC 0533





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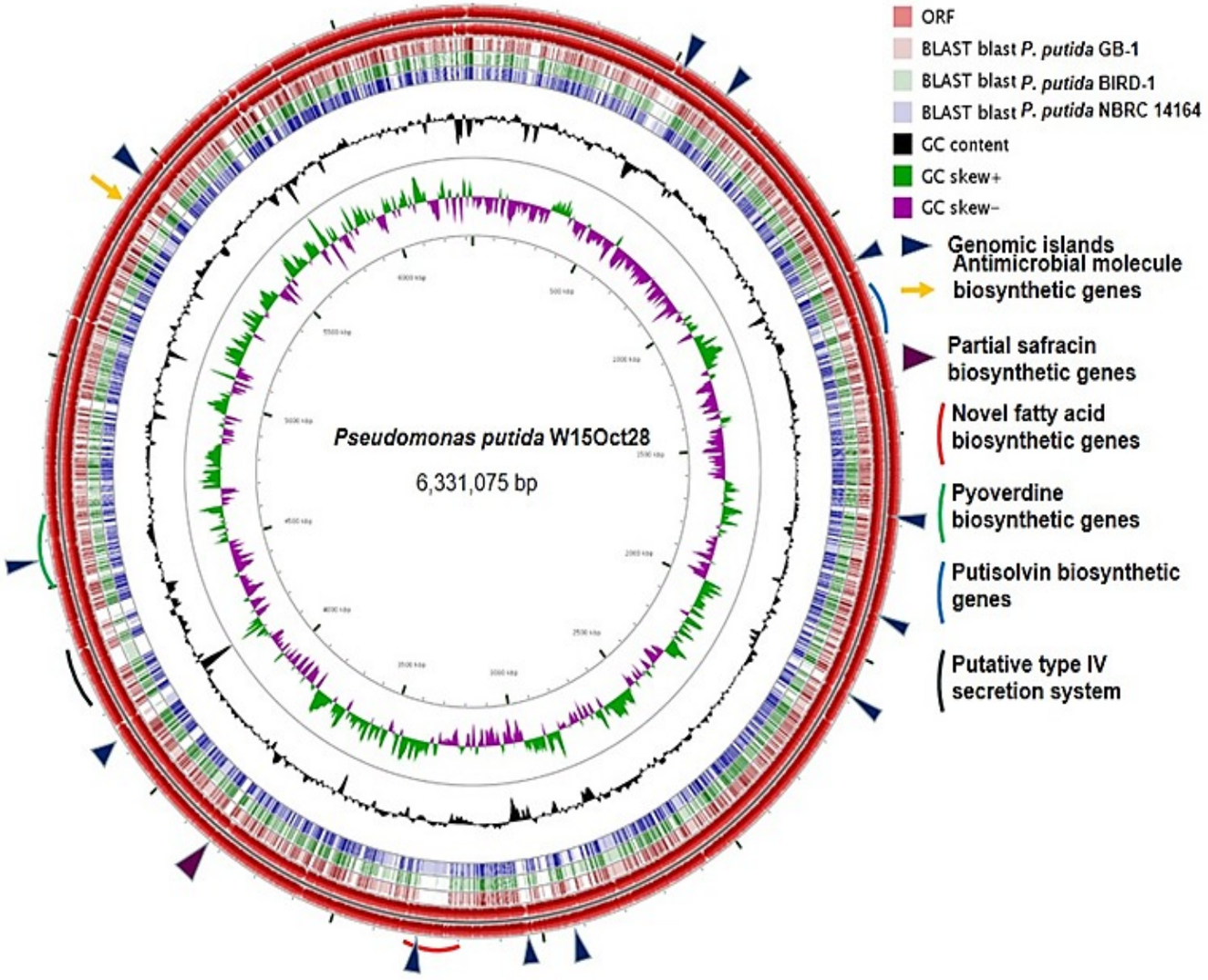
WHOLE GENOME SEQUENCING

New approach for IP Protection



Element	Biochemical Process	Microbial genes
Nitrogen	Nitrogen fixation	nifD, nifH, nifK
	Protein depolymerization	apr, npr, sub
	Urea catabolism	ureA, ureB, ureC
Phosphorous	Phosphate ester cleavage	phoA, phoD, phoX, ACPase, glpQ, ushA, appA, phyA, phyB
	Phosphonate breakdown	phnJ, phnX
Sulfur	Sulfate ester cleavage	aslA, asfA
	Sulfonate breakdown	ssuD

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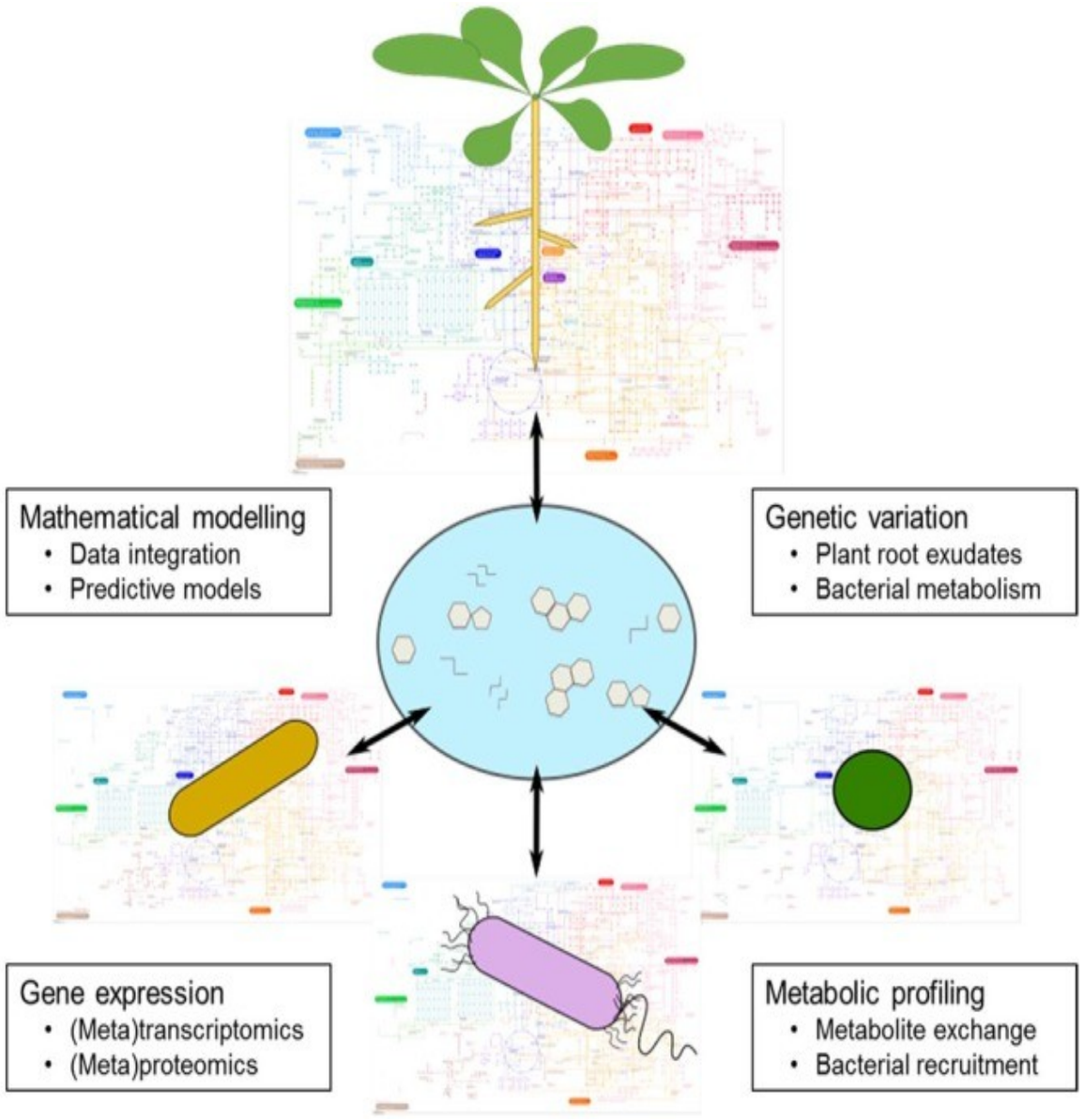
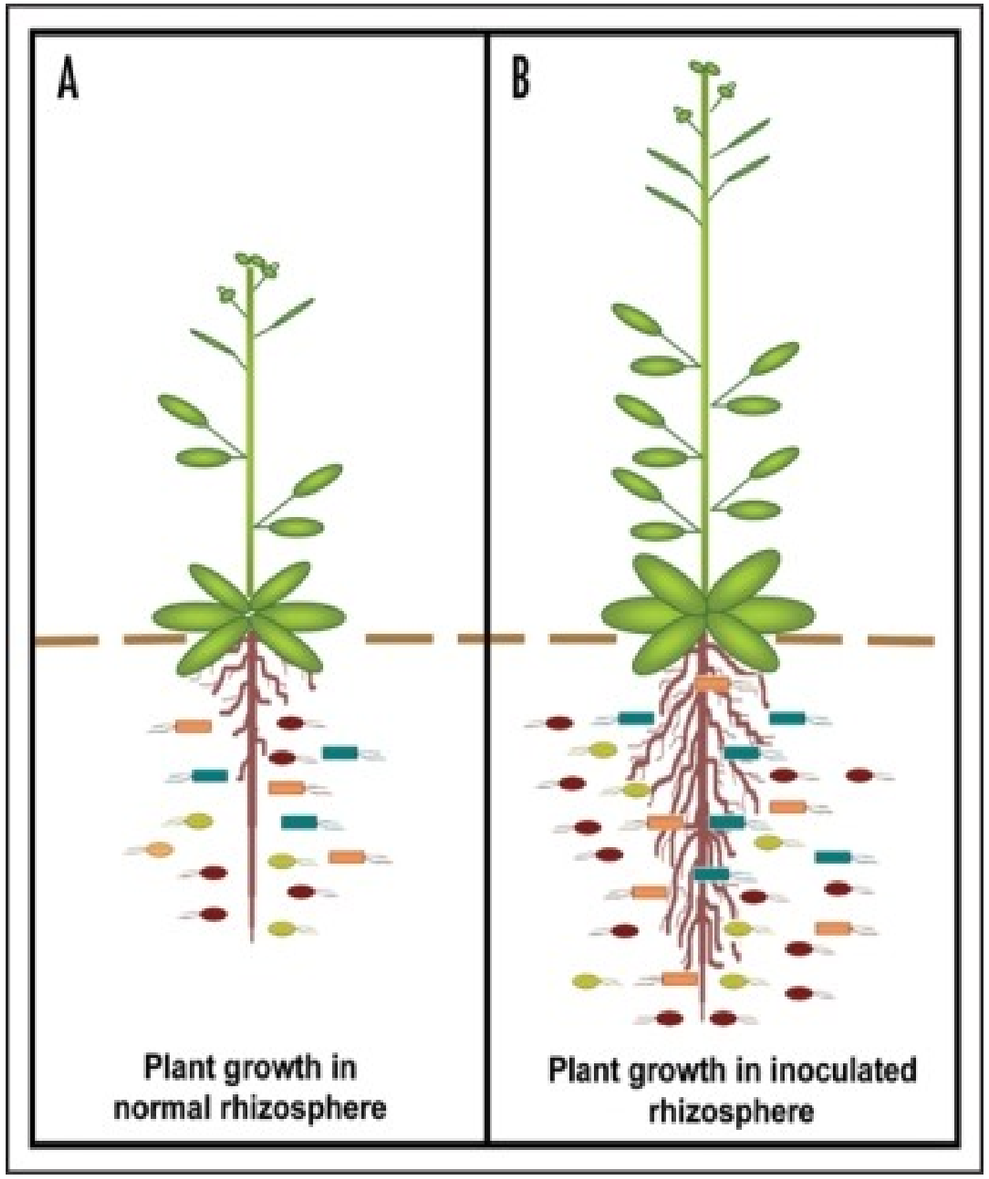
OMIC Studies





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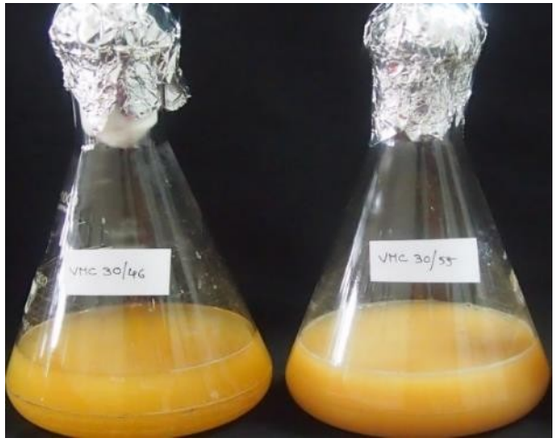
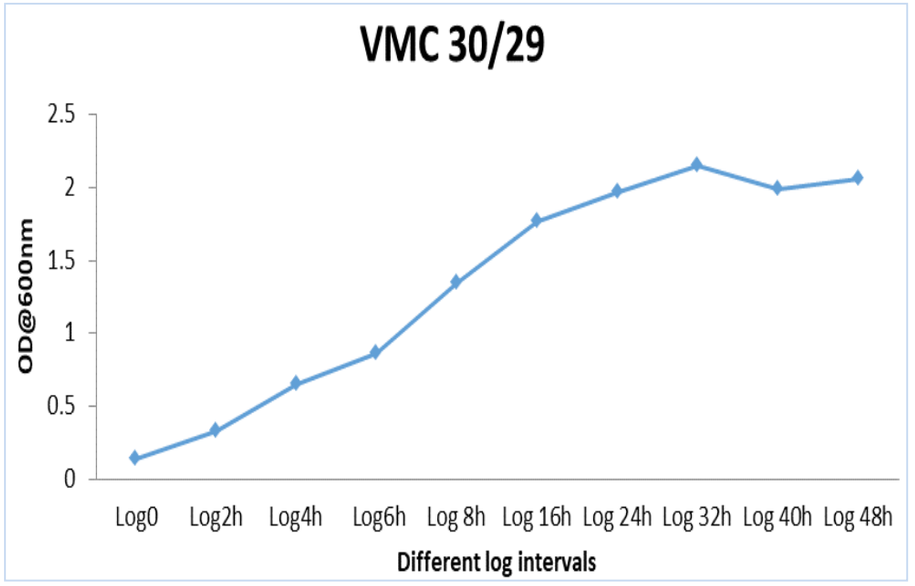
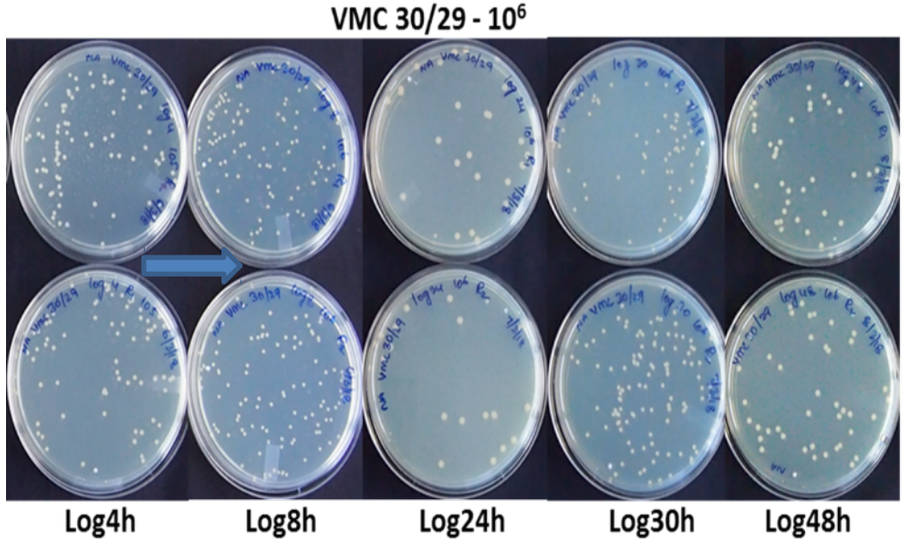
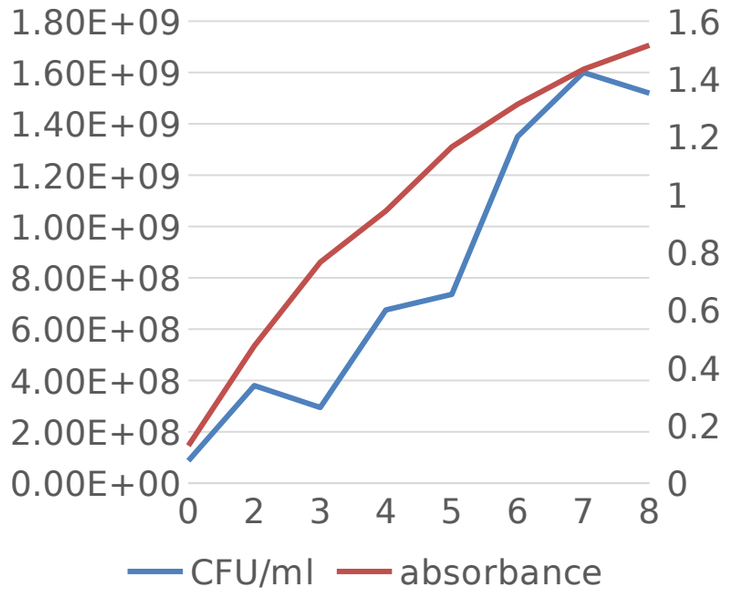
Growth curve studies









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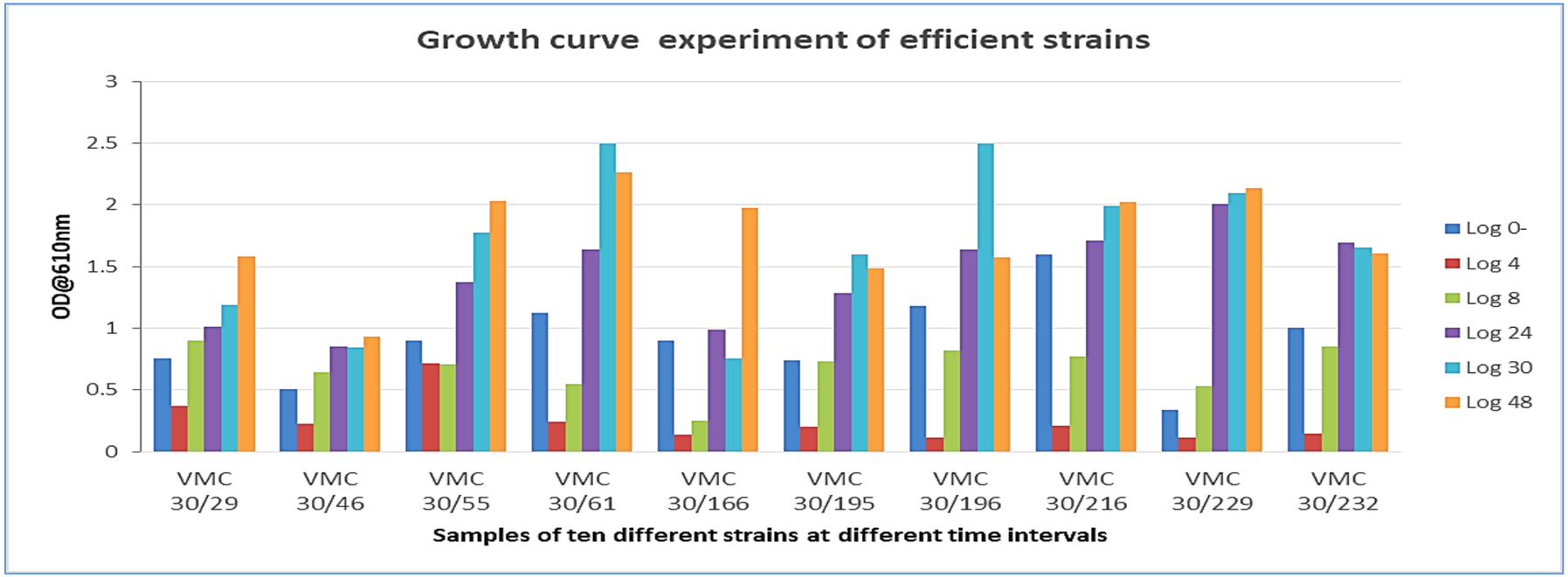
Growth curve studies of 10 strains at flask level fermentation



Microbial count (CFU/ml)

Time interval	VMC 30/29	VMC 30/46	VMC 30/55	VMC 30/61	VMC 30/166	VMC 30/195	VMC 30/196	VMC 30/216	VMC 30/229	VMC 30/232
Log 0-	2.00E+08	1.37E+09	4.12E+08	3.55E+07	2.10E+08	1.00E+09	1.00E+07	2.45E+09	4.60E+07	1.89E+09
Log 4	4.50E+07	1.60E+08	1.19E+08	4.22E+07	4.30E+06	5.00E+07	2.10E+06	3.24E+06	2.12E+06	4.21E+06
Log 8	5.10E+08	1.06E+08	6.35E+08	2.00E+08	9.80E+06	9.40E+07	7.80E+07	1.24E+07	3.42E+07	3.24E+07
Log 24	1.20E+08	2.17E+09	3.15E+08	1.37E+09	6.50E+08	1.60E+09	1.70E+07	4.42E+09	1.70E+09	8.63E+09
Log 30	5.40E+08	3.07E+09	3.50E+08	2.75E+09	4.30E+08	1.80E+09	1.10E+08	3.24E+09	1.52E+09	7.25E+09
Log 48	2.30E+08	6.75E+07	1.48E+09	2.15E+09	4.40E+08	2.10E+09	2.70E+07	2.02E+09	1.10E+09	7.41E+09

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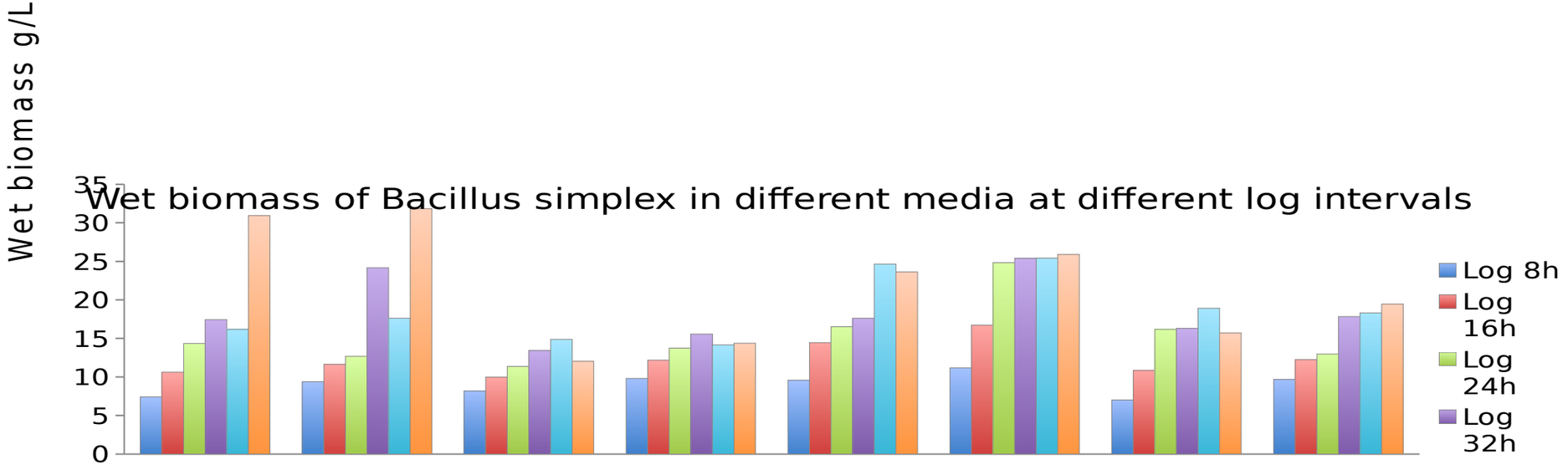
Media and Inoculum optimization studies for *Bacillus simplex*

Time intervals	Microbial count cfu/ml							
	NBG		MNB		BSM		TYM	
	1%	10%	1%	10%	1%	10%	1%	10%
Log0+	6.80E+06	9.80E+07	6.75E+06	1.25E+08	7.55E+06	1.11E+08	9.60E+06	8.55E+07
Log 8h	4.25E+08	6.23E+09	5.95E+08	4.50E+10	2.40E+08	3.20E+09	5.10E+09	2.01E+10
Log 16h	6.55E+09	8.60E+08	9.35E+09	2.35E+09	6.65E+08	2.60E+08	1.42E+10	7.25E+09
Log 24h	2.25E+10	3.50E+09	3.70E+10	6.85E+09	4.45E+10	**1.65E+09**	6.30E+10	4.05E+10
Log 32h	3.70E+09	2.30E+09	7.95E+10	8.30E+09	4.05E+10	9.50E+08	1.53E+11	1.00E+10
Log 40h	3.70E+09	5.45E+09	3.95E+09	3.90E+09	2.65E+09	1.90E+09	3.80E+10	9.60E+09
Log 48h	3.70E+09	5.45E+09	1.00E+09	5.00E+08	1.65E+09	1.40E+09	9.50E+08	1.00E+09



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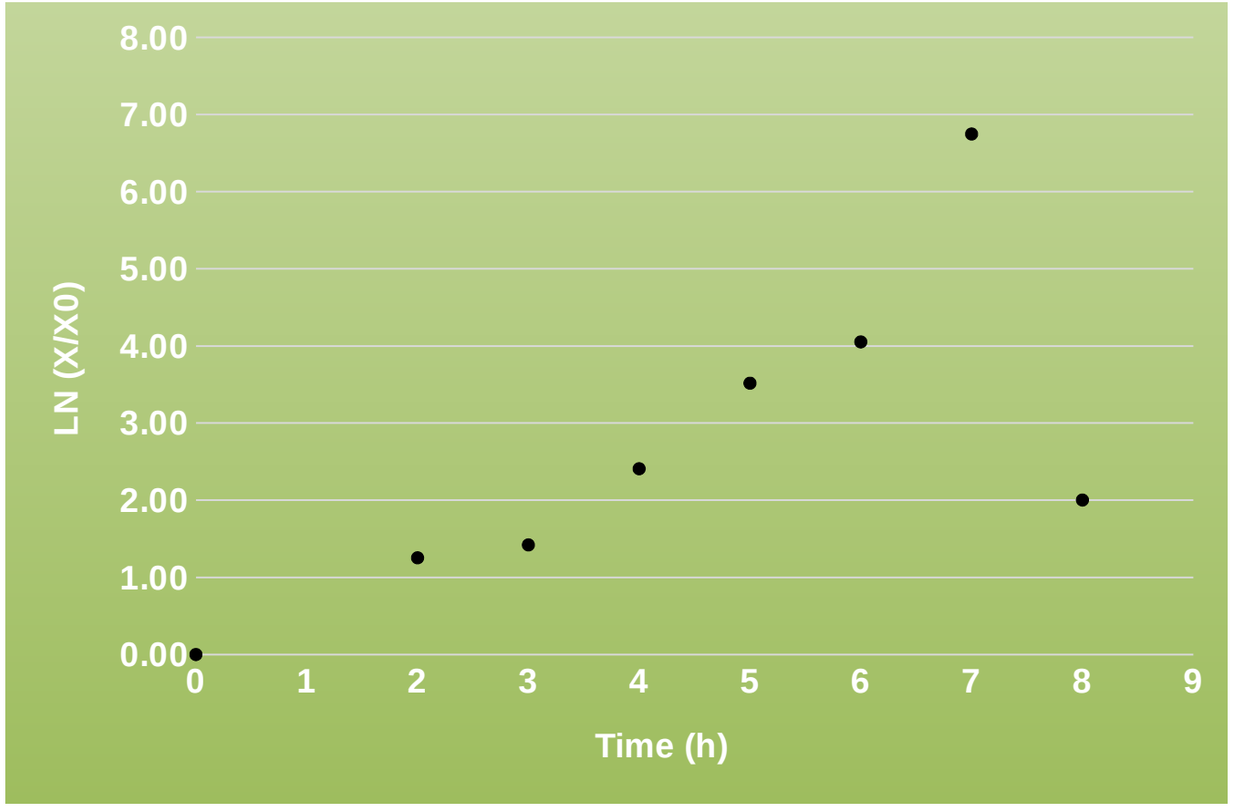
SET UP **best conditions for growth**

KINETIC parameters

Medium	Depending on strain
Inoculum (L)	1
Medium Volume (L)	9
Temperature (°C)	30
Dissolved Oxygen (%)	80
Stirring (RPM)	110
Air (L/min)	10 lpm

VMC 30/196

14 L Fermentation		
Time	**Fermentation Timing**	**Viable Count**
(hh:mm)	(h)	(CFU/ml)
09:45	0	1,35E+07
11:45	2	4,73E+07
12:45	3	5,59E+07
13:45	4	1,50E+08
14:45	5	4,55E+08
15:45	6	7,77E+08
16:45	7	1,15E+10
17:45	8	1,00E+08



Wet Biomass (g/L)	Dry Biomass (g/L)	Total Dry Matter (g/L)
13,48	1,94	17,3

Yield data need to be confirmed on a bigger scale

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Large scale fermentation-1 KL

VMC 30/29					
Date (M/D/Y)	**Time (H:Mi)**	**Sampling (Hr)**	**Turbidity (OD600)**	**pH**	**Viable Count (Cells/ml)**
Pre – seed culture					
2/12/2018	5.30PM	Log0	0.131	6.79	4.70E+07
3/12/2018	7.30AM	Log14h	1.24	7.4	6.00E+08
14L seed inoculum					
3/12/2018	8.15 AM	Log0	0.217	6.6	7.70E+07
3/12/2018	11.00AM	Log3h	0.996	7.33	7.45E+08
3/12/2018	12.00PM	Log4h	1.321	7.64	
3/12/2018	12.30PM	Log5h	1.513	7.87	5.15E+10
1000 L fermentation					
3/12/2018	1.30PM	Log0	0.003	6.76	3.50E+06
3/12/2018	2.30PM	Log1h	0.017	6.83	3.50E+06
3/12/2018	3.30 PM	Log2h	0.104	6.92	3.85E+07
3/12/2018	4.30PM	Log3h	0.47	7.21	7.10E+07
3/12/2018	5.30PM	Log4h	0.909	7.52	1.75E+08
3/12/2018	6.30PM	Log5h	1.276	7.82	3.75E+08
3/12/2018	7.00PM	Log5.5h	1.53	7.95	6.30E+08

wet biomass	9.0kg from 1000L	1.70E+10
Lyophilized powder	900g from 9 kg	1.85E+11

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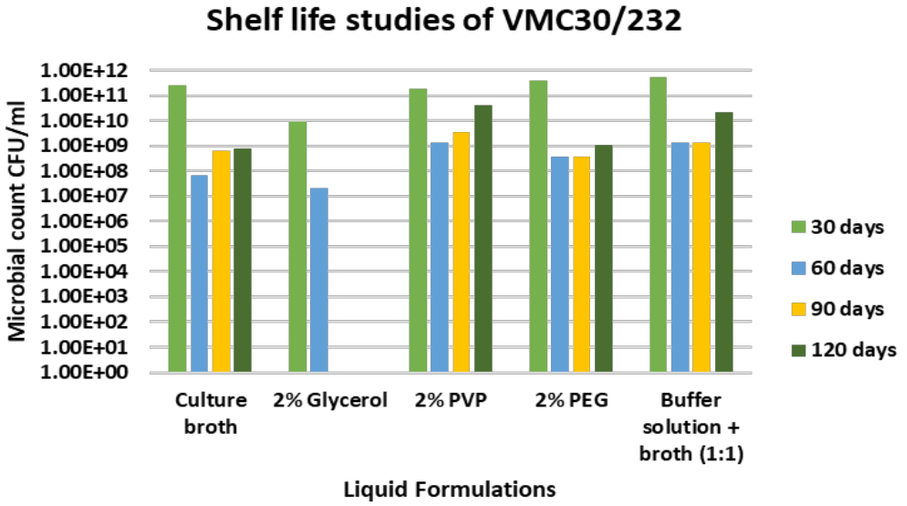
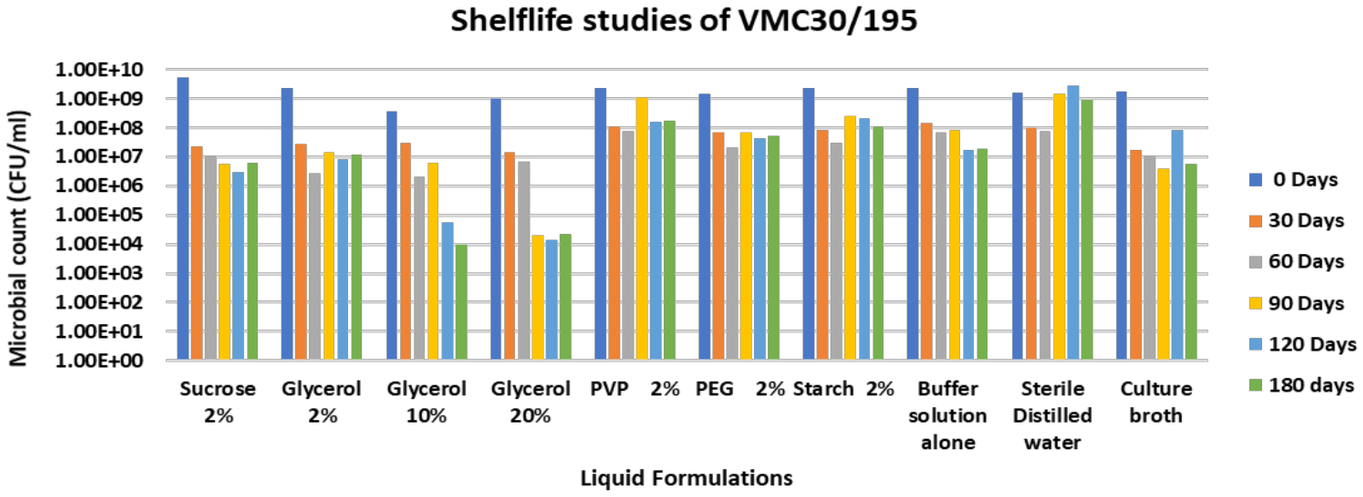
HYORO PLUS

Liquid formulation studies

Formulations	Microbial count					
	0 Days	30 Days	60 Days	90 Days	120 Days	180 days
Sucrose 2%	5.20E+09	2.35E+07	1.05E+07	5.50E+06	3.05E+06	6.50E+06
Glycerol 2%	2.29E+09	2.80E+07	2.75E+06	1.40E+07	8.15E+06	1.20E+07
Glycerol 10%	3.55E+08	3.10E+07	2.05E+06	6.30E+06	5.50E+04	1.00E+04
Glycerol 20%	1.00E+09	1.40E+07	7.00E+06	2.00E+04	1.45E+04	2.15E+04
PVP 2%	2.38E+09	1.11E+08	7.90E+07	1.06E+09	1.52E+08	1.74E+08
PEG 2%	1.42E+09	6.85E+07	2.10E+07	7.05E+07	4.40E+07	5.05E+07
Starch 2%	2.30E+09	7.95E+07	3.05E+07	2.40E+08	2.13E+08	1.12E+08
Buffer solution alone	2.22E+09	1.49E+08	7.10E+07	8.05E+07	1.80E+07	1.95E+07
Sterile Distilled water	1.60E+09	9.80E+07	7.80E+07	1.39E+09	2.72E+09	8.85E+08
Culture broth	1.75E+09	1.80E+07	1.10E+07	3.85E+06	8.40E+07	5.80E+06





Stabilizers tested	Buffer solution g/L
Sucrose 2%	
Glycerol 2%	NaCl ----- 8
Glycerol 10%	KH_2PO_4 ----- 2
Glycerol 20%	KCl ----- 2
PVP 2%	Na_2HPO_4 ----- 2
PEG 2%	pH ----- 6.9
Starch 2%	
Buffer solution	
Sterile Distilled water	
Culture broth	

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Phenomics

High throughput image analysis

High-throughput, multi-spectrum image analysis to detect morphometric and physiological parameters

Scanalyzer™ unit with different inlays for multiwell plates (left) and trays (right)

In-house HTS phenomic system for model plants

- **UV (fluorescence)**: to analyze the photosynthetic efficiency

- **Visible - RGB**: morphology, architecture, digital biomass, green and yellow index

- **NIR (Near Infra-Red)**: plant water content

OUTCOME Phenotype characterization of nutritional, hydrological, physiological state of plants

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Phenomics

High throughput image analysis

High-throughput, multi-spectrum image analysis to detect morphometric and physiological parameters

https://www.youtube.com/watch?v=xj3-r9sJyZM

In collaboration with ALISA, Metapontum

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PRIMARY SCREENING

Controlled environment

On Field testing

Plant growth chamber

Biological incubator

BEST APPLICATION METHODS, TIMING, RATES

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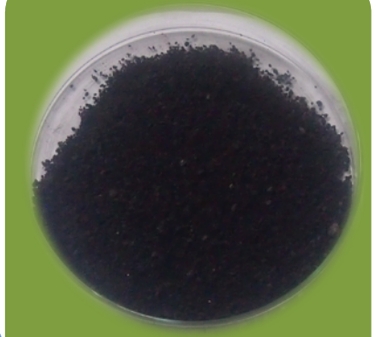
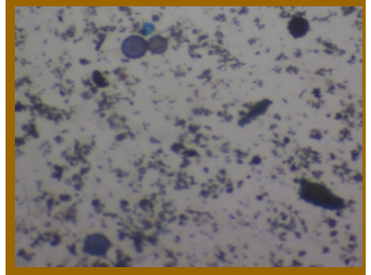
MYCORRHIZA-NOVEL FORMULATION

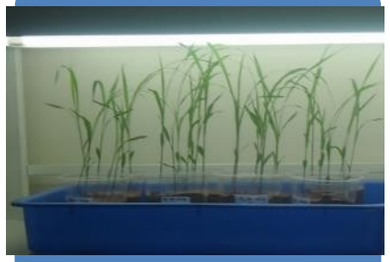
PROTOTYPING



Testing Different formulations

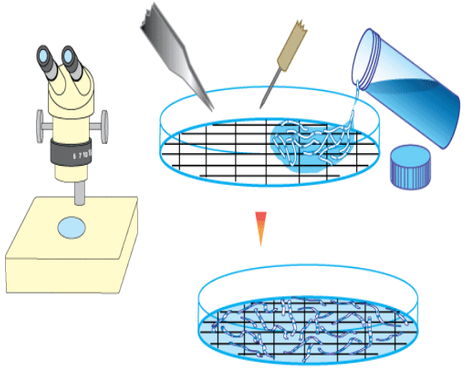


Spore count by wet sieving method and microscopic observation



Testing on sorghum by Soil application

Root colonisation



REALIZATION OF THE BEST PROTOTYPES

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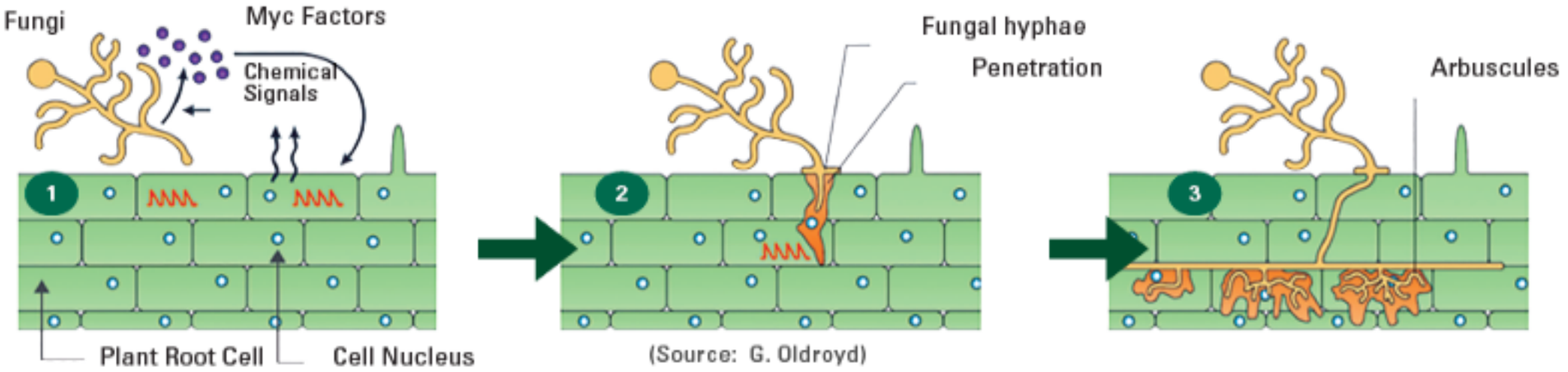
MYCORRHIZA

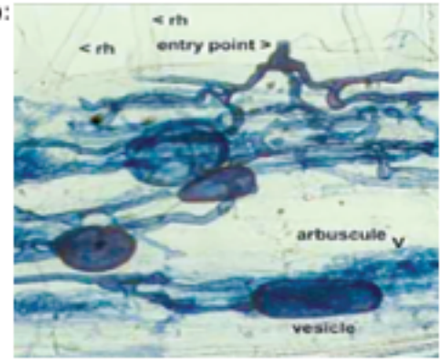
Mycorrhizae: HOW IT WORKS

1. The spores of mycorrhizal fungi germinate after receiving the chemical signal from the roots
2. Endomycorrhizal Fungi colonize the intercellular spaces of roots
3. The Arbuscular Mycorrhizal Fungi (AMF) disseminate through the growth of hyphae inside the roots and soil

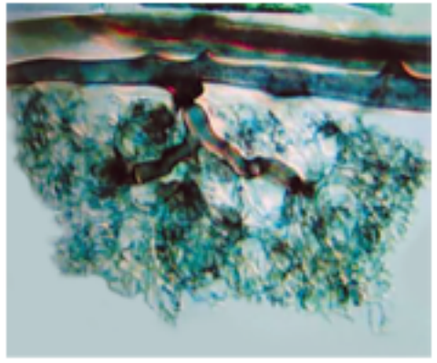


Endomycorrhizae: magnification of spores

(Photo: G. Bécard)



Endomycorrhizae: detailed structures ovoid (vesicles) and branched (Arbuscules)
(Photo: J. Deacon)



Endomycorrhizae: magnification of Arbuscules (Photo: M. Amaranthus)

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HYORO MY+

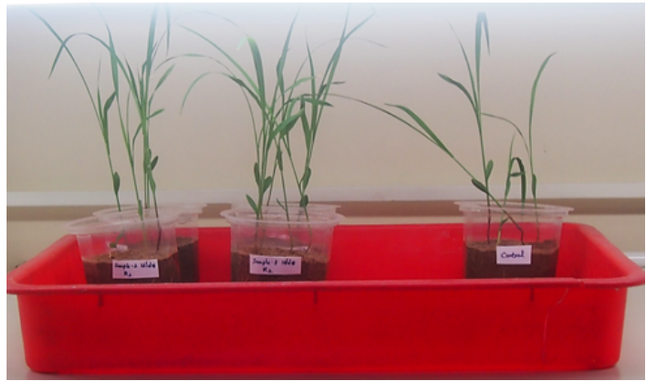
Mycorrhiza Root Colonization Studies

Treatments (45 DAT)	Average Root length	Average Shoot length	plant wet biomass/plant
F1(A)-250 spores/g	8.00	23.67	0.84
F1(B)-166 spores/g	12.00	27.33	0.56
F1(C)-100 spores/g	**16.00**	**37.00**	**1.80**
F2(A)-300 spores/g	9.67	15.33	0.96
F2(B)-200 spores/g	8.33	17.67	1.24
F2 (C) -150 spores/g	18.00	47.00	2.14

Treatments	% of colonization	% of Arbuscule colonization	% of spore colonization	Spore count
F1(C)-100 spores/g	**100%**	**58.00**	**32**	**163**
F2 (C) -150 spores/g	**100%**	**66.00**	**40**	**779**
F2(D) -120 spores/g	**100%**	**57.00**	**23**	**130**
Sample 01	100%	22.00	7.00%	153

Highest root biomass observed in F2(D)120 spore/gm compared to control after 45 DAT

Sorghum seedlings after 45 DAT of Mycorrhiza



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THANK YOU
FOR YOUR ATTENTION

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