Production Of Ammonia In Rhizosphere Bacteria Isolated From Chickpea Field

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Introduction

Ammonia (NH_3) is the foundation for the nitrogen (N) Fertilizer industry. It can be directly applied to soil as a plant nutrient or converted into a variety of common N fertilizers. Special safety and management precautions are required. Ammonia is undoubtedly one of the essential chemicals in the agriculture world as a fertilizer and in the chemical industry as a starting reagent for production of other chemical. (Ithisuphalap et. Al.,2019).Ammonia is present in soil water and Air and it is an important source of nitrogen for plants.
Nitrogen promotes plant growth and improve fruit and seed production resulting in a greater yield.
Ammonia occurs naturally in the environment it is found in relatively low nontoxic concentration in soil, air and water and provides a source of nitrogen for plant.
Rhizobacteria are root associated the form symbiotic relationship with many plants plant growth promoting rhizobacteria (PGPR) are a group of free living bacteria .that live in the plant Rhizosphere, aggressively colonize the root system and have been studies as plant growth promoters for increasing agricultural production.
Plant microbe interaction have been utilized to improve plant growth for the production of wood, fiber, Bio-Fuels and key molecule.
In the era of sustainable agricultural production. Plant-Bacteria interactions in the Rhizosphere play a pivotal role in transformation, mobilization, Solubilization etc.
Keeping in view the importance of ammonia production by bacteria for sustainable agriculture, the present study was conducted to investigate prevalence of ammonia producing rhizobacteria from chickpea rhizosphere from Nagpur area.

Objectives

There is almost no information Available on production of ammonia from rhizobacteria especially from the Nagpur area.
Keeping this in view the present investigation was conducted with following objectives .
❑ To study the production rate of ammonia from rhizobacteria at different pH.

Methods

The rhizospheric soil samples (eight) were collected from fields growing Chickpea (C. arietinum L.) from Nagpur, India. All bacterial strains were isolated on their respective media.

Cleaning and washing of glassware's.

Collection of Rhizosphere soil or collection of soil sample.

Pull up the chickpea plant in a circular motion, tap off the excess of soil and collect the soil which is stick on the root surface. Collect that soil into a plastic bag. Total 8 sample was collected named as S2, S3, S4, S5, K1, K2, K4, and K5.

Isolation of bacteria

Total 164 bacteria straine were isolated from rhizospheric soil sample. Soil sample were serially diluted in distilled water and inoculated on different media like, nutrient agar medium for total heterotrophs, macconkey agar for coliform. And after incubation at 28-37°C for 24-48hrs . bacteria colonies were isolated bacterial cultures.
Characterization of bacteria for plant growth promoting (PGP) traits.
Bacteria isolates were characterized for PGP traits employing standard procedures. The following traits were analysed.

Ammonia production

Bacteria isolates were screened for the production of Ammonia in peptone water. Freshly grown culture were inoculated in 10 ml peptone water in different tube and incubated for 48-72 hrs. After 2-3 day's Nessler's reagent (0.5ml) was added in each tube development faint yellow to dark brown color indicated that production of ammonia.

PH Tolerance

For determining pH tolerance of the isolated bacteria they were inoculated in nutrient brother with varying pH (5, 6 7,8 and 9) and incubated 48-72 hrs at 37 C observation on bacterial growth were made after 3 days.

Acknowledgements

Success is an essential of hard work, discipline, sincerity focused approach and encouragement guidance so with warm gratitude. Here with I acknowledge all those whose guidance and encouragement made this poster possible.
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Results

The plant Rizosphere is a versatile and dynamic ecological environment of intense micro plant interaction for harnessing essential micro and macro nutrients from the limited nutrient pool.
Ammonia (NH_3) is undoubtedly one of the essential chemicals in the agriculture world as a fertilizer. And in the chemical industry as a strong reagent for production of other chemicals for this reason, ammonia is the second most produced chemicals with the production of 200 million tonnes annually . The production of ammonia from the bacteria have the broad impact of the society.
In the present investigation there are Rizosphere bacteria was isolated from chickpea field. After the isolation the production of ammonia were observed at different ph level. It is a great finding for the agriculture field production of ammonia is one of the important attributes in Rizosphere bacteria population that has promise to replace chemical fertilizers.

Isolation of Rhizobacteria from soil sample

From 8 Rhizospheric soil sample isolated different type of colonies and inoculated it on another fresh plate and make a masterplate. Total 164 bacterial strain isolated from that soil sample.

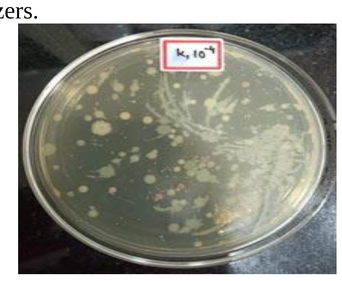

Fig no 1:- Growth of soil sample k1 at 10-4 dilution

pH Tolerance

The bacteria isolates from chickpea field displayed tolerance to variable range of pH of the 164 bacterial isolates studied for their tolerance to pH range, 156(95.1%) of displayed tolerance to a wide range of pH 5-9 (Table no 1) and neutral pH 7 very high of the isolates exhibited growth. However, majority (95.1%) of the isolates showed Tolerance to wide range of pH 5-9.

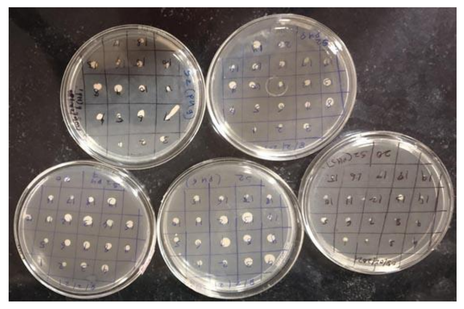

Fig No 2-The colonies of Rhizospheric bacteria in different pH

Production of Ammonia

Was observed predominantly among isolates from chickpea field . In early study production of ammonia was detected in (74.1%). Bacteria isolates from rhizospheric soil of chickpea of conventional farm in the Nagpur area.
The effect of pH on the growth of Rizosphere and production of ammonia Rizosphere were tested for their ability to grow at 5-9 ph. At the different pH concentration the production was also different. The results are given in (table no 1) there are 164 isolated Rizosphere bacteria from chickpea field tested, from that 122(74.4%) isolated produced ammonia and 38(23.1%) were highly ammonia producers moderate and low level of ammonia production was noted in 39(23.7%) and 45(27.4%), respectively additionally around 98% of isolated showed tolerance to wide range of pH 5-9.

The Nessler's reagent method use for detection of ammonia. Most common Nessler's reagent is a solution contacting K2HgI4 and KoH. Iodide and mercury ions react with ammonia under alkaline conditions to produce a reddish -brown Complex. It is worth noting that
(1) Mercury ions in Nessler's reagent are toxic and thus the reagent should be used carefully .
(2) The life time of Nessler's reagent is relatively short (around three weeks).
(3) The water used to prepare the Nessler's reagent solution must be free ammonia (ultrapure water) and
(4) The reaction time of ammonia with Nessler's reagent also affects the accurate quantification of NH_3 with a reaction time from 10 to 30 min being recommend.
Solution pH is an important factor for the quantification of ammonia. The effect of pH of the reaction medium on NH_3 detection. The effect of pH on ammonia detection with Nessler's reagent the method were largely insensitive to pH at pH values.
Nessler's reagent was added in tube development of faint yellow to dark brown colour indicated that production of ammonia.

Fig No 3:- Test for ammonia production dark brown coloration high ammonia production

Fig No 4:-Test for ammonia production brownish yellow coloration moderate ammonia production.

Fig No 5:-light yellow coloration no ammonia production

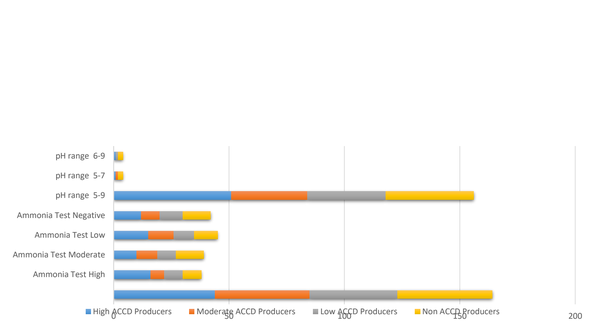

Fig no 6:-production of ammonia by rhizospheric bacteria in different pH level

Organism No.		Ammonia				pH	Range	
		High	Moderate	Low	Negative	5-9	5-7	6-9
High ACCD Producers	44	16	10	15	12	51	1	1
Moderate ACCD Producers	41	6	9	11	8	33	1	0
Low ACCD Producers	38	8	8	9	10	34	0	1
Non ACCD Producers	41	8	12	10	12	38	2	2
Total	164	38	39	45	42	156	4	4

Table No 1:- Rate of ammonia related to PH

Conclusion

The rhizosphere is the part of the soil ecosystem where plant roots, soil and the soil biota interact with each other. These interaction are often of benefit to plants, improve soil fertility and enhance the degradation of soil contaminants. extensive use of chemical fertilizers has led to widespread degradation of agricultural soil. Production of ammonia is one of the important attributes in rhizospheric bacterial population that has promise to replace chemical fertilizers. The present study was undertaken to find out production of ammonia among rhizospheric bacteria isolated from chickpea field. Of 164 isolates tested, 122 (74.4%) isolates produced ammonia and 38 (23.1%) were highly ammonia producers. Moderate and low level of ammonia production was noted in 39 (23.7) and 45 (27.4%), respectively. Additionally, around 98% of isolates showed tolerance to wide range of pH 5-9. Further studies are in progress on these bacteria in promotion of different crops under wide range of pH.





Augmentation of *Triticum durum* (var.poshan) by a rhizoshperic yeast

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INTRODUCTION

- Phosphate solubilizing microorganisms (PSM) play an important role in converting the insoluble P in soluble form which can be easily utilized by plants.
- These microorganisms enhance the crop productivity and offer a better substitute to the harmful chemical fertilizers and supplements.
- PSM include bacteria, fungi and actinomycetes.
- Phosphate solubilizing yeast have not been extensively investigated yet but have the potential to be utilized as a bio-inoculant in agriculture for the development of biofertilizers (S. -F. Fu et al. 2016)

OBJECTIVES

- Isolation of phosphate solubilizing microbes from the rhizosphere of *Triticum durum* (var.Poshan).
- Assessment of other plant growth promoting traits of isolated PSM.
- Effect of selected isolate on plant growth parameters of durum wheat.

METHODOLOGY

Sample collection and isolation of phosphate solubilizing microorganism (PSM).(Nautiyal 1999)

↓

Selection of potential PSM through Phosphate solubilizing index (PSI) (Nakayan et al. 2013)

↓

Morphological Identification of Isolate

↓

Assessment of other plant promoting traits of selected isolate
1. IAA production (Nakayan et al. 2013)
2. Ammonia production (Cappuccino and Sherman,1992)
3.Zinc solubilization (Kamran et al. 2017)
4. Cellulase activity (Nakayan et al. 2013)
5. Catalase activity (S. -F. Fu et al. 2016)

↓

Assessment of plant growth promotion by yeast (plate germination assay) (Amprayn et al. 2011)

RESULTS

Isolation of PSM and morphological identification

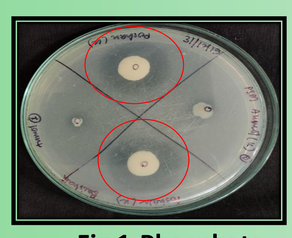


Fig 1-Phosphate solubilization by yeast
3.2±0.05 PSI.

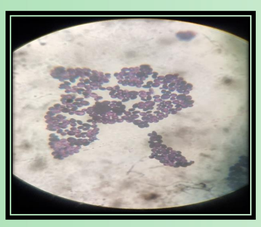
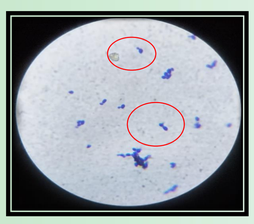


Fig 2-Microscopic view of budding yeast



Phosphate Solubilization Index of yeast (PY1)

No. of Days	Diameter of Colony (mm)	Diameter of Zone (mm)
Day 1	4	7
	4	7
	3	6
Day 2	5	10
	5	8
	4	9
Day 3	6	14
	6	12
	5	13
Day 4	7	18
	7	19
	6	18
Day 5	9	20
	9	22
	8	20
Day 6	10	22
	10	23
	10	21

PSI =Colony diameter+ Clear zone diameter
Colony Diameter

= 10 + 22
10

= 3.2

Plant growth promoting traits of yeast

Fig 3-Zinc solubilizing by yeast

Fig 4-Cellulase activity by yeast

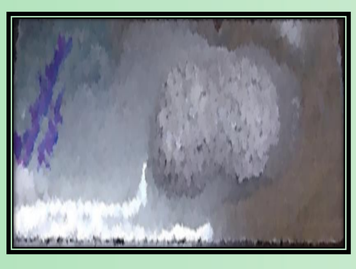
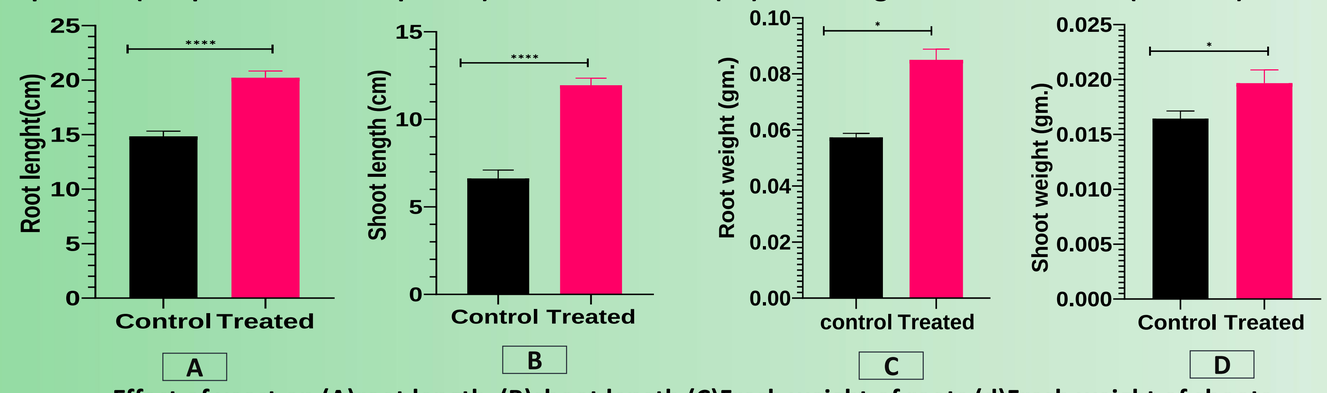


Fig 5-Catalase activity by yeast

PGP traits	(+/-)
IAA production (ug/ml)	+ (7.93±0.24)
Ammonia Production (ug/ml)	+ (5.8±0.07)
Zinc solubilization	++
Cellulase activity	++
Catalase Activity	++

Assessment of plant growth promotion by PY1

Influence of yeast on seed germination and wheat plant growth parameters. Values are means of three replicates (ten plants in each replicate)± standard errors (SE) according to Student's t-test ($P < 0.05$).



Effect of yeast on (A)root length (B)shoot length (C)Fresh weight of roots (d)Fresh weight of shoots

	Germination %	Root length (cm)	Shoot length (cm)	Fresh wt. of roots (gm)	Fresh wt. of shoots (gm)
Control	73±0.66	14.84±0.47	6.7±0.4	0.05±0.002	0.016±0.0006
Treated	83±0.88	20.22±0.61	11.94±0.4	0.085±0.003	0.019±0.001

CONCLUSION

- Phosphate solubilizing Yeast (PY1) isolated from the rhizosphere of durum wheat was showing multiferous PGP traits including IAA production, ammonia production, zinc solubilization, cellulase and catalase activity.
- Seeds treatment with PY1 showing enhanced effect on different growth parameter as compared to control.
- Therefore our results indicated that isolated yeast PY1 have potential to be utilized as a bio-inoculant in agriculture for the development of biofertilizers.

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PLANT GROWTH ENHANCING SUBSTANCES BY SOLID STATE FERMENTATION

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ABSTRACT

Various agroindustry by-products are generated viz., paddy husk, peanut shell, corn cob, sawdust, bagasse, wheat straw, pressmud, etc. These agroindustry by-products are just burnt or disposed to the landfills. They can be converted into value-added products viz., compost, animal feed, single cell protein, enzymes, etc. by solid state fermentation using plant growth promoting rhizobacteria. The plant growth promoting rhizobacteria produce plant growth substances. The work here describes solid state fermentation using bagasse as a substrate for production of plant growth promoting substances by *Herbaspirillum* sp. viz., indole acetic acid, enzyme - chitinase, iron chelating compounds - siderophores, solubilisation of mineral - potassium, etc. The indole acetic acid production was 20 (µg/ml), the zone of clearance which indicated chitinase and siderophores production was 1.4 and 1.9 cm respectively on 20th day. The potassium solubilisation was also more on 20th day which was 1.1 cm. These plant growth promoting substances can be used for the plant growth. The production of plant growth substances by solid state fermentation using *Herbaspirillum* sp. will be eco-friendly, economical, easy and also help in the management of bagasse by converting into value-added product. Further pot and field experiments to study the effect of plant growth substances on the plant growth need to be carried out.

Keywords: Agriculture, Economical, Eco-friendly, Sustainable, Vermicompost

INTRODUCTION

India produce nearly 40 million matrix tons (MMT) of bagasse and nearly 3 tons of bagasse is produced by one industry. Bagasse is ecofriendly and easily available in large quantity. Bagasse is either incinerated or disposed directly to the landfills. It can be converted to different value-added products by solid state fermentation. Development of biotechnological products containing plant-beneficial microorganisms needs research.

MATERIALS & METHODS

Isolation, characterization and identification of plant growth promoting rhizobacteria from the rhizosphere region

Collection and processing of the samples

Isolation, characterization and identification of PGPR

Production of enzymes by the isolates

Source of the agroindustry by-product – bagasse

***In-situ* production of plant growth promoting substances by *Herbaspirillum* sp.**

Substrate for the SSF

Inoculum preparation and SSF

RESULTS

Isolation, characterization and identification of PGPR

- ✓ Total three isolates were obtained which were *Pseudomonas* sp. (isolate No. 3), *Alcaligenes* sp. (isolate No. 4) and *Herbaspirillum* sp. (isolate No. 5).
- ✓ The isolate no. 5 was motile, Gram -ve short rod (Figure 1).
- ✓ Comparing with Bergey's Manual of Determinative Bacteriology, the isolate no. 5 belonged to *Herbaspirillum* sp.

Table 1: Morphological characteristics of the isolate No. 5.

Morphological Characters	Isolate No. 5
Size (mm)	12
Shape	Irregular
Color	White
Margin	Undulated
Elevation	Flat
Opacity	Opaque
Consistency	Smooth
Gram character	Gram - ve short rods
Motility	Motile

Day	Plant growth promoting substances			
	Chitinase	Siderophore	Potassium solubilization	IAA
0	1.9 ± 0.00	-	0.6 ± 0.00	8±0.00
5	0.9 ± 0.00	1.4 ± 0.01	0.9 ± 0.00	10±0.01
10	0.9 ± 0.02	2.0 ± 0.01	0.9 ± 0.01	17.2±0.03
15	1.2 ± 0.00	2.3 ± 0.00	1.0 ± 0.01	20±0.01
20	1.2 ± 0.01	2.1 ± 0.00	1.2 ± 0.01	21±0.00

The data represents average of triplicate. The zone of clearance is in mm and IAA production is in µg/ml.

DISCUSSION

- ➢ There is a report on citric acid production (Yadegary *et al*., 2013) and coconut aroma (da Penha *et al*., 2012) through SSF with bagasse as substrate.
- ➢ Also, work focused on production of gibberellic acid by SSF with bagasse as substrate (Rodrigues *et al*., 2009). Study on production of biocontrol fertilizer from brewer's spent grain by SSF had been carried out (Qiu *et al*., 2019).
- ➢ There are very few reports on *in-situ* production of plant growth promoting substances by *Herbaspirillum* sp. using bagasse as substrate.
- ➢ There is a report on paddy husk as carrier for *in-situ* production of plant growth promoting substances by *Burkholderia gladioli* and *Bacillus subtilis* (Gunjal *et al*., 2015).
- ➢ More study needs to be carried on *in-situ* production of plant growth promoting substances by *Herbaspirillum* sp. The effect of the leachate from SSF needs to be checked by performing further field experiments.

CONCLUSIONS

❖ The *in-situ* production of plant growth substances by *Halomonas* sp. will be very eco-friendly and economical.

❖ This will help in the plant growth which will ultimately benefit the farmers. Also, it will help in the management of bagasse by converting it into value-added product i.e.., plant growth promoting substances.

❖ This is a biological approach and reduces the use of chemical fertilizers.

❖ The field experiment needs to be carried on large-scale to check the effect of leachate on the plant growth.

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Registration No: 1.5

Efficacy of native Rhizobium isolates on growth and yield of summer groundnut

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Introduction/Rationale/Objective

- Groundnut is one of the key remunerating crop in Gujarat and have importance in state GDP.
- Nitrogen is one of the major nutrients required for groundnut growth and productivity, and found most commonly deficient in soil to meet the demand and thereby reducing yields.
- Groundnut accommodates *Rhizobium*- A potent symbiotic nitrogen fixing bacteria in the root nodules, which is involved in plant growth promotion by multiple traits.
- Tribal small and marginal farmers can not afford costly nitrogen fertilizers and mainly dependent on BNF support for sustainable groundnut production and conserve soil nitrogen resources.
- *Rhizobium* is very important for sustainable groundnut production, hence a systematic study regarding diversity and efficacy of native *Rhizobium* population in summer groundnut from middle Gujarat was carried out first time with following objectives.
  - Isolation, characterization and evaluation of PGP traits of native *Rhizobium* spp. from summer groundnut cultivated area of middle Gujarat.
  - Efficacy of selected native *Rhizobium* isolates for Plant Growth Promotion of summer groundnut in field.

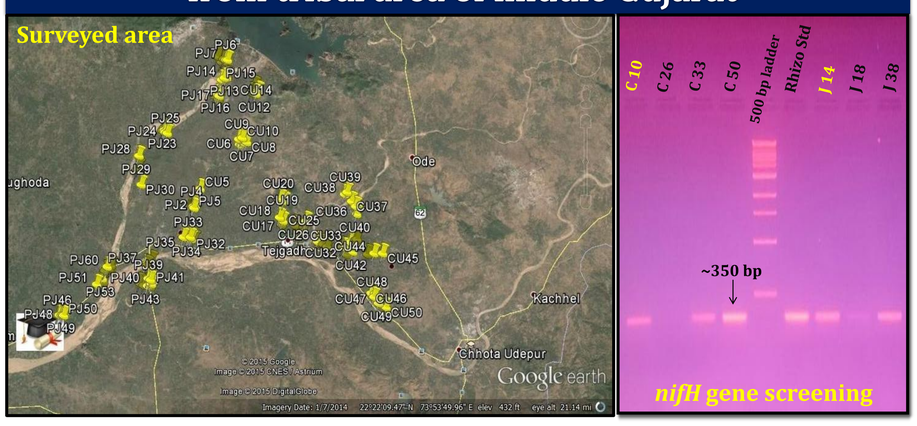
Materials & Methods

- A GPS based sampling survey was undertaken and more than 100 samples (Root nodule and soil) were collected from farmers' fields cultivating summer groundnut in middle Gujarat.
- Isolation of *Rhizobium* spp. was carried out on CRYEM agar plate using methodology given by Vincent (1970).
- Representative native *Rhizobium* isolates were identified through morphological, colonial and molecular approaches (Halt et al., 1994) and characterized further for additional plant growth promotion traits in laboratory *viz*. phosphate and potash solubilization, IAA production (Sawar and Kremer, 1995), lytic enzyme production, antifungal activity using standard methodology (Bai et al., 2002).
- Selected isolates were tested in field (RBD; Treatments: 10; Replications: 3) in summer groundnut *cv*. GJG 31 comprising different combinations of Nitrogen and FYM for enhancement of plant growth.
- At harvest, yield and plant growth parameters *viz.* pod and biomass yield, plant height, number of pods/plant, seed index were recorded.

Results

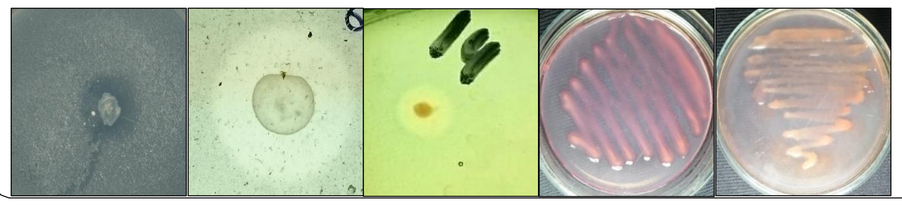
- From 100 samples, total 138 isolates obtained and **14 were screened** out based on morphological and cultural characters.
- **PCR detection of *nif*H** gene revealed, **5** isolates *viz*. **C 10, C 33, C 50, J 14** and **J 38** showed intense band of ~350 bp.
- Isolate **C 10** designated as **GNR I** and identified as ***Rhizobium huautlense* (Accn. No: KU836508)** while isolate **J 14** designated as **GNR II** and identified **as *Rhizobium giardinii* (Accn. No: KU836509)** based on 16S *r*RNA gene sequencing analysis.

Isolation and characterization of native *Rhizobium* from tribal area of middle Gujarat

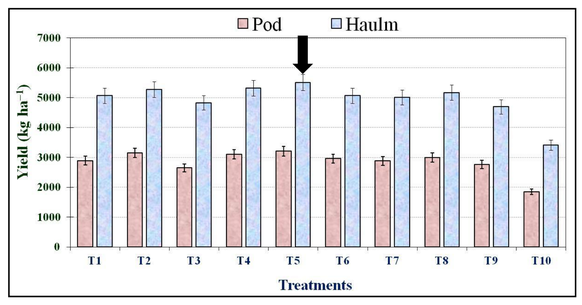


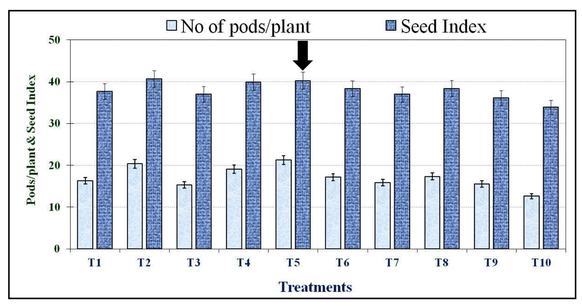
PGP Traits Characterization

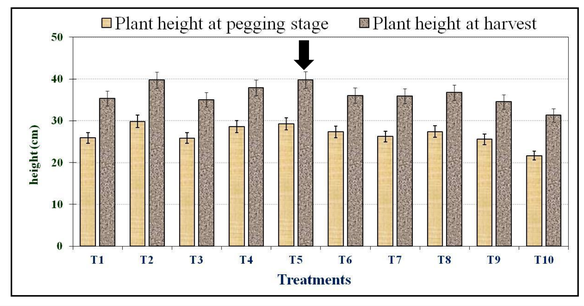
Isolate No	Nutrient solubilization		Siderophore production	Enzyme production		
	P	K		ACC Deaminase	Lipase	Cellulase
C 10	+	+	+	+	-	+
C 33	-	-	+	-	+	-
C 50	+	+	+	-	-	-
J 14	+	+	+	+	-	+
J 38	-	-	+	-	+	-



Efficacy of native *Rhizobium* isolates on yield and growth attributes of summer groundnut *cv*. GJG-31 in field (Year: 2017-19)







Treat. No.	Treatments details	Treat. No.	Treatments details
T_1	FYM @ 5 ton/ha	T_6	T_1 + *Rhizobium* standard
T_2	N @ 25 kg/ha	T_7	T_3 + AAU GNR-1
T_3	N @ 12.5 kg/ha	T_8	T_3 + AAU GNR-2
T_4	T_1 + AAU GNR-1	T_9	T_3 + *Rhizobium* standard
T_5	T_1 + AAU GNR-2	T_{10}	Control

Note: Phosphorus applied @50 kg/ha through SSP in T_2, T_3, T_7, T_8, T_9
Rhizobium inoculation@5 ml/kg of seed (seed treatment)

Conclusions

- **From 138 isolates, two promising N fixing cultures *Rhizobium huautlense* (Accn No: KU836508) and *Rhizobium giardinii* (Accn No: KU836509) have been screened out, which is also having additional benefits of P & K solubilization, IAA, siderophore & ACC deaminase production making them proficient PGPR.**
- **Field efficacy of native *Rhizobium* isolates revealed that different yield and growth attributes of groundnut were significantly influenced by the treatment of native *Rhizobium giardinii* AAU GNR 2 along with FYM, recorded the highest groundnut pod yield 3,207 kg/ha, which is 80 % higher over control with saving of 25:50 kg/ha of N:P fertilizer.**
- **Investigation outcome provided efficient native N2 fixing *Rhizobium* isolates from tribal area to support livelihood of famers by curtailing use of NPK fertilizers and improving soil health with sustainable agro-ecosystem.**

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6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
(3-4, September 2021)





PGPR – BOON TO AGRICULTURAL PRACTICES

REGISTRATION NO. – 1.8

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Introduction

The rhizosphere is the narrow zone of soil specifically influenced by the root system. This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria. Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria that colonize in the plant roots and promote plant growth. A major threat to crop plants are fungal phytopathogens. Rhizobacteria induce resistance via the salicylic acid-dependent SAR pathway (Systemic Acquired Resistance). Genera *Pseudomonas* and *Bacillus* of rhizobacteria are well known for their ability to trigger ISR (Induced Systemic Resistance) and also for their antagonistic effects. PGPRs are often seen as potential candidates that provide various micronutrients to crop plants by solubilizations, which decrease micronutrient deficiencies. PGPRs have been used as effective bioinoculants, biocontrol agents, mineral solubilizers, etc. Out of different ecofriendly approaches, plant growth promoting rhizobacterial (PGPR) strains may acts as an efficient nematode biocontrol. Microbes of the rhizomicrobiome play key roles in nutrient acquisition and assimilation, improved soil texture, secreting, and modulating extracellular molecules such as hormones, secondary metabolites, antibiotics, and various signal compounds, all leading to enhancement of plant growth.

PGPR strains	Agriculture crops	Nematodes
Bacillus subtilis	Tomato	*Rotylenchulus reniformis*
Azotobacter chroococcum	Tomato	*Meloidogyne incognita*
	Brinjal	*Meloidogyne javanica*
Pseudomonads stutzeri	Turmeric	*Meloidogyne incognita*
Bacillus velezensis and Bacillus mojavensis	Soybean	*Heterodera glycines*

Table 1: Effects of PGPR inoculation on plant parasitic nematodes biocontrol

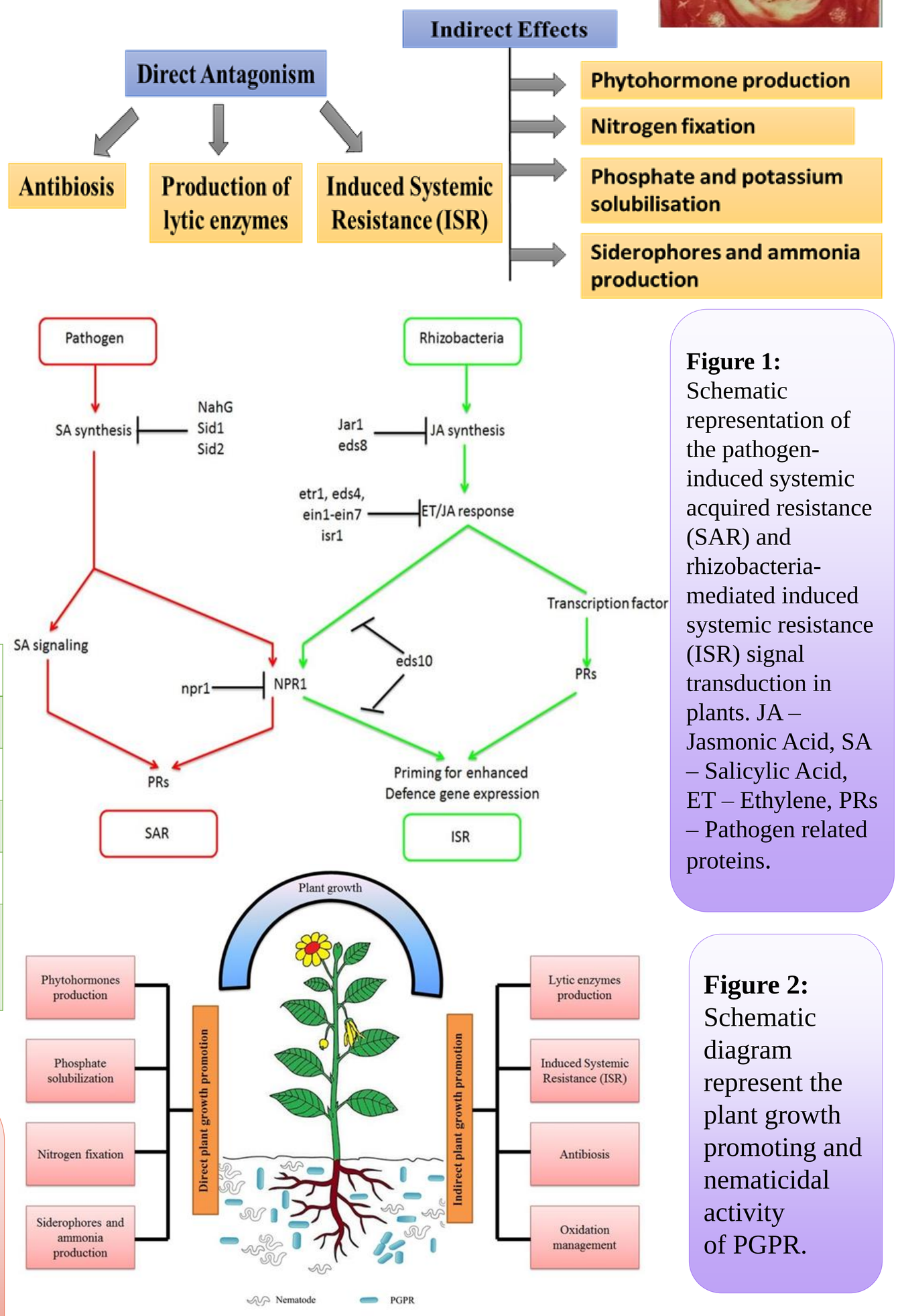

Figure 1: Schematic representation of the pathogen-induced systemic acquired resistance (SAR) and rhizobacteria-mediated induced systemic resistance (ISR) signal transduction in plants. JA – Jasmonic Acid, SA – Salicylic Acid, ET – Ethylene, PRs – Pathogen related proteins.

Figure 2: Schematic diagram represent the plant growth promoting and nematicidal activity of PGPR.

Mechanism of PGPR in nematode Suppression

The mechanism of nematode suppression can be categorized manly in two major ways –
1. direct antagonism by producing enzymes, toxins and other metabolic products and
2. indirect effect by regulating nematode behaviour, altering root diffusates and inducing the production of repellents by host that adversely affects the host recognition, alteration the nematode feeding site development or sex ratio inside the root tissue, promoting plant growth, competing for essential nutrients and inducing systemic resistance

Conclusion

Evolution is pragmatic, random and relentless, and we should expect to discover many additional and sometimes surprising relationships that are beneficial to crops, and therefore global food production. Resistance-inducing and antagonistic rhizobacteria might be useful in formulating new inoculants, offering an attractive alternative of environmentally friendly biological control of plant disease and improving the cropping systems into which it can be most profitably applied.

Roadmap to Commercialization

The development of PGPR-based inoculants is not strictly defined but generally includes the following steps:
(1) Isolation of the bacteria from roots or other plant tissues.
(2) Laboratory and controlled growth environment screening.
(3) Field screening for a range of crops, geographic locations, planting dates and soil types.
(4) Evaluation of the possible combinations of strains and/or signals.
(5) Consideration of the management practices (e.g., agrochemical use and rotation)
(6) Refinement of the product.
(7) Experiments confirming absence eco-toxicological effects
(8) Product delivery formulation – e.g., peat, granular, liquid or wettable powder.
(9) Registration and regulatory approval of the product.
(10) Product available on the market.

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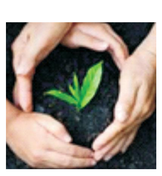
There is no acknowledgment.





EXPLORING SIDEROPHORE PRODUCING MICROBES ISOLATED FROM MANGROVES

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Naran Lala College of Professional and Applied Sciences, Navsari

Bhumi B. Patel

INTRODUCTION

•Iron is an essential element for most of the growth and development processes of every living organisms. Iron is the fourth most abundant element in the earth's terrestrial environment. This element exists in two readily inter-convertible oxidation states, Fe(II) and Fe (III).

•Most microorganisms have evolved adaptive mechanisms that can solubilize iron. One such strategy is the production of siderophore.

•Mechanism of siderophore is that, the bacterial cell release the siderophore & siderophore bind with ferric iron & bacterial cell convert ferric iron into ferrous iron. Ferrous iron release and then incorporated into plant cell to increase the plant growth.

Siderophore Greek word → **"Iron Carrier"**

•Siderophores are small, low molecular weight, metal chelating agents which are produced by plants and microorganisms in Fe-limiting conditions. Siderophores are secondary metabolites that are secreted into extracellular environment where they chelate ferric iron with high affinity.

•Siderophores are divided into main 4 families:

(1) Hydroxamate, (2) Catecholate, (3) Carboxylate, (4) Mixed type

AIM & OBJECTIVES

AIM: Exploring siderophore producing microbes isolated from mangroves

OBJECTIVES:

1. Isolation, screening and characterization of siderophore producing microbes.
2. Determination of siderophore type and its quantification.
3. Optimization of siderophore production.
4. Extraction of siderophore.
5. Determination of antimicrobial activity of siderophore.
6. Application of siderophores on plant growth enhancement.

MATERIALS & METHODS

[1] Sample collection from mangrove site

↓

[2] Isolation of microorganisms

↓

[3] Screening of siderophore producer
(Schwyn & Neilands, 1987)

↓

[4] Estimation of siderophore production

↓

[5] Characterization and typing of siderophores
(iron perchlorate test, tetrazolium test, csaky test, arnow's test, chemical test and spectrophotometric assay)
[Atkin *et al*., 1970, Csaky (1948), Vogel & Shenkar (1992), Snow (1954)]

↓

[5] Quantification of potential siderophore producer
(Payne S.M. 1993-1994)

↓

[6] Optimization of siderophore production
(pH, temperature, N-source, C-source, organic acid & salt concentrations)
[Tailor & Joshi (2012), V. K.S. *et al*., (2017), Sayyed, Vani & Shaikh (2016), Chaudhary *et al*., (2017)

↓

[7] Extraction of siderophore
[Arnow (1937), Rioux *et al*., (1983)]
(TLC) [Lemos *et al*., (1988)]

↓

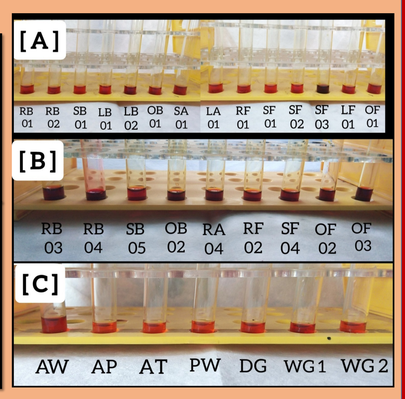
[8] Antimicrobial activity of siderophore
[Vincent *et al*., (1982)]

↓

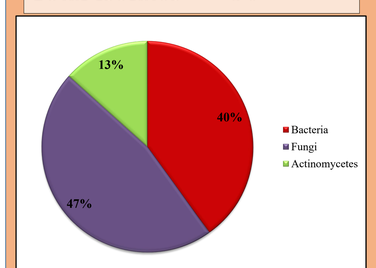
[9] Application of siderophore as bioinoculant
[Dimpka *et al*., (2009)]

RESULTS

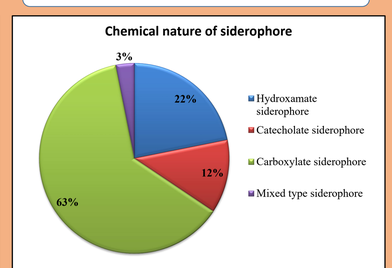
Sample Collection:



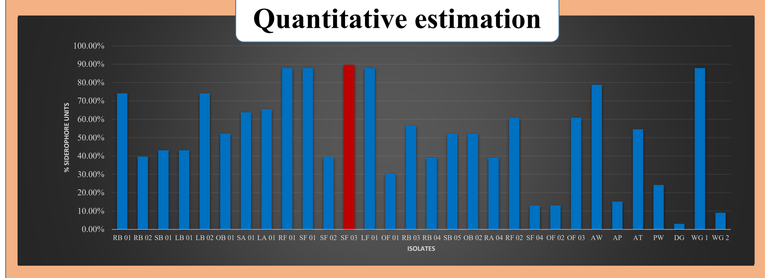
Total isolates → 30



Chemical characterization



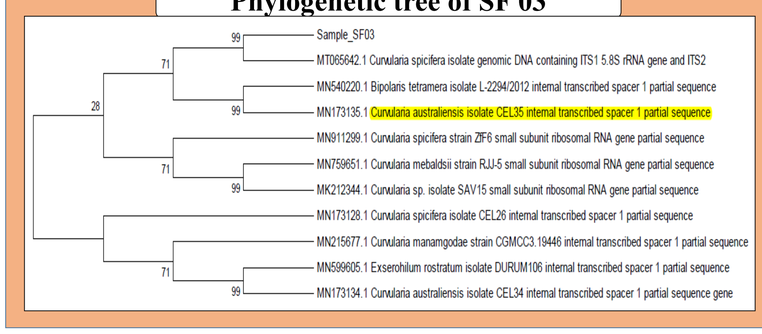
Quantitative estimation



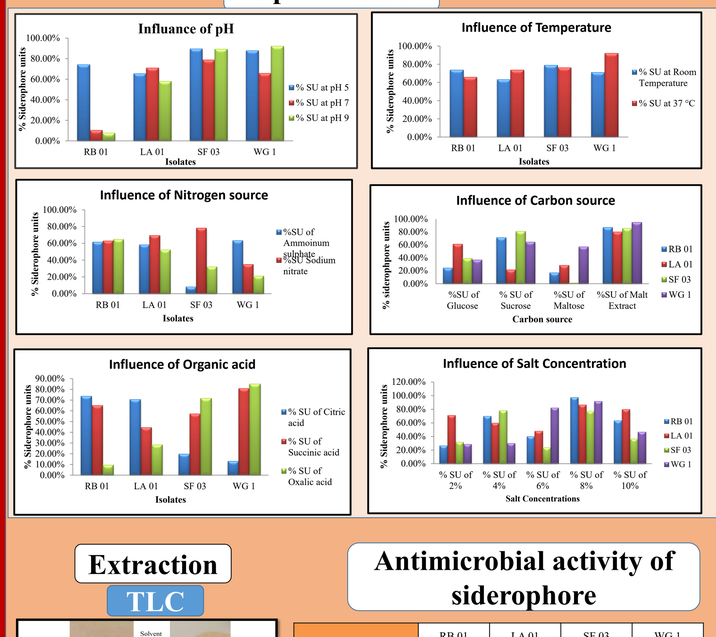
4 isolates were selected on the basis of siderophore production for further study & SF 03 was molecularly identified as ***Curvularia australiensis.***

ISOLATES	SIDEROPHORE %	CHEMICAL NATURE OF SIDEROPHORE
RB 01	74.13 %	Carboxylate type siderophore
LA 01	65.51 %	Carboxylate type siderophore
SF 03	89.65 %	Catecholate type siderophore
WG 1	87.87 %	Carboxylate type siderophore

Phylogenetic tree of SF 03

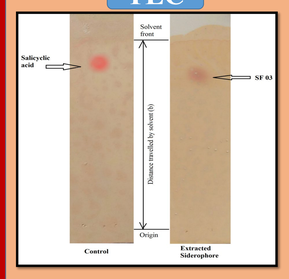


Optimization



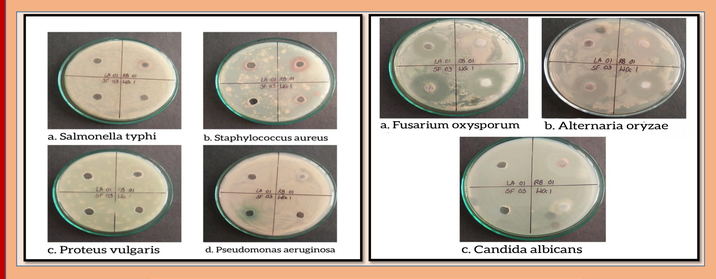
Extraction

TLC



Antimicrobial activity of siderophore

PATHOGEN	RB 01	LA 01	SF 03	WG 1
	Zone of diameter (mm)			
BACTERIA				
Salmonella typhi	19.66 ± 0.57	14.33 ± 1.15	14.66 ± 1.15	00
Staphylococcus aureus	18.33 ± 1.52	12.5 ± 1.32	14.33 ± 2.08	00
Proteus vulgaris	21.5 ± 0.50	00	00	00
Pseudomonas aeruginosa	16.33 ± 1.52	00	12.83 ± 2.02	00
FUNGI				
Fusarium oxysporum	00	14.33 ± 1.15	16.33 ± 1.52	18.33 ± 4.72
Alternaria oryzae	26.5 ± 4.09	00	20 ± 2	23.33 ± 2.08
Candida albicans	17.33 ± 3.78	00	00	00



Application as bioinoculant

Plant Variety	Pots	Incubation Time	Shoot Length	Root Length	No. of Leaves
Spinacia oleracea (spinach)	Control	20 days	13 cm	4 cm	3
	Test		18 cm	6 cm	7
Trigonella foenumgraecum (fenugreek)	Control	7 days	5.5 cm	1.5 cm	26
	Test		8 cm	5 cm	32
Vigna radiata (moong)	Control	7 days	10 cm	3.5 cm	10
	Test		14 cm	7 cm	12

Conclusion

•As a vital element, iron is needed by every living organism for their growth and development. Under iron deficient condition, microorganisms produce siderophore and scavenge iron from the environment.

•One highest siderophore producing fungal isolate was selected for plant growth promotion. Catecholate type of siderophore producing isolate, SF 03 was shown very rare. The isolate SF 03 was molecularly identified as *Curvularia australiensis.*

•It was found to be quite effective in promoting plant growth enhancement, in terms of enhanced root & shoot length as well as number of leaves of *Spinacia oleracea* (spinach), *Trigonella foenumgraecum* (fenugreek) and *Vigna radiata* (moong).

•Therefore, it is suggested that the use of this potential strain as a potent bioinoculant can be beneficial for vegetable plants, marine plants and other crops.

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TITLE THE PRESENTATION
IMPACT OF FINGER MILLET (*Eleusine coracana*) ENDOPHYTIC BACTERIA ON PLANT GROWTH PROMOTION AND METAL (Zn AND Fe) SOLUBILIZATION

AUTHORS

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IMPACT OF FINGER MILLET (Eleusine coracana) ENDOPHYTIC BACTERIA ON PLANT GROWTH PROMOTION AND METAL (Zn AND Fe) SOLUBILIZATION

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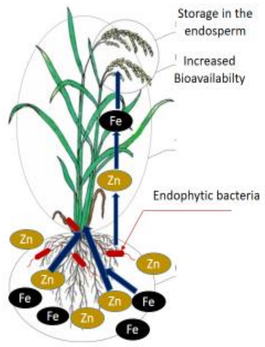
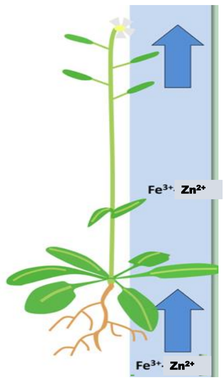
INTRODUCTION

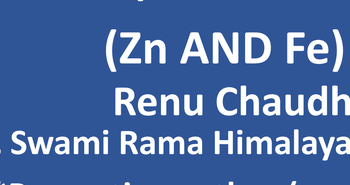
Finger millet is one of the oldest staple, imperative food and fodder crop of Uttarakhand and sustainable food source for combating hunger in changing world climate.
Enriched with nutrition and health benefits.
Tolerant to harsh environmental conditions.
Rapid rise in micronutrient malnutrition in food is due to deficiency of micronutrients in soils/ crops.
In Indian soils, Fe/Zn deficiency is high as 13/47 % respectively.
Endophytes through PGP traits, uptake and mobilize the micro nutrients from soil could biofortify grains of crop plants.
Fe/Zn uptake capacity and fortification in plants - an important approach to supplement the grains to combat micronutrients deficiency.





MATERIALS AND METHODS

- Isolation of biofunctional endophytic bacteria from finger millet plan



Cultivars of finger millet used in the experiment:

✓ PRM-1
✓ VL-352
✓ VL-348

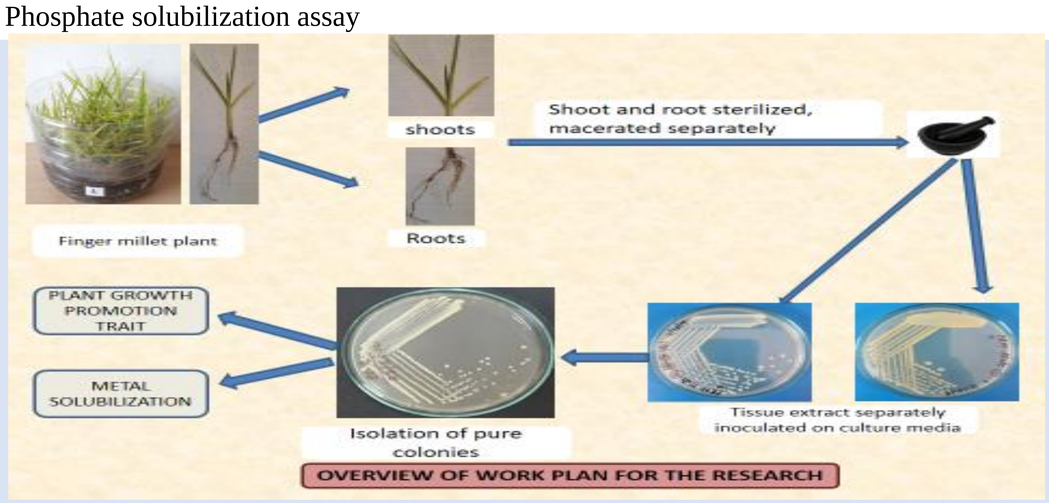
- Morphological and biochemical characterization of isolated endophytes.
- Screened isolates for plant growth promotion traits and micronutrients solubilization (zinc and iron)

Screening of plant growth promotion traits-

- IAA
- Ammonia production
- HCN production
- Siderophore production

Screening of metal (Zinc and Iron) solubilization

- Zinc solubilization assay
- Iron solubilization assay
- Phosphate solubilization assay



RESULTS

- Bacterial endophytes isolated and biochemically characterized after surface sterilization of the plant tissue (root, shoot and leaf). 55 bacterial endophytes isolated from finger millet plant tissues. The isolated isolates were named according to the variety name.
- Bacterial isolates produced indole acetic acid in varying amount. 16 isolates produced IAA in the range between 8-302 (µg/ml) PRM-4da Produced significantly highest concentration of IAA (302 µg/ml), (Fig.1)
- Bacterial isolates solubilized zinc and iron, zinc solubilizing media amended with ZnO, $ZnCO_3$, ZnP and ZnS, zinc solubilizing index were calculated and ranged between : 22 -51 mm. (Fig.2). While for iron the ferric phosphate and ferric oxide rich medium were used. (Fig.3)
- Many bacterial isolates also exhibited ammonia production (Fig.4) and produced siderophore (Fig.5)
- Germinated seeds treated with efficient micronutrients solubilizing endophytes. (Fig.6)

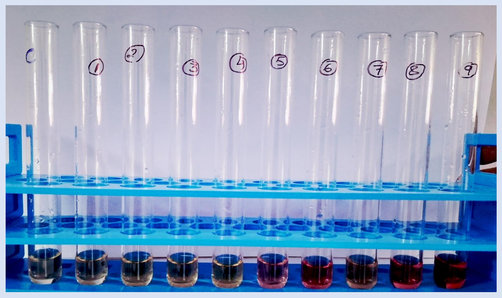


Figure 1: INDOLE ACETIC ACID

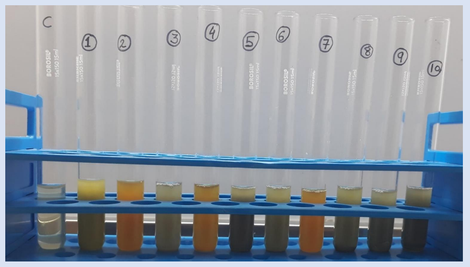


Figure 4: AMMONIA PRODUCTION

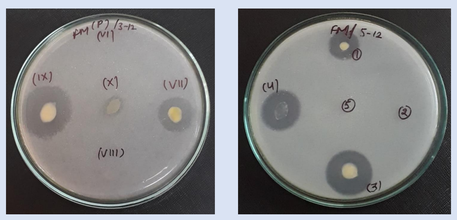


Figure 2: ZINC SOLUBILIZATION ASSAY

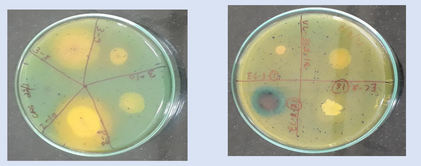


Figure 5: SIDEROPHORE PRODUCTION

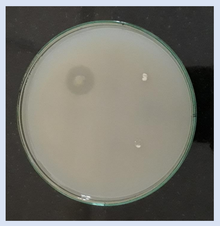


Figure .3: IRON SOLUBILIZATION ASSAY

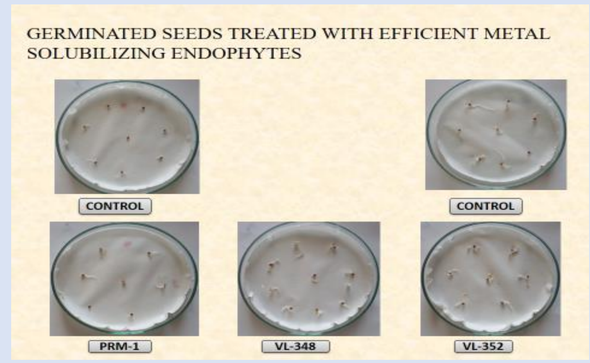


Figure 6: SEED GERMINATION

CONCLUSION

The selected bacterial isolates solubilized zinc, iron, excreted ammonia and produced indole acetic acid and siderophores. Under laboratory conditions, potential endophyte treated seeds showed better germination of finger millet as compared to control.
These isolates will be helpful in the transportation of metal from soil to the plant and also promoting higher plant growth.

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Dinesha R, Srinivasana V, Hamzaa S, Sarathambala C, Gowdaa A, Ganeshamurthyb AN, Guptac SB, Nairc VA, SubilaaKP, Lijinaa A, Divyaa VC (2018) Isolation and characterization of potential Zn solubilizing bacteria from soil and its effects on soil Zn release rates, soil available Zn and plant Zn content. J.Geoderma 321:173-186

Registration No.: 1.11

Efficacy of Plant Growth Promoting Rhizobacteria as a Bioinoculant for Vegetable (Tomato)

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Abstract
It is a well known fact that in India 70% of the population is directly or indirectly involved in agriculture and allied activities. Since independence agriculture has got a status of primary sector but always treated by all the government as a secondary sector or even below that. Bio-fertilizers are microbial inoculants consisting of living cells of microorganisms like bacteria, algae and fungi alone or in combination which may help in increasing the crop productivity, stimulating plant growth or in decomposition of plant residues. Tomato seeds of two varieties are inoculated with PGPR in 4 sets and 1 set as control for each variety. Control plant is with no bacterial treatment. All such control and experimental sets are sown in seed bed in proper way. At 33 days after seedling transplantation there was substantial difference in shoot height which were clearly visible due to inoculation of PGPR in experimental sets. Seeds treated with PGPR promotes early plant growth.

The study concluded that the plant growth promoting rhizobium (PGPR) has shown positive effect on entire plant body. A visible effect on growth and cluster has been observed. The plant has shown substantial improvement at first picking time. The fruit of PGPR plants are high in weight nearly 8% to 15% as compared to control. The fruit has a good shine and colour and has also shown very good toughness for transportation and durability. The use of PGPR as bio-inoculants is definitely having remarkable effect on increase in revenue to farmers which ultimately leads to benefit of farming community and thereby boosting our national income.
(Key words: PGPR, Microbial inoculants, Tomato, and Quality Improvement)

Introduction

It is a well known fact that in India 70 % of the population is directly or indirectly involved in agriculture and allied activities. Improvement in agriculture productivity is largely depend on quality and quantity of agri inputs such as seed ,fertilizers and pesticides.
Vegetables are an important part of human dietary systems. They contain several important nutrients including vitamins, antioxidants etc. and affect immensely the human health. During cultivation most of the vegetable crops are attacked by various insect pests, pathogenic microorganisms there by causing severe damages, leading to huge yield loses. Therefore agriculturists apply large number of fertilizers and pesticides to manage insect pests and to enhance crop productivity which will damage soil fertility and destroy soil biota.
In nature there are a number of useful soil microorganisms which can help plants to get nutrients. Their utility can be enhanced with human intervention by selecting efficient organisms, culturing them and adding them to soil directly or through seed. The cultured microorganisms prove beneficial for crop growth and soil fertility. This microorganisms packed in some carrier material for easy application. PGPR are such microorganisms which enhance nutrient availability to crop plants by process like fixing atmospheric nitrogen or dissolving insoluble phosphorus present in the soil. And also impart better health to plant and soil, enhancing crop yields in a moderate way.
Present study focused on use of Plant Growth Promoting Rhizo bacteria as a bio inoculants for vegetable crops for sustainable improvement in growth and quality of crops.

Materials & Methods

Isolation of Plant Growth Promoting Bacteria
Rhizospheric soil was suspended in 50 ml of buffer solution (phosphate buffer 10Mm Ph 7.0) shaken vigorously 1 hr on a gravatory shaker and serially diluted up to 10^{-5}. Bacterial strain isolated by plating soil dilution on agar plate, Jensen's plate (Norris & Chapman, 1968), King's B agar plate (King, 1954) for *Bacillus*, *Azatobacter,* & *Pseudomonas* respectively. The plates were incubated at 30°C for 2-7 days. The bacterial colonies were chosen based on colony morphology. Clones picked up and streaked on the respective plates.

Identification
Pure isolates of PGPR from rhizospheric soil was characterized by using the criteria described in Bergey's Manual of Systematic Bacteriology (Bergey's Manual of Determinative Bacteriology, 1984). The PGPR strain was identified on the basis of colony morphology, and biochemical characteristics.

Method of Inoculation
I have selected two varieties of hybrid tomato seeds. One is Namdhari Seed (815) and another is Mahyco (S-72). Both the varieties are commercially accepted in almost all the areas where tomato acerage is substantial. Tomato seeds of both the varieties are inoculated with PGPR in 4 sets and 1 set defined as control for each variety.

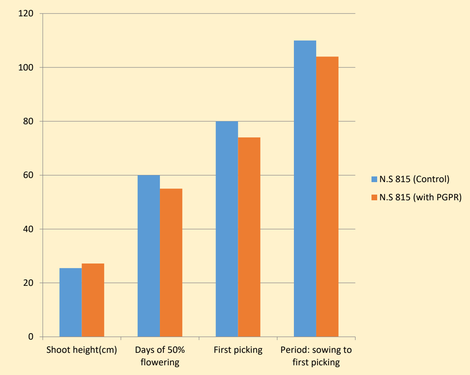
Slurry Method
Mix inoculum with sterile water to form a slurry. Then pour slurry on the seed and mix well. If done properly, the seed will absorb water leaving an even dry coat of inoculums. The seeds should be sown immediately after inoculation in a pot.

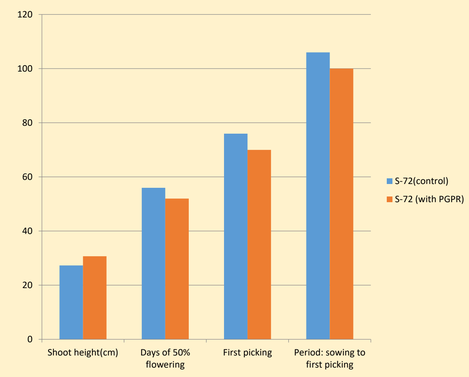
Result and Discussion

At 33 days after seedling transplantation there was substantial difference in shoot height, colour of leaves, flowering which were clearly seen due to inoculation of PGPR in experimental sets. Seeds treated with PGPR promotes early plant growth.

Comparative chart of result variety wise is shown in following table.

Sr.no	Particulars	N.S 815 (Control)	N.S 815 (with PGPR)	S-72(control)	S-72 (with PGPR)
1	Crop	Hybrid tomato	Hybrid tomato	Hybrid tomato	Hybrid tomato
2	Shoot height(cm)	25.5	27.2	27.3	30.7
3	Colour of leaves	Light green	Green	Green	Dark Green
4	Days of 50% flowering	60	55	56	52
5	First picking	80 days	74 days	76 days	70 days
6	Period: sowing to first picking	110 days	104 days	106 days	100 days
7	Soil pH	6.5	6.5	6.5	6.5
8	Temperature at the time of sowing	32°C	32°C	32°c	32°C





Conclusion:

From above mentioned data it is very clear that Plant Growth Promoting Rhizobium (PGPR) has shown positive effect on entire plant body. I have seen visible effect on growth and cluster of fruits working with PGPR mechanism as compare to control. The fruit of PGPR plant is very good in shine and colour. The use of PGPR as a bio inoculant is definitely having remarkable effect on increase in revenue to farmers which ultimately leads to benefit of farming community and thereby giving boost in increase of our national income.

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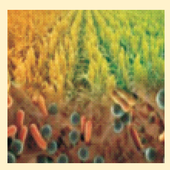


Plant Growth promotion by Siderophore producing *Achromobacter Xylosoxidans*

1.12

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Introduction

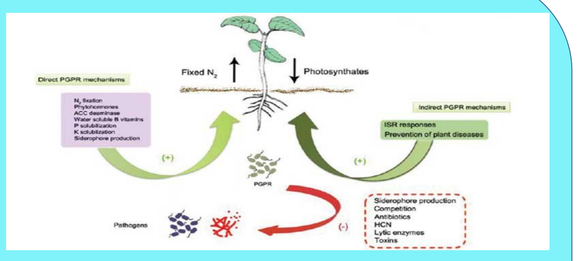
SIDEROPHORES → Role of Microbes

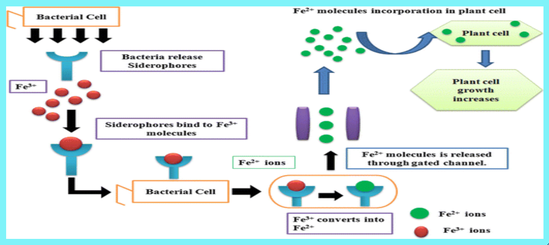
CHARACTERISTICS

- Expression under low Fe+3
- Low Molecular weight
- Molecules (Approx 1000Da)
- Specific Binding to Fe
- Functional Moieties
  - Catecholates
  - Phenolate
  - Hydroxamate

Role of Microbes

- Iron sequestration
- Iron Storage
- Iron transport





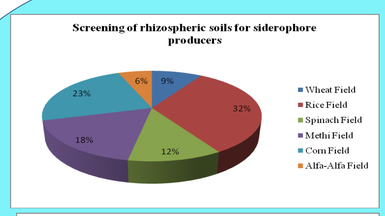
Objectives

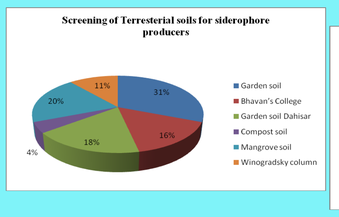
- Screening and procurement of siderophore producers from Soil & Aquatic ecosystems by primary and secondary screening methods
- Optimization of siderophore production using Plackett Burman design method.
- Characterization studies of siderophore produced using HPTLC, FTIR and Mass spectroscopy.
- Identification of potenial siderophore producer by 16s r RNA method
- Assessing the plant growth promoting ability of siderophore producing *Achromobacter xylosoxidans*

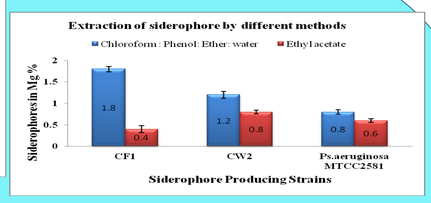
Materials & Methods

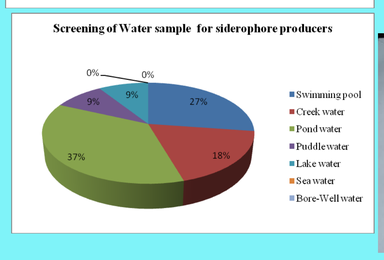
Screening	• Soil samples collected: Compost pit, salt pan, garden soil, mangroves,Wheat, Alfa-Alfa, Spinach & Corn field soil. • Water sample: Swimming pool (Andheri Recreation), Pond water (Bhavan's Andheri), Sea water (Bhayander), Creek water (Mahim). • Selective enrichment using Chrome Azurol -Sulphonate agar media was performed. • Positive cultures were Gram stained & maintained on NA for further analysis.
Quantification methods	• Csaky's Method for determination of Hydroxamate –type of siderophores • Arnow method for determination of Catechol-type siderophore • Payne's Method for quantification of total siderophores
Media Optimization studies	• Optimization of media using Plackett & Burman method • In present study Mannitol,,Ammonium sulphate, Succinic acid , pH & Iron concentration were selected as the independent variables. These variables were investigated & 8 experimental trials were set up along with standard modified succinate media
Culture Identification Studies	• Culture identification was done by classical biochemical & 16s rRNA method
Chemical Characterization	• Characterization studies of siderophore produced using HPTLC, FTIR and Mass spectroscopy
Plant Growth Promotion Activity	• Assessing the plant growth promoting ability of siderophore producing *Achromobacter xylosoxidans* by performing experimental studies on *Tricosanthes anguina*

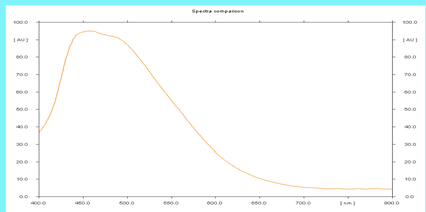
Results

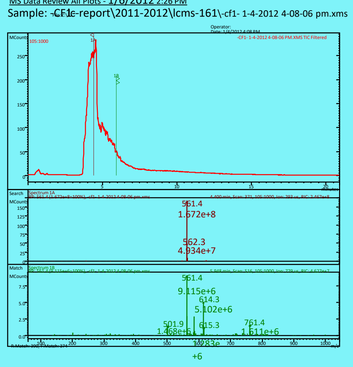


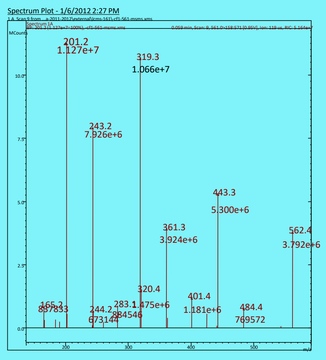


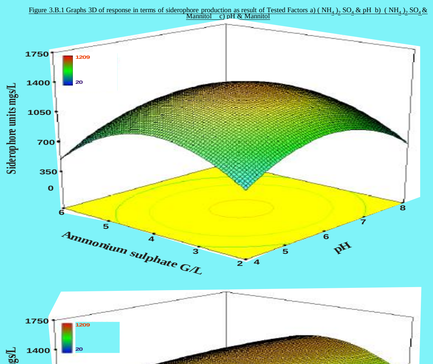




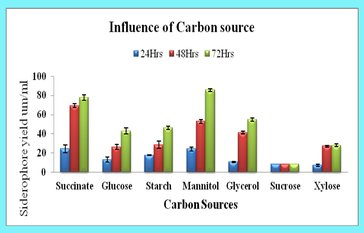


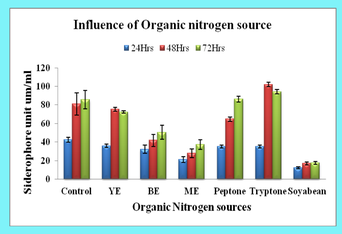


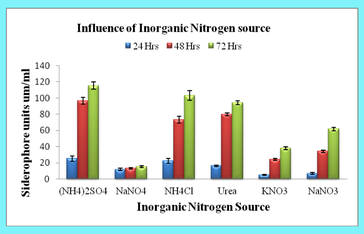


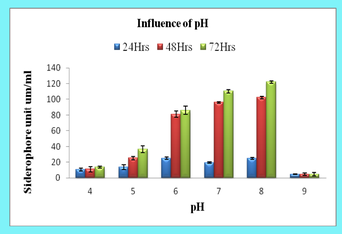


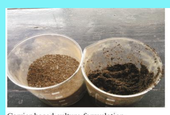
Optimisation study for CF1 isolate









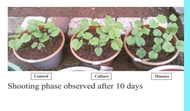

Carrier based culture formulation


Seeds of *Trichosanthes anguina*


Seeds of *Trichosanthes anguina* after germination


Shooting phase observed after 2 days


Shooting phase observed after 5 days



Shooting phase observed after 10 days


Linear growth observed in manure & culture system as compared to shunted growth seen in control


Curling of the stem supported with help of thread


Extensive branching seen in culture system after 2 weeks


Control seen shunted after 25 days of growth


Budding observed at the axile position


Flowering observed after 35 days of growth


Leaves of manure plants showing infection


Leaves of culture plants seen to be healthy & non infected

Fruits produced by Culture and Manure systems


Fruits of culture system showing good length & fuller in weight having dark green outer skin clearly indicating higher concentration of chlorophyll content due to increased availability of iron .


Fruits of manure system showing good length & reduced weight having light green outer skin indicating less concentration of chlorophyll.


Harvest of the fruits obtained from manure & culture system.

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Conclusion

- 36 isolates were obtained from screening of 18 different samples ranging from Rhizospheric soil, Terrestrial Soil and Aquatic niches.
- 10 isolates were selected on their ability to produce siderophore in a range of 40- 70% SU yield.
- The chemical characterization of CW2 isolate produced siderophore can be summarized to contain hydroxamate as well as catecholate siderophore .
- Further the statistical method of Plackett Burman worked to be an powerful tool for optimization of siderophore from CW2 raising the yield to 1.2grams /L and yielding an 90% increase as compared to its original yield .
- Isolate CW2 was identified as *Achromobacter xylosoxidans* by conventional biochemical & molecular methods.
- *Achromobacter xylosoxidans* based bio-fertilizer and bio-fungicide was effective as it was capable of not only enhancing the plant growth but also the quality of the fruit was free from infestations and contains high nutritional content of iron finding applications in herbal medicinal preparations.

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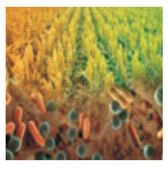
❑ UGC-Minor Research Project, part of the work was funded.

6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
(3-4, September 2021)



Pseudomonas monteilli (MN759447) , a promising siderophore producer from *Dalbergia sissoo* Roxb. Forest ecosystem

Pragati Srivastava and Manvika Sahgal*

Department Of Microbiology,C.B.S.H., G B Pant University Of Agriculture and Technology, Pantnagar-263145

THEME 1:PGPR and microbe for plant growth support
R.G.No:1.13

Introduction:

Issue:

Dalbergia sissoo Roxb. (family Fabaceae) is a large deciduous multipurpose timber tree. This high value cash tree is facing heavy mortality rate in its natural as well as plantation forests, induced by poor soil fertility. Iron scarcity in *Dalbergia sissoo* Roxb. leads to IDIC (Iron deficiency induced chlorosis) which makes it more susceptible to fungal pathogens and lepidopteron attack. Since *Dalbergia sissoo* Roxb. provides valuable timber as well as enriches soil nitrogen, its large scale decline incurs huge economic loss. Several rhizosphere dwelling bacteria secrete ferric iron chelating agents, siderophores under iron deficient conditions. The siderophore positive bacteria are significant as plant growth promotion and bio-control agents.

This pioneer species is getting depreciated due to heavy mortality rate

Phytopathogenic Fungi
- ***Polysporus sp***
- ***Ganoderma sp***
- ***Fusarium solani***
- ***Rhizoctonia solani***
- ***Fomes lucidium***
- ***Phellinus gelvus***

Causes
- **Inappropriate seed storage conditions**
- **Monoculture one of the major cause of shisham decline**
- **Insects attack**
- **Biotic Factors**
- **Abiotic factors**

AIM:

•Isolation , screening and identification of best siderophore producing bacteria from *Dalbergia sissoo* Roxb. forest ecosytem.
•Optimization of physico-chemical parameters for enhanced siderophore production
•Identification of siderophore compounds in selected siderophore producing bacteria.
•Checking the antagonistic activity of the siderophore producing bacteria againt fungus isolated from the Shisham rhizopshere.

Methodology:

1-Collection of soil sample from Agroforestry research centre,GBPAU&T ,Pantnagar, 28°58′N 79°25′E / 28.97°N 79.41°E

Qualitative estimation via CAS(Chrome Azurole Assay) (Schwyn and Neilands 1987; Oliveira *et al*., 2006)

Quantitative estimation :
% siderophore Unit= Siderophore= Ar-As/Ar*100
(Set *et al*., 2017).
Where, Ar =Absorbance of the reference (CAS Reagent);
A s= absorbance of the sample at 630 nm.

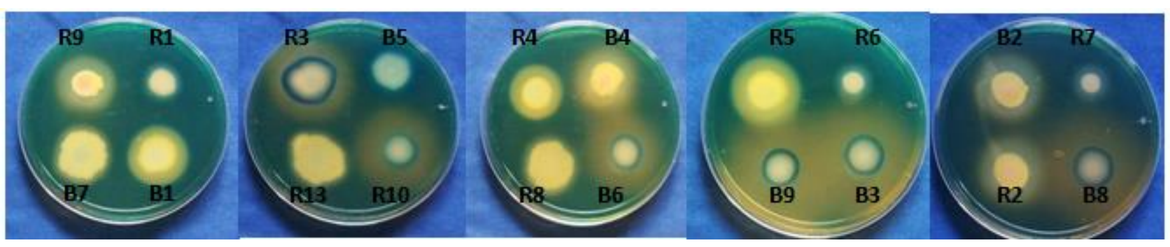


Figure1:Siderophore production by bacterial isolates from *Dalbergia sissoo* Roxb. plantation forest ecosystem in a CAS agar Plate Assay

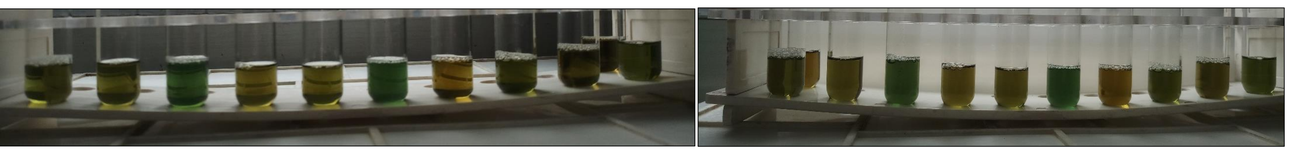


Figure2:Quantitative estimation of siderophore via CAS Assay
2-16S rDNA Sequencing for the identification of the strains isolated from *Dalbergia sissoo* Roxb. forest ecosytem.
3-Optimization of physico-chemical parameters for enhanced siderophore production in 10 isolates
- Effect of pH
- Effect of iron concentration
- Effect of carbon source
- Effect of nitrogen source
- Effect of heavy Metals

4-Second step optimization via RSM(Response Surface Megthodology) of promising siderophore producer *Pseudomonas monteilli*

Figure 3:Box-Behnken Design for siderophore Production (% SU)

- Box-Behnken design equation to analyze the correlation between the dependent and independent factors.

$$Y=\beta_O+\Sigma^{n}_{i=1}\beta iXi+\Sigma_{i=1}^{n-1}\Sigma_{j=i+1}^{n}\beta_{ij}X_iX_j+\Sigma_{i=1}^{n}\beta_{ii}X^2i\ldots\ldots$$

where ,Y is the response (dependent variable), Xi,Xj,Xk… are independent variables

Table1:Box-Behnken Actual Design

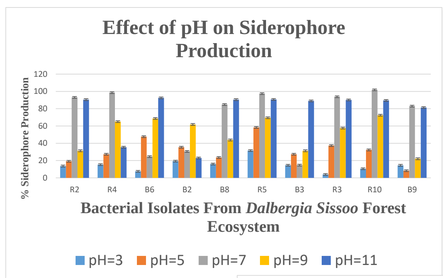
S.No	Factor 1 A-pH	Factor 2 B concentration of Fe µm	Factor 3 C Ammonium Nitrate (g/l)	Response 1 % siderophore Unit				
				SID 1	SID 2	SID3	SID 4	SID 5
1	9	10	10	72.59	51.97	69.01	33.48	26.99
2	7	10	5	71.51	5.5	4.56	23.87	90.01
3	9	5	5	74.81	19.54	50.01	74.01	57.94
4	7	10	15	70.95	18.62	50.02	36.62	45.09
5	9	10	10	72.56	49.46	69.61	33.46	26.89
6	9	5	15	53.24	6.2	65.02	79.89	33.45
7	9	10	10	72.59	50.96	68.01	34.01	27.01
8	11	10	15	62.55	15.92	6.7	20.99	32.27
9	9	10	10	72.59	52.00	68.9	33.47	26.01
10	7	5	10	65.96	15.03	55.65	73.34	50.03
11	11	5	10	54.18	54.18	53.03	78.91	30.68
12	11	10	5	69.89	57.04	5.3	72.34	31.85
13	9	15	5	70.15	69.54	20.01	57.05	55.25
14	11	15	10	72.35	57.05	20.04	48.15	31.01
15	9	15	15	72.95	41.89	55.01	43.11	41.89
16	9	10	10	72.59	51.98	68.09	33.45	26.03
17	7	15	10	75.55	44.39	55.01	25.01	75.01

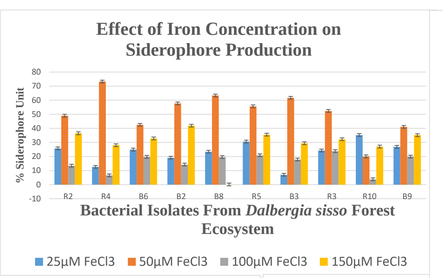
5-**Structure Determination of the Siderophore**
6-Determination of siderophore mediated in vitro inhibition of three fungus isolated from *Dalbergia sisso* Forest ecosystem

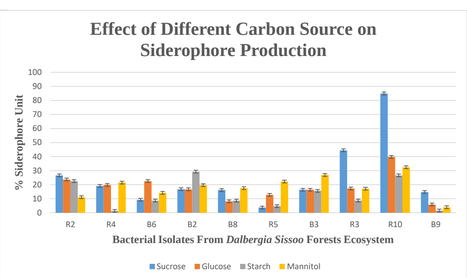
Results:

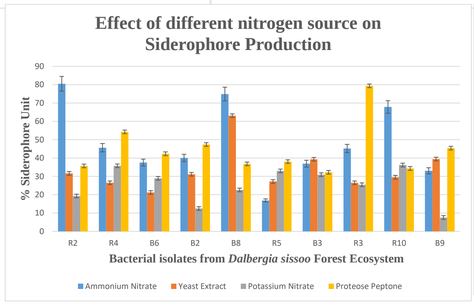
Table2: Identification of the Siderophore Producing Bacteria through 16S rDNA Sequencing and Qualitative and Quantitative Estimation of Siderophore Production in Bacterial Strains Recovered from *Dalbergia sissoo* Plantation Forest Ecosystem

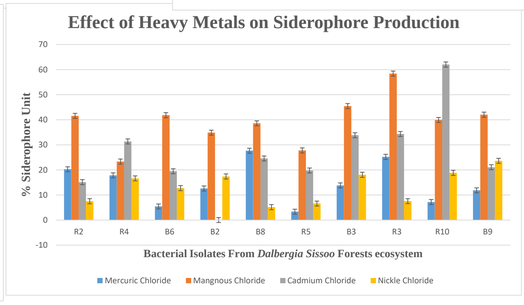
Strain	Identified Strain	Quanitative assay%SU	Qualitative estimation	% similarity	Acession no.
R3	*Pseudomonas constantinni*	72.23±0.05	++	92.75	MN759444
R2	*Pseudomonas benzenevorans*	73.33±0.5	+++	90.95	MN759445
B9	*Pseudomonas chlororaphis*	86.32±0.005	+++	98.41	MN759442
B6	*Pseudomonas lini*	70.79±0.01	++	97.61	MN759443
B8	*Pseudomonas monteilli*	91.36±0.005	+++	93.13	MN759447
B3	*Pseudomonas azotoformans*	82.66±0.05	+++	97.68	MN759446
B2	*Pseudomonas cedrina sub sp cedrina*	71.91±0.005	++	83.94	-*
R4	*Pseudomonas paralactis*	68.33±0.01	++	86.15	-*
R5	*Streptomyces lavendulae*	88.33±0.57	+++	90.62	MN759448
R10	*Burkholderia territorii*	80.33±0,5	+++	86.73	-*











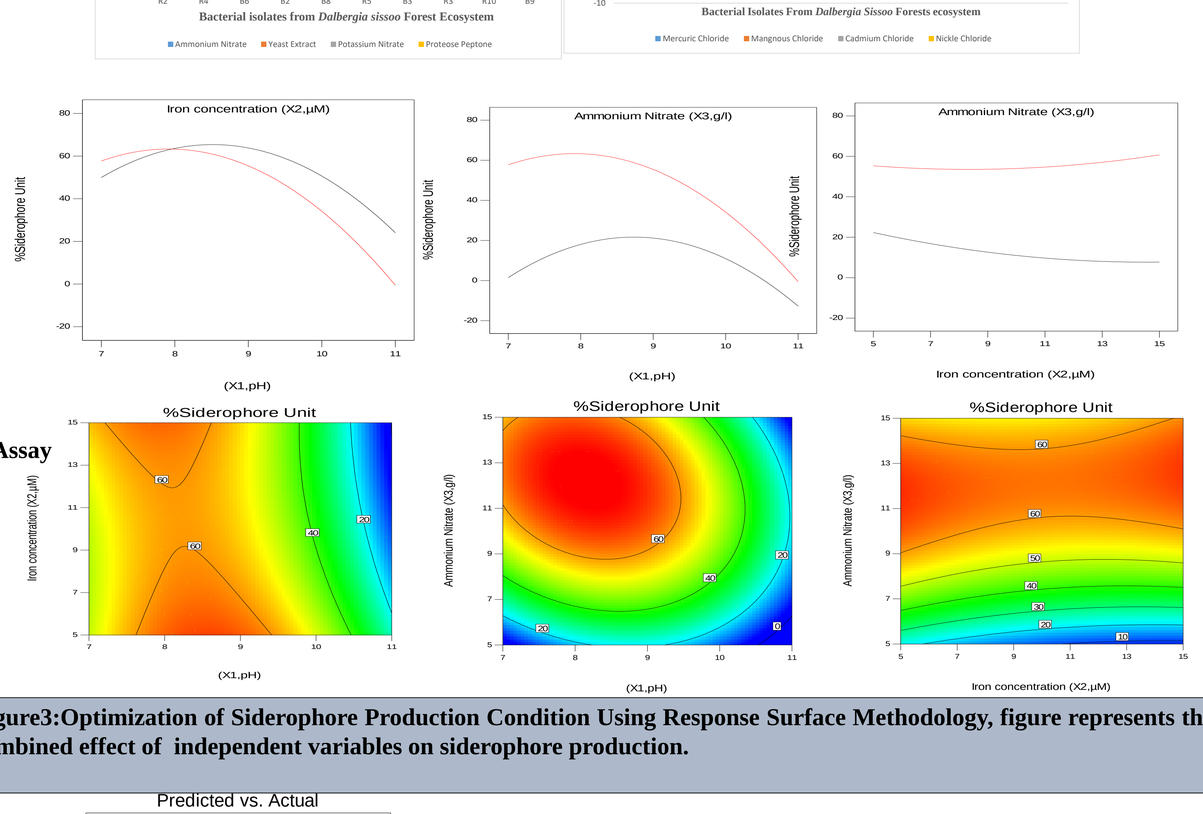
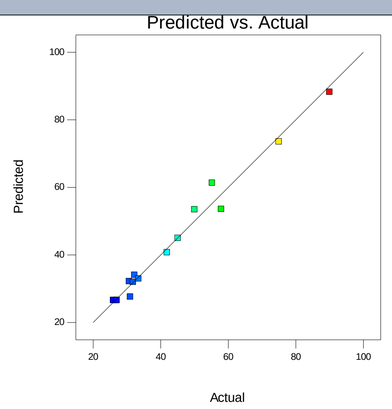


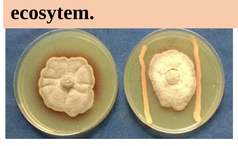
Figure3:Optimization of Siderophore Production Condition Using Response Surface Methodology, figure represents the combined effect of independent variables on siderophore production.



Color points by value of % SU in *Pseudomonas monteillii is 90.1% SU*

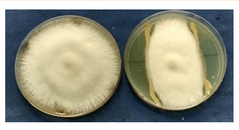
- **pH=below 7,Fe=9µM,NH_4NO_3= below 5g/l for siderophore production**
- **pH=7,Fe=5-15µM,NH_4NO_3=15g/l for growth**

Figure 4:Four representative examples of in vitro dual culture assays against fungus isolated from *Dalbergia sissoo* Roxb. forest ecosytem.



Aspergillus caliodoustus/ Pseudomonas monteili

Talaromycesverruculos Pseudomonas monteilli



Fusarium oxysporum Pseudomonas monteilli

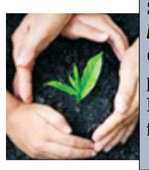
Talaromyces pinophilus Pseudomonas monteilli

Table 3:Compounds detected in LC-MS in strain *Pseudomonas monteillii*

S.No	Compound Name	Chemical Formula	Molecular Weight
1	2,2,6,6 Tetremethyl 4-piperdinyl heptanoate	$C_{16}H_{31}NO_2$	269.234
2	2(S)-2[1 tert-Butoxycarbonyl amino]-5-(cyclohexyloxy)-5 oxo pentanoic acid	$C_{16}H_{27}NO_6$ *a pseudomoninederivative	329.183319

Conclusion:
***The availability of siderophore producing bacteria in the rhizosphere region is worthy of importance in agriculture, providing iron to the plants and preventing growth of phyto-pathogens which are iron dependent**
***Since application of siderophore producing bacteria as bio-inoculant is of immense importance in crop field as well as tree based ecosystem to improve yield and maintaining the fertility of the soil, the findings of this study are significant.**

Acknowledgement: Authors thanks Dr Laksmi Tewari (Head of Department), Department of Microbiology GBPUA&T, Pantnagar Uttarakhand for providing facilities and working environment. We would like to express our gratitude to the expert of AgroforestryDr Salil Tewari (Head of Department), Department of Genetics & Plant Breeding, GBPUA&T, Pantnagar Uttarakhand .



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E-Poster 1.14 Theme 1

In Vitro, seed germination and plant growth promoting applications of pigment produced by *Rhodococcus kroppenstedtii*

Chaudhari Varsha Madhukar

PSGVPM's SIPatil Arts, GBPatel Science and STKVS Commerce, College, Shahada , Dist-Nandurbar.

Introduction- The worldwide increase in population increases the demands of food production. But environmental damage causes problems in agriculture and depletion of food. Plant Growth-Promoting Rhizobacteria (PGPR) include a highly diverse variety of soil bacteria, which when grown in association with a host plant, results in stimulation of growth of the host plant due to increased mobility, uptake, and enrichment of nutrients in the plant. Thus PGPR has many beneficial effects on the soil environment. *Rhodococcus kroppenstedtii* is one of the novel organism isolated from the soil sample collected from soyabean field, produces red pigment. The pigment extracted from isolate was found to enhance the germination of wheat (*Triticum aestivum*), and mung (*Vigna radiata*) seeds and hence enhances the growth of plant.

Objectives- The objective of the present study was to evaluate the efficacy of pigment extracted from *Rhodococcus* on the seed germination and plant growth promotion abilities of different seed.

Method-

- Surface sterilization of healthy seeds with 0.1 % $HgCl_2$ solution 2-3 min.
- Washed repeatedly with D/W.
- Seeds coated with 1 mg ml^{-1} of pigment solution and dried in shade
- Seeds incubated for 3-4 days in sterile plate with moistened filter paper,
- Uncoated seeds were used as control.
- The effect of pigment on the germination was determined.
- Similarly the influence of pigment on plant growth promotion abilities was experimented by coating the seeds with concentrated pigment solution in trial pots of soil in laboratory and seeds without pigment coating were treated as control in each case.




Results- The experimental data obtained indicated that germination of Mung and wheat seeds were observed in seeds coated with concentrated pigment; growth was greatly enhanced with better germination in experimental test as compared to control seeds. Similarly, increase in the morphological properties- root length and shoot length of plants were observable in the pots containing seeds coated with the pigment as compared to untreated seeds which served as control. This data clearly indicated that pigment enhanced germination efficiency and growth in plants.

Conclusion- *Rhodococcus kropenstedtii* is one of the plant growth promoting Rhizobacteria was found to produced red pigment having potential seed germination and plant growth promotion activities.

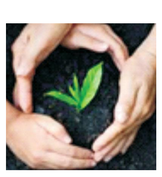
Reference- Bernard R. Glick (2012) Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindwi Publication

Rachel Backer, J. Stefan Rokem , Gayathri Ilangumaran , John Lamont, Dana Praslickova, Emily Ricci, Sowmyalakshmi Subramanian and Donald L. Smith (2018) Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. Frontiers in Plant Sciences,

Acknowledgement-

Author is greatly thankful to Department of Microbiology, PSGVPMandal's ASC College for providing facility to this work.





The effect of different medium components on the solubilization of phosphate was determined using the Packett-Burman design. It was observed that glucose and sodium nitrate had significant effect on phosphate solubilization. Considering the Plackett-Burman results, the response surface methodology was used for optimization of these medium components along with tri-calcium phosphate on P-solubilization. The analysis from RSM revealed that the optimum values for the tested variables were 1.5% glucose, 0.005% sod

Registration No. 1.15

Optimization of phosphate solubilization efficiency of *Talaromyces trachyspermus* by Plackett-Burman and Response Surface Methodology.

Smriti Chouhan* and Dr. Anil Prakash Department of Microbiology, Barkatullah University, Bhopal (M.P.) email: smritischauhan50gmail.com

INTRODUCTION

Phosphorus is one of the essential components needed for the metabolic activities and for the growth of any plant. Phosphate solubilization by plant growth promoting microorganisms is the important factor for development of sustainable agriculture system. In this study we aimed to assess optimization of phosphate solubilizing efficiency of *Talaromyces trachyspermus* by Plackett-Burman and Response Surface Methodology.

METHODOLOGY

Phosphate solubilization by fungus in NBRIP Medium

↓

Optimization of phosphate solubilization by OVAT mehtod

↓

Selection of most affectable variables on phosphate solubilization by Plackett-Burman method

↓

Optimization of concentration of variables by Response surface methodology

↓

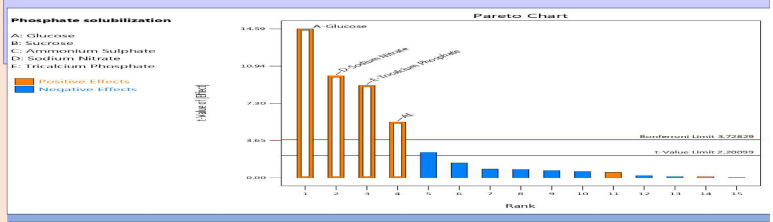
Large scale production of fungal biomass in optimized medium and extraction of Organic acids

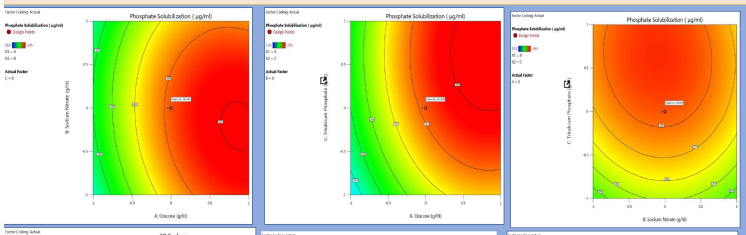
↓

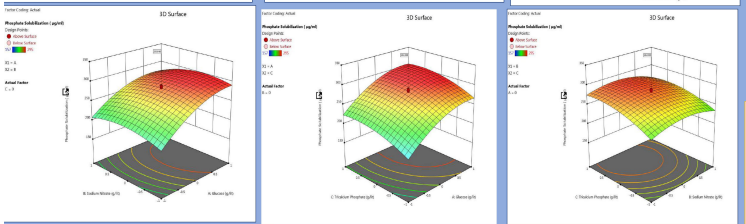
Detection of organic acids from High Resolution Orbitrap Liquid Chromatography Mass Spectrometer analysis

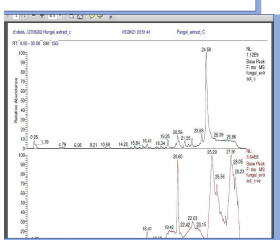
RESULTS AND DISCUSSION

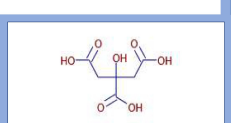
Analysis of Packett-Burman design, it was observed that glucose and sodium nitrate had significant effect on phosphate solubilization. RSM revealed that the optimum values for the tested variables were 1.5% glucose, 0.005% sodium nitrate and 1% tricalcium phosphate. Phosphate solubilization of 295 µg/ml was observed as comparison to original level of 157µg/ml, which was a 1.87 fold increase. High Resolution Orbitrap Liquid Chromatography Mass Spectrometer analysis confermed that citric acid and lactic acid, gallic acid and palmitic acid were the major acids found to be responsible for enhancing the P solubilization.

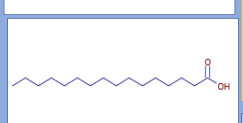


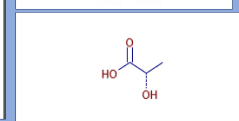


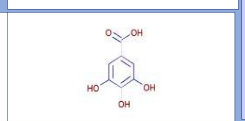


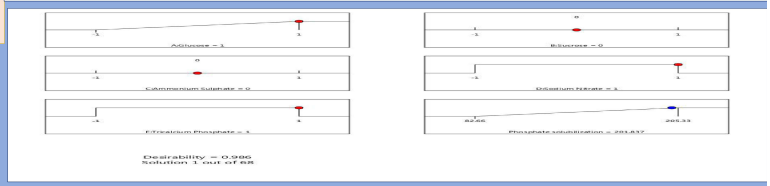


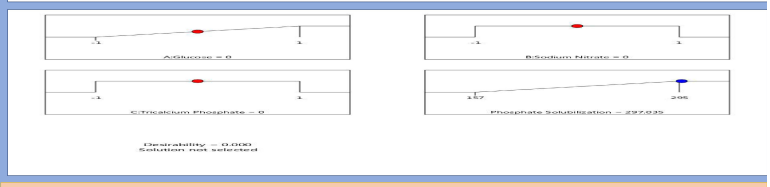












CONCLUSION

Here we show that native fungal isolate *Talaromyces trachyspermus have the potential to be a* sustainable alternative to the problem of phosphorus fixation. We recommend future research to evaluate the efficiency of isolates under field conditions.

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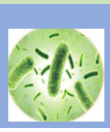

1.16

EVALUATION OF RHIZOSPHERE FUNGI FROM MEDICINALLY IMPORTANT PLANTS SHOWING PLANT GROWTH PROMOTING TRAITS

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INTRODUCTION:

❖Fungi inhabiting the rhizospheric zone of Medicinal plants are one of the most propitious groups of microorganisms.

❖ Providing ecological fitness to their host plant by plethora of mechanisms involving solubilization of mineral phosphates and other nutrients, production of phytohormones, vitamins, enzyme, siderophores and by synthesis of antibiotics compounds etc. (Lugtenberg and Kamilova2009;Sarma *et.al*, 2015)

❖Presently, however, only a small subset of potential microbial strains could be definitively attributed to phytotherapeutic properties of medicinal plant (Strobel *et.al*, 2004; Miller *et.al*,2012)

❖Their relative contribution to the recognized valuable bioactivity of medicinal plants is not clear as of yet.

❖This is the first study focused on rhizosphere soil fungal diversity of medicnal plant *Butea monosperma*, *Gmelina arborea*, *Celosia argentea* and *Tinospora cordifolia* showing PGP traits.

OBJECTIVES:

▪To isolate and identify the fungi present in the rhizosphere soil samples of selected medicinal plants.

▪ To analyze isolates having plant growth promoting traits.

MATERIALS AND METHODS:

Medicinal plants selected for the rhizosphere study:

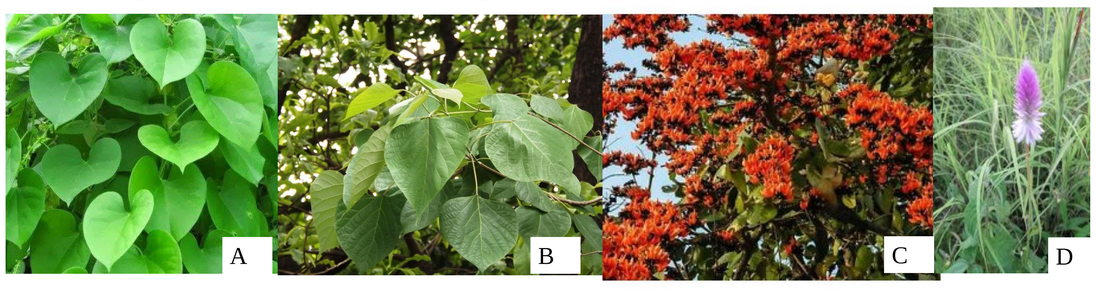


Fig1. Showing (A.)*Tinospora cordifolia* , (B)*Gmelina arborea*, (C) *Butea monosperma*, (D) *Celosia argentea*

Fig2: Map showing different rhizosphere soil sample collectesd sites at Barkatullah University, Bhopal Madhya Pradesh(latitude 23°12' 01.83" N and longitude 77° 27'12.50" E).

•Randomized soil sample was collected from rhizosphere soil of medicinal plant of University campus.

•Isolation and purification of fungi morphotypes was carried out according to Cappuccino and Sherman,2014.

•The morphotypes were identified based on their morphological features following the monographs of Alexopulos *et.al*,1996.

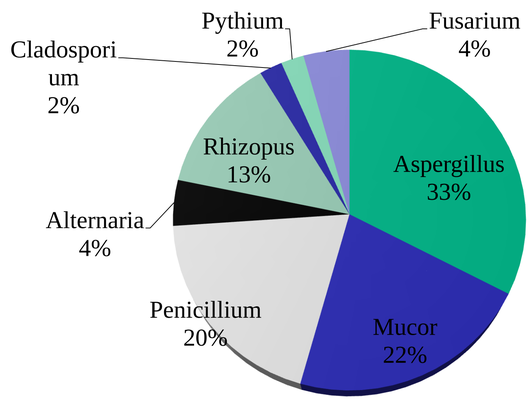
• PGP characteristic such as phosphorus solubilization, siderophore production, IAA production determined according to Tamilarasi *et.al*, 2008.

RESULTS:

Table1. Abundance of morphotypes in rhizosphere of meldicinal plants selected for the study.

S.No.	Genus	Medicinal plant				Total morphotypes
		Butea monospermea	*Gmelina arborea*	*Celosia argentea*	*Tinospora cordifolia*	
1.	*Aspergillus*	3	8	3	1	15
2.	*Mucor*	0	3	2	5	10
3.	*Penicillium*	6	1	0	1	8
4.	*Alternaria*	1	0	1	0	2
5.	*Rhizopus*	0	2	1	3	6
6.	*Cladosporium*	0	0	1	0	1
7.	*Pythium*	0	0	1	0	1
8.	*Fusarium*	0	2	0	0	2
	Total	10	16	9	10	45

Fig4. Total percent abundance of fungi of different genera in 4 medicinal plants.



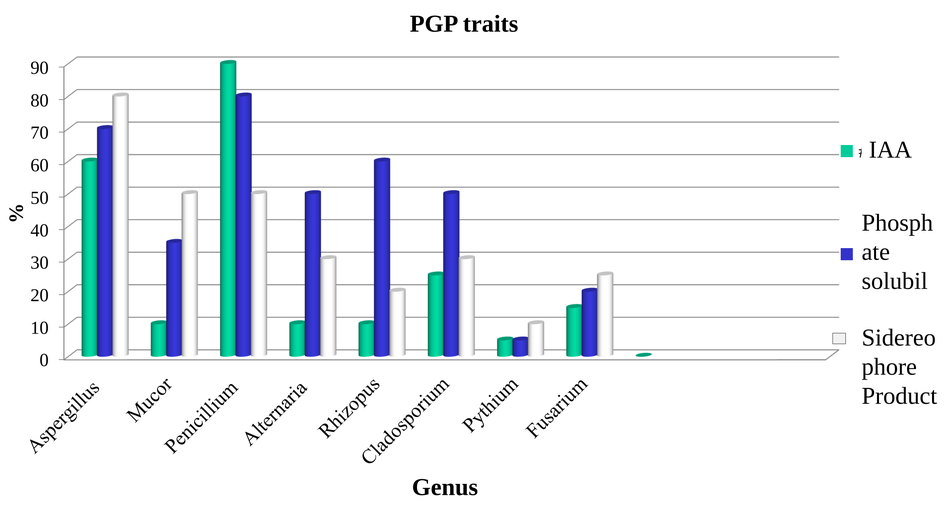


Fig5. PGP characteristic percentage of different genus isolated from medicinal plants rhizosphere..

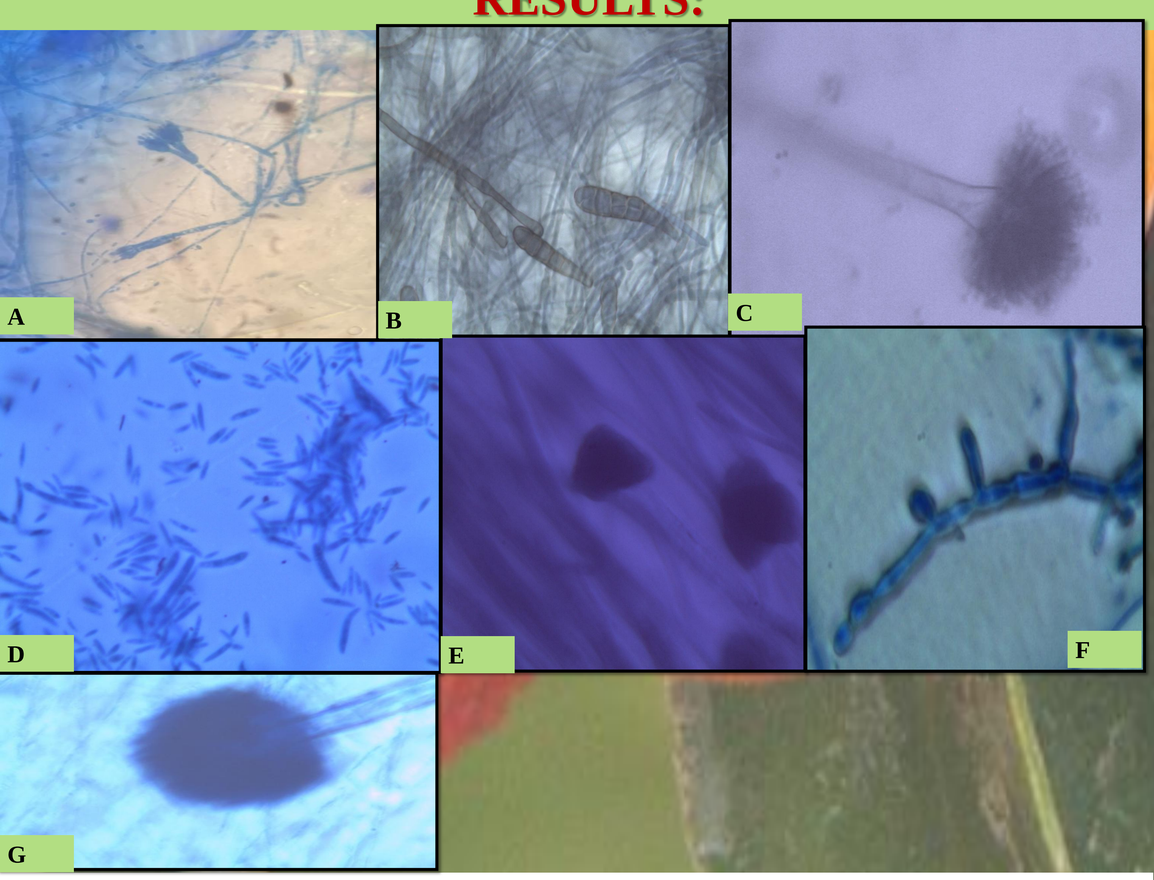


Fig3: Microphotographs of morphologically identified fungal genus under Light microscope at 40X magnification

A-*Penicillium* sp,**B**-*Alternaria* sp.**C**-*Aspergillus* sp;**D**-*Fusarium*, **E**-*Mucor*,**F**-*Cladosporium*,**G**-*Rhizopus*

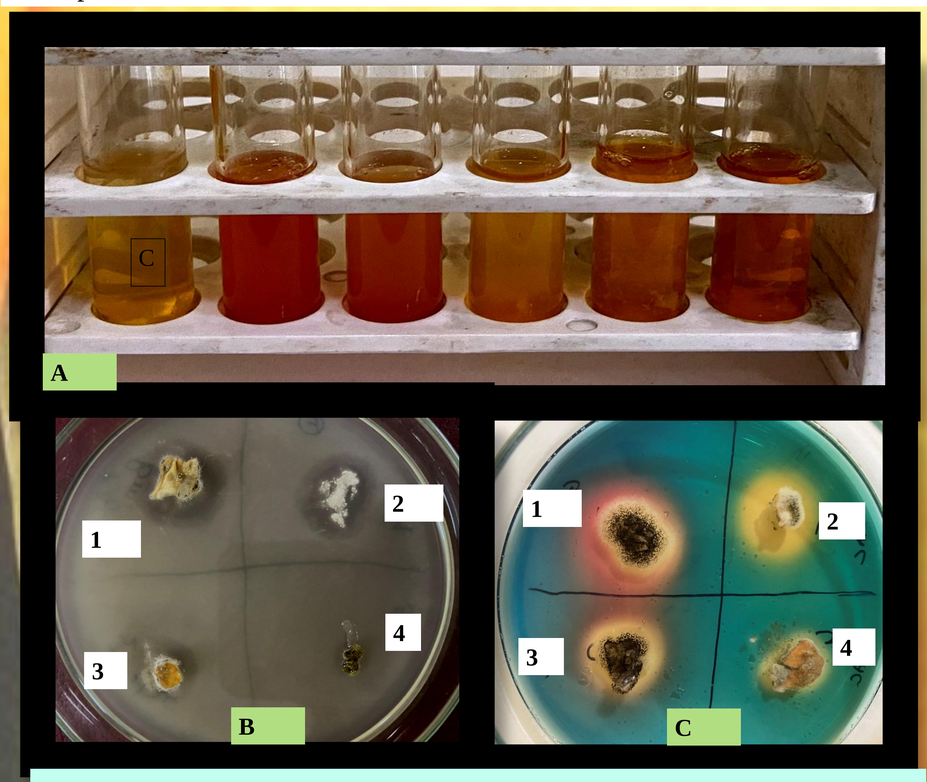


Fig 6. Showing fungi morphotypes

A. IAA production on peptone broth

B. Phosphorus solubilization on Pikovaskya agar

C. Siderophore production on CAS agar

DISCUSSION:

- In the present study rhizosphere soil of 4 medicinal plant possess diverse groups of genus. Higher abundance of *Aspergillus* genus(33%) followed by *Mucor*(22%) and *Penicillium(20%)* was found.
- This study signifies PGP potential of isolated 45 morphotypes which may provide multifaceted beneficial effects on plant growth and health and bioactive metabolite enhancement.Miller,2012 reported positive effect of bioinoculation of PGP isolated from medicinal plants.

CONCLUSION

- Total 45 morphotypes was isolated from four medicinal plants rhizosphere out of which *Aspergillus* and *Penicillium* genus posses higher percentage of plant growth promoting (PGP) traits.Therefore have potential for the development of bioinoculants for the medicinal plant .
- These properties not only promote growth but give insights of host plant fungi interaction and phytochemical modulation with the use of “omic” technology.

ACKNOWLEDGEMENT:

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Characterisation of plant growth promoting bacteria isolated from volcanic soil.

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Background

- **Abiotic stresses dominantly affects the production of agricultural crops at a global scale each year.**
- **PGPR are typically incapable of improving plant fitness under abiotic stress conditions in local habitats.**
- **Apart from genetic engineering and molecular marker assisted breeding technologies, use of alternate technologies like utilization of 'exotic' plant growth promoting bacteria (PGPB) for ameliorating abiotic stress is gaining importance.**

Objective

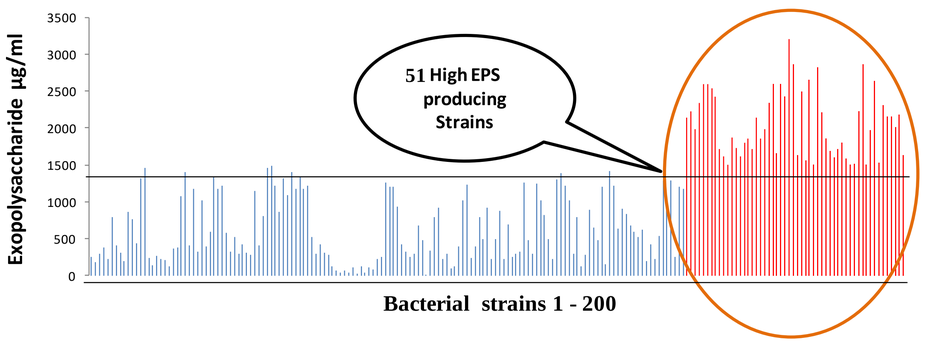
- **Exploitation of extreme environment (volcanic soil) for isolation of PGPR in favor of plant growth promotion.**

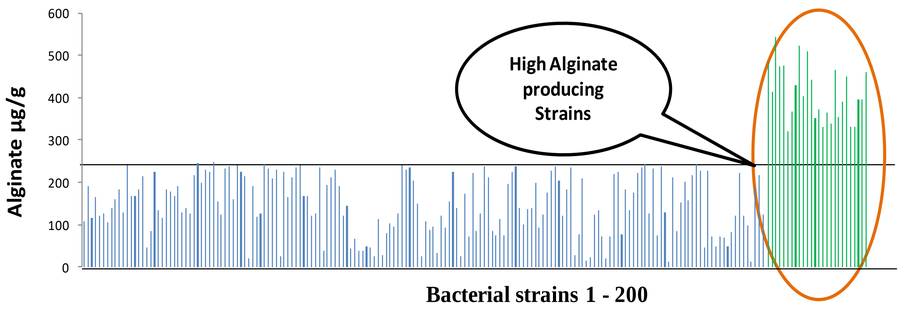
Materials and Methods

- **Screening of isolates having high exo- polysaccharide and alginate production along with abiotic stress tolerance ability.**
- **Temporal quantitative estimation of survivability and PGP attributes under abiotic stresses of selected strains.**
- **Evaluation of bacterial isolates for their ability of plant growth promotion under *in vivo* condition.**
- **16S rRNA gene PCR amplification for characterization of potentially selected isolates.**

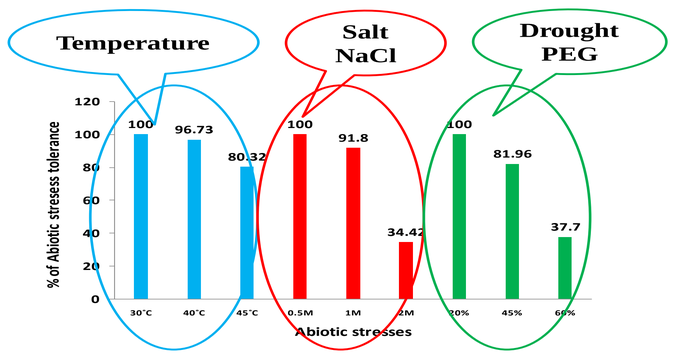
Results

S. No.	Location	District	Country	Coordinates	source	Year of sampling
1	Valcano mud baratanga,	North and Middle Andaman district	India	12°07'N 92 °47'E	Mud	April 2010
2	Smith island	North and Middle Andaman district	India	13° 18.532′ N; long. 93° 04.314′ E	Soil	April 2010
3	Lime stone cave, Baratanga	North and Middle Andaman district	India	12.9200° N, 92.9000° E	Stone	April 2010
4	Uttara,		India		Sand	April 2010
5	lichen adhered stone from a cave, baratanga;	North and Middle Andaman district	India	12°07'N 92°47'E	stone	April 2010
6	Alkaline soil sample, Banthara;	Lucknow	India	26°41'56"N 80°49'55"E	Soil	April 2010
7	Sippy ghat	Andaman	India	11.6683° N, 92.7378° E	soil	April 2010





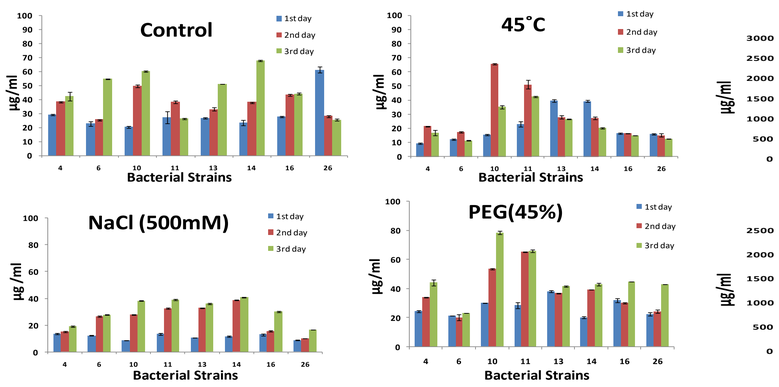
Screening of bacterial isolates based on exo-polysaccharide and alginate production



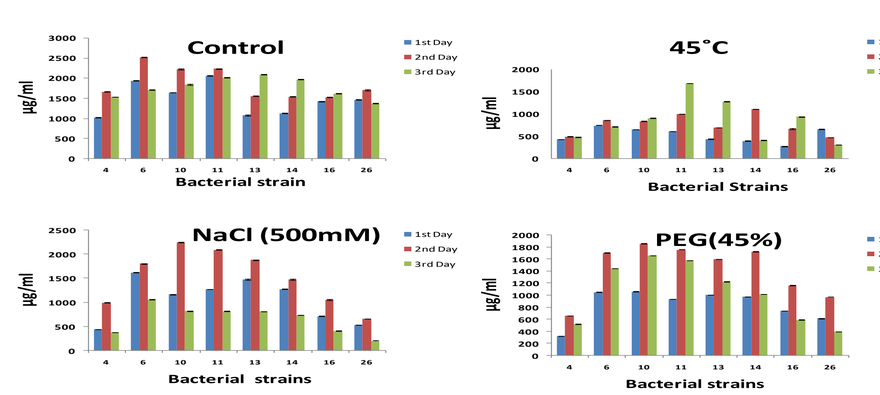
Screening of bacterial isolates for abiotic stresses tolerance

CFU of the selected strains in presence of different stress conditions

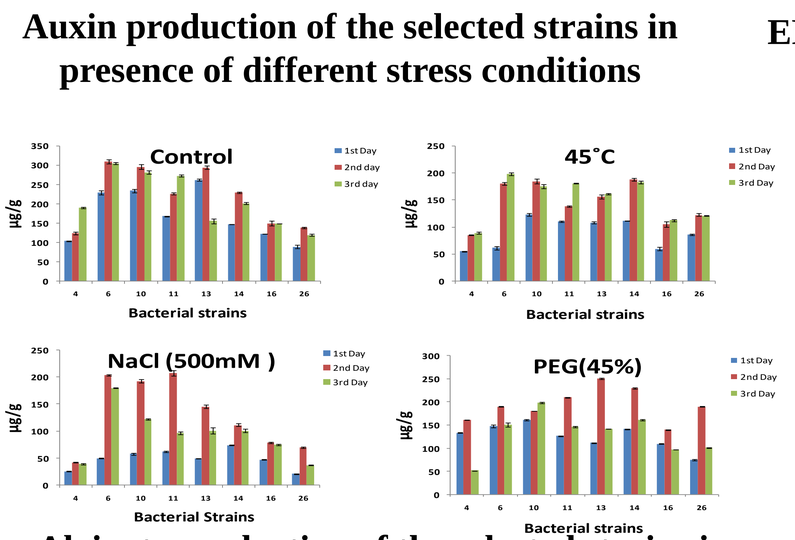
Phosphate solubilization of the selected strains in presence of different stress conditions



Auxin production of the selected strains in presence of different stress conditions

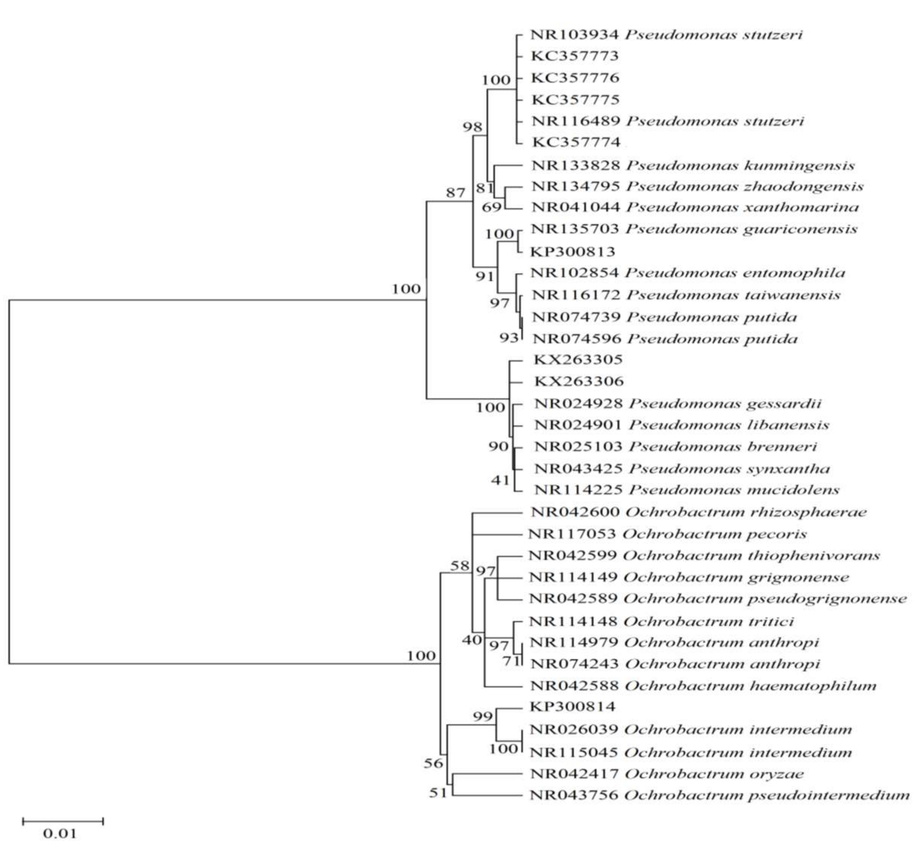


EPS production of the selected strains in presence of different stress conditions



Alginate production of the selected strains in presence of different stress conditions

Plant growth promotional activity of bacterial strains on *Zea mays*



Phylogenetic relationship based on the 16S rRNA gene

Conclusions

- **Exotic *Pseudomonas* spp. and *Ochrobactrum* sp., isolated from extreme environment exhibiting tolerance towards temperature , salt and drought stress with multiple plant growth promoting (PGP) attributes.**
- **Results based on the beneficial plant–microbe interactions indicate that it is possible to develop *Ochrobactrum* sp.(NBRISH6) as bioinoculant for stress environment as phytostimulator to impart abiotic stress tolerance in plants.**

6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
(3-4, September 2021)





Screening of phosphate solubilizing microorganisms from diverse soil samples: A promising approach as biofertilizers

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Aim

The present work was based on following objectives : to isolate efficient indigenous microorganisms from various rhizospheric soil samples having phosphate solubilisation efficiency and to quantify their phosphate production rate .

Introduction

Phosphorus is one of the major nutrients, second to nitrogen, required by plants for growth and productivity. It plays remarkable role in photosynthesis, sugar production, nucleic acid synthesis, and promotes N2 fixation in legume and energy production. A greater part of soil organic and inorganic phosphorus(approx 95-99%) is present in the form of insoluble phosphates that is bound by Al or Fe in acid soils, or Ca and Mg in alkaline soils which cannot be utilized by the plants easily. Phosphate solubilizing microorganisms can increase soil phosphate solubility and availability by production of organic acids and enzymes, that solubilize insoluble phosphate and make available to the plants.

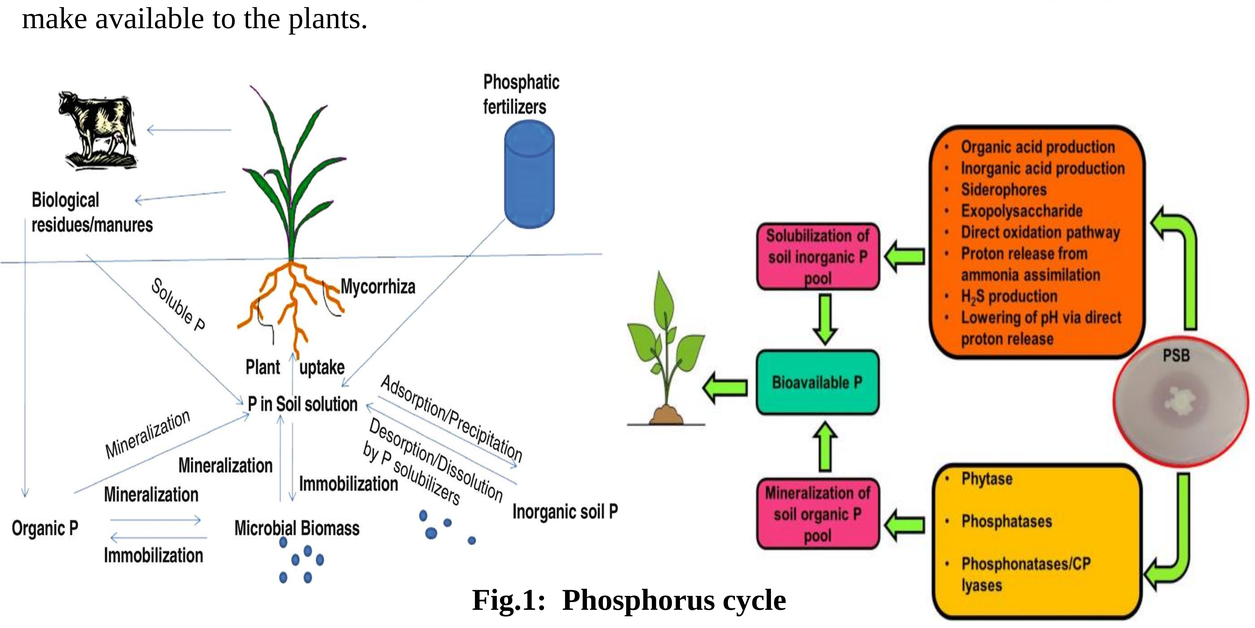

Fig.1: Phosphorus cycle

Materials and Methods

Screening of Phosphate Solubilizing Microorganisms(PSM)

- Enrichment of various rhizospheric soil samples in Pikovskaya's broth.
- Enrichment followed by isolation on modified Pikovskaya's agar medium containing bromophenol blue.

Identification of PSM

- The colony morphology of the isolated microbes was examined after growth on Pikovskaya's agar medium at 28˚C for 7 The morphological, cultural and biochemical characteristics of bacteria were used for identification of isolates using Bergey's Manual of Systematic Bacteriology, while fungal identification was based on the study of colony characteristics and microscopic features. . The microscopic characters were identified using trinocular microscope(Labovision LABEX –AXL).

Qualitative estimation: Determination of The phosphate solubilization efficiency (PSE)

- The phosphate solubilization efficiency (PSE) of all isolates was evaluated by measuring the zone of solubilizatiion on modified Pikovskaya's agar medium containing bromophenol blue .
- PSE was calculated as the ratio of **: PSE= Colony diameter + Halo zone / colony diameter.**

Quantitative Estimation

- The quantitative estimation of phosphate solubilization by the selected isolates was performed spectrophotometrically at 660nm by Olsen et al method, using Shimadzu U/Vis 1800 Spectrophotometer.

Results

Identification of phosphate solubilizing microbes:- The colony morphology of the isolated microorganisms were examined after growth on modified Pikovskaya's agar medium at 28˚C for 7 days and their colony morphology, colony color and their microscopic characters were recorded.
Most of the isolated phosphate solubilizing microorganisms were species of the genera of *Bacillus,Pseudomonas,Streptomyces,Aspergillus,Penicillium,Trichoderma* and an unidentified yeast (P1).

Phosphate solubilization activity:- From the 34 isolates screened, six fungal species (F1,F2,F3,F4,F5 and F6) and 1 yeast (P1) were evaluated and their phosphate solubilization efficiency was calculated by measuring colony diameter and zone of solublization on Pikovskaya's agar medium. PSE value for P1 was found to be 2.8 and PSE values for fungal isolates was in range of 1.5 to 2.3. Phosphate solubilizing capacity of P1 was found to be 36.92 µg/ml as determined by Olsen et al method after 15 days of incubation at 28˚C in Pikovskaya's broth.

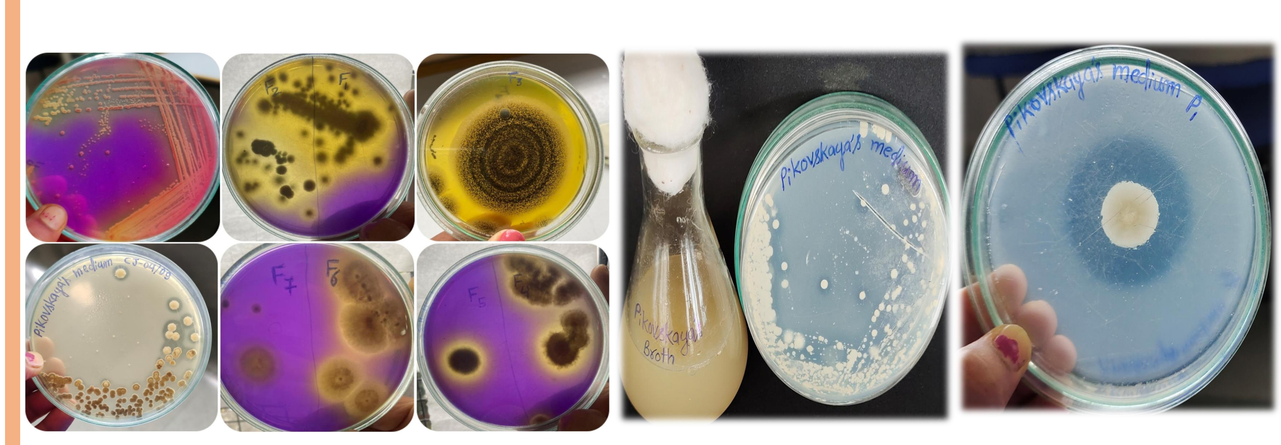

Fig.2: Zone of solubilization by microbial isolates on Pikovskaya's agar medium

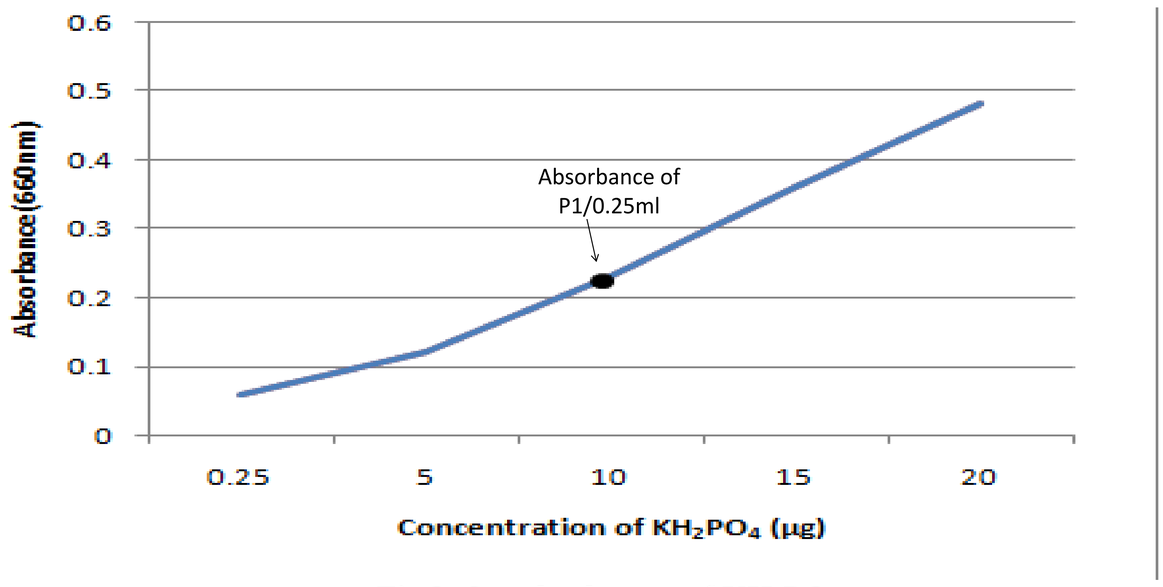

Fig.3: Standard curve of KH_2PO_4

Conclusion

From our research work we can conclude that isolate P1 can be utilised as an efficient biofertilizer strain after studying certain other PGPR attributes. The present work is an attempt to explore species which are responsible to solve the problem of soil fertility. Isolating efficient strains of PGPR submitting it to regional centers of organic farming, making farmers aware about these efficient strain, giving farmers information about benefits of organic farming, gradually motivating them to turn towards organic farming is need of society and our present work is a step towards it.

Looking for further research of isolates:

- IAA production
- Cytokinins production
- Gibberlins production
- Antibiotics
- Siderophores
- Cellulase production

Acknowledgement

The authors express thanks to the management and Principal of PMB Gujarti Science College, Indore, for providing instrumental and technical support .We are also thankful to teaching and non teaching staff of Department of Microbiology,

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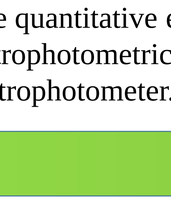
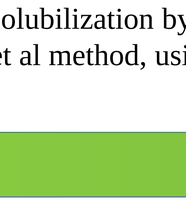
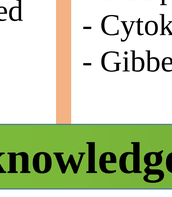
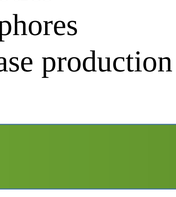


ISOLATION AND CHARACTERISATION OF FEATHER DEGRADING MICRORGANISM(S) FROM KHANDESH REGION

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ABSTRACT

This present investigation is intended to isolate and characterize feather degrading microorganism from soils of Khandesh region. Proteolytic enzymes which hydrolyze insoluble keratins more efficiently than other proteases are called keratinases produced keratinolytic bacteria. Keratinase producing microorganisms have the important industrial applications in fermentation technology. These protein byproducts may be used as animal and livestock feed, and as leather filling agents. Keratinase can be used for preparation of vaccine for Dermatophytosis, for pharmaceutical enhancement of the nail treatment. They are also useful for the degradation of prion and prion like proteins. Microorganisms that were isolated and tested for their capability to grow on feather meal agar (FMA). Influence of various parameters on enzyme activity of the organisms was investigated. pH 7 was optimum for maximal enzyme activity than pH 5 and pH 9. The optimum temperature for enzyme activity was 37^0 C. The effects of metal ions Zinc, $CaCl_2$ and $MgCl_2$ were found to be the activators and $HgCl_2$ was the inhibitor for the enzyme.

Key words- keratinase, enzyme activity, fermentation, proteolytic .

INTRODUCTION

Keratinases are specific type of protease enzymes, the degradative substrate being "keratin". Keratinases (EC 3.4.21/24/99.11) are a particular class of serine or metallo type proteolytic enzymes that display the capability of degrading insoluble recalcitrant keratin substrates. Reduction of cysteine bridges may have a significant influence on keratin degradation and sulfitolysis. In general, protease has wide range of industrial application and it is reported that proteases count for nearly 65% of the world enzyme market. Commercial proteases are mostly produced from various bacteria and it was reported that about 35% of the total microbial enzymes used in detergent industry are the proteases from bacterial sources. Plants, animals and microbes are the main sources for protease production. The preferred sources of proteases are microbes because of their rapid growth and the ease with which they can be genetically manipulated to generate new enzymes with altered properties. Keratin degradation is an age old phenomenon associated with dermatomycosis; certain fungi such as Aspergillus, Ctenomyces and genus Streptomyces group from actinomyces were recognized as keratinase producers. It was mainly a domain of medical mycologists. However, its biotechnological and environmental importance came to light with the first report on the isolation and characterization of a feather degrading bacterium Bacillus licheniformis PWD-1. This work mainly focused on the feather recycling and feather meal production, where they established KerA from B.licheniformis as a potential keratinase. For years to come, KerA was exhaustively characterized including its sequence and expression in various heterologous hosts.

Properties of Keratinase:-

1. Keratinase are all serine protease.
2. Their molecular weight generally ranges from 30 to 50 kDa.
3. Most keratinase enzymes are active at P^H 7 to 8, other at odd values.
4. Keratinase is found to have broad substrate specificity and it can hydrolyzed noy only keratin but also large variety of insoluble proteins. Ex:-BSA, Collagen, casein.
5. Keratinase enzyme characterized by good there stability.
6. Different keratinase produces by different organism have E.C. No Ex:- Bacillus licheniformis has E.C.3.4.99.11.
7. Enzyme is fairly stable at low tempreture .
8. Isoelectric point is 7.23.

METHDOLOGY

Isolation of Microorganisms

Samples (soil) were taken from the poultry farm in the town of Khandesh Region (Maharashtra). Serial dilution for each sample was prepared by adding 1 gm of the soil sample to 9 ml of sterile saline. Then serial dilution up to 10^{-9} was done using sterile saline. All dilutions were placed on Nutrient agar medium and minimal medium incubated at 37^0C for 24 hours.

IMViC Test, spore staining, catalase test, starch hydrolysis, casein hydrolysis, proteolytic activity were performed as per standard protocols

Preparation of substrate

White chicken feathers were used in this study to prepare the pure feather meal powder. They were first washed extensively under tap water to remove blood and any dust particles. Then abundant with distilled water. All the materials were later oven – dried at 75^0c for 8 hours. The dried keratin materials were chopped into pieces not exceeding 1.5 cm in length, and then milled to 60 – mesh particles size. The powder was kept at room temperature and used for further studies.

Screening for keratinolytic bacteria

Among the different bacterial colonies obtained on the spread plated agar plate. Five different morphologically different bacterial colonies were identified and each inoculated into a sterile feather meal agar plate. The inoculated plate was then incubated at 37^0c for 48 hours. The strain that shows high zone of clearance was observed.

Enzyme production

1) The inoculums can be prepared into a medium.
2) This culture is distributed in five conical flasks.
3) The different bacterial colonies obtained on the nutrient agar plate. Five different bacterial colonies were identified and each inoculated onto a distributed culture.
4) The culture is incubated at 37^0c for 7 days.
5) After incubation this culture is transferred into centrifuged tube and centrifuged at 5000rpm at 10^0c for 20 minutes and the supernatant served as crude extracellular keratinase, which is used without further purification.

Estimation of protein concentration

The protein content was determined according to the Folin's Lowry method.

Protein detection

1) Take 5 test tubes and naming as test 1, test 2, test 3, test 4, and test 5.
2) Then add 2 ml of enzyme crude extract in each test tube.
3) The test tube with 2 ml distilled water serves as blank.
4) Add 4.5 ml of reagent I and incubate at room temperature for 10 min.
5) After incubation add 0.5 ml of reagent II and incubate at room temperature for 30 minutes.
6) Measure the absorbance at 660 nm and plot the graph.(Test against O.D)

Enzyme Activity

Keratinase activity was determined by measuring the hydrolysis ability on keratin 200 µl of enzyme extract was added to 800 µl of keratin solution (5 mg/ml) in 50 mM phosphate buffer (P^H 7.5). After 60 minute reaction at 50^0c, equal volume of 15% Trichloroacetic acid (TCA) solution was added to stop the reaction. Absorbance at 450 nm was then measured. (After 5 minute centrifugation at 10,000 g. one unit of activity was defined as the amount of keratinase that caused an increase in absorbance of 0.01 at 450 nm within 60 minute reaction at 50^0c.

Effect of P^H on enzyme activity

200 µl of enzyme crude extract
Added to 800 µl of keratin solution in 50 mM phosphate buffer with different P^H
Incubation at 55^0c for 60 minute
After incubation equal volume of 15% TCA solution was added to stop the reaction
Absorbance at 450 nm was then measured.

Effect of Temperature on enzyme activity

200 µl of enzyme crude extract
Added to 800 µl of keratin solution in 50 mM phosphate buffer with different P^H
Incubation at 4^0c, 37^0c, and 55^0c for 60 minute
After incubation equal volume of 15% TCA solution was added to stop the reaction
Absorbance at 450 nm was then measured.

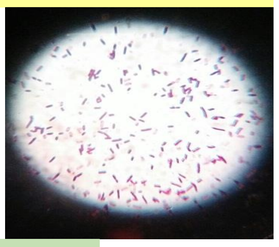
Effect of Metal Ions on enzyme activity

200 µl of enzyme crude extract
Added to 800 µl of keratin solution in 50 mM phosphate buffer with different metal ions at 3 mM concentration
Incubation at 55^0c for 60 minute
After incubation equal volume of 15% TCA solution was added to stop the reaction
Absorbance at 450 nm was then measured.-

RESULTS AND CONCLUSION

Isolation and colony charactarisation:-

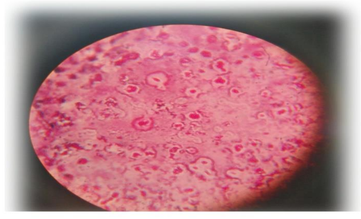
Total of five microbial cultures were isolated from soil sample of different regions. Microbial isolates were selected on the basis of different morphology appear on nutrient agar plates. Selected cultures are maintained on minimal agar plates. All bacteria were **Gram Positive**.



MICROBIAL ISOLATES AND GRAM STAINING

Culture No.	Indole	Methyl red	Vogesproskaur	Citrate utilization
M1	Negative	Positive	Positive	Negative
M2	Positive	Negative	Positive	Positive
M3	Positive	Positive	Positive	Negative
M4	Positive	Positive	Negative	Positive
M5	Positive	Positive		

Culture No.	Observation
M1	Positive
M2	Positive
M3	Positive
M4	Positive
M5	



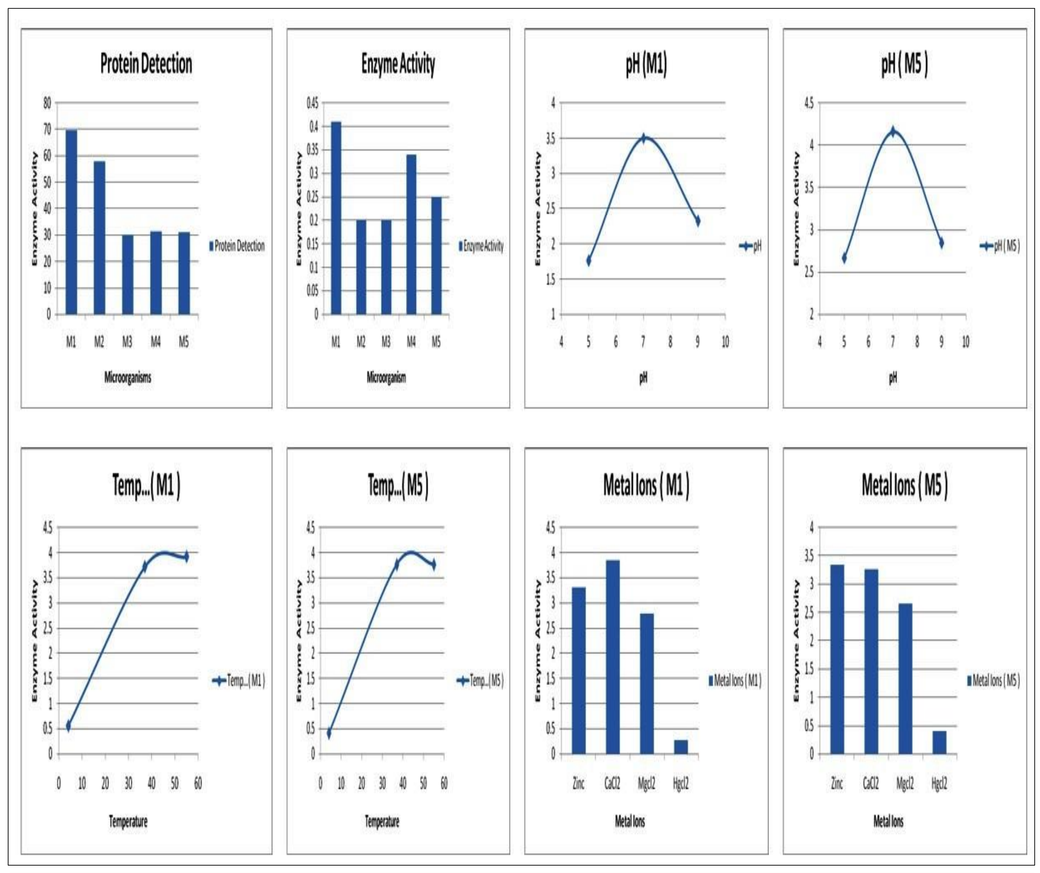
Spore Staining

Catalase test:-Catalase test was performed, bubbles were observed test is positive.

Starch hydrolysis was performed the result is zone of clearance is observed and test is positive.

Casein hydrolysis was performed the result is zone of clearance is observed and test is positive

Screening of keratinolytic producing bacteria The given isolated bacteria show keratinolytic activity on keratin agar. Zone of clearance were observed.



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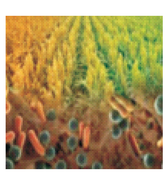

1.21

ISOLATION OF PLANT GROWTH PROMOTING BACTERIA FROM FERMENTED PANCHAGAVYA AND THEIR EFFECT ON *Vigna radiata*

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INTRODUCTION

- Panchagavya is a mixture of five cow products viz. cow dung, urine, milk, curd and ghee, mentioned in ancient Indian scripts.
- Microbial fermentation of the panchagavya resulted in a rich repertoire of compounds and microbes that are beneficial for plant growth.
- Panchagavya bacteria increase the growth as well as development of plant by amassing the accessibility of mineral nutrients, solubilizing phosphorus, and phytohormones production.
- The production of growth regulators by microorganisms delivers benefits to the plant with the facilitation of root system expansion, which increases the absorption of water and nutrients and improves plant survival.

MATERIALS AND METHODS

Preparation of panchagavya

Fresh cow dung (10 g) and ghee (1 g) were mixed thoroughly in a plastic container. The mixture was kept for 3 days and mixed twice a day.

•On 4th day cow urine (6 ml), cow milk (4 ml) and cow curd (4 ml) were added to the mixture.

•This mixture was kept for 15 days for fermentation mixed twice a day for 10 minutes.

Plant growth promoting traits of RK-1 & RK-7

•RK-1 and RK-7 isolated from fermented panchagavya which showing plant growth promoting activities.

•RK-1 was studied for GA production and RK-7 for phosphorus solubilization and their effect on the growth of *Vigna radiata* was tested.

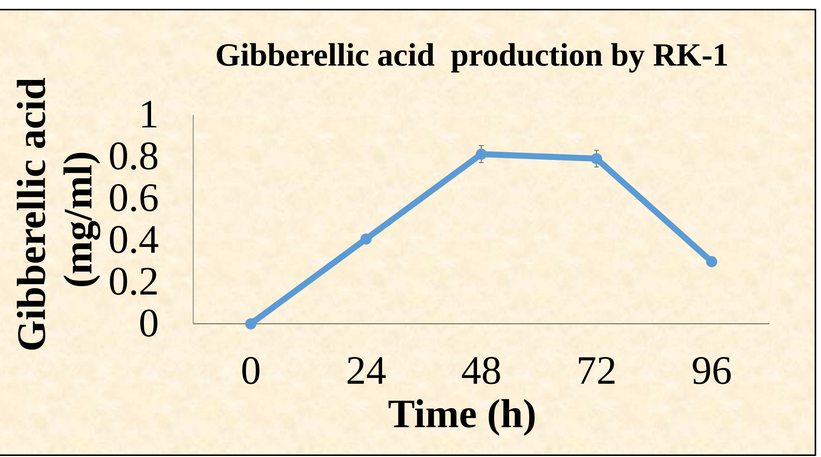
Gibberellic acid production by RK-1.

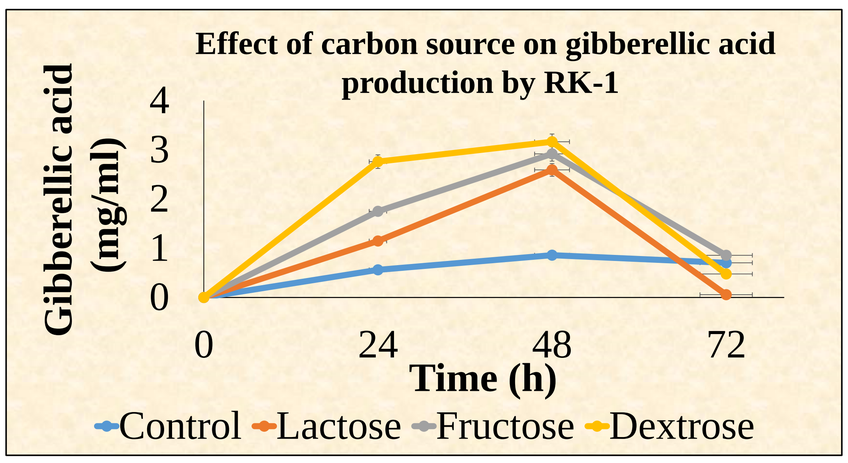
The effect of carbon (dextrose, lactose and fructose; 2% w/v) and nitrogen sources ($NaNO_3$, urea, and glycine; 0.5% w/v)) on the production of gibberellic acid was studied.

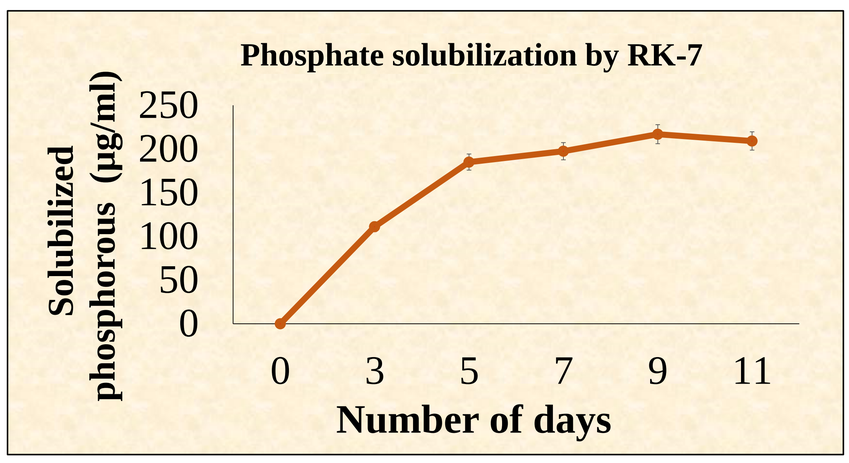
Spectrophotometric estimation of gibberellic acid was performed using the method by (Graham and Thomas, 1961).

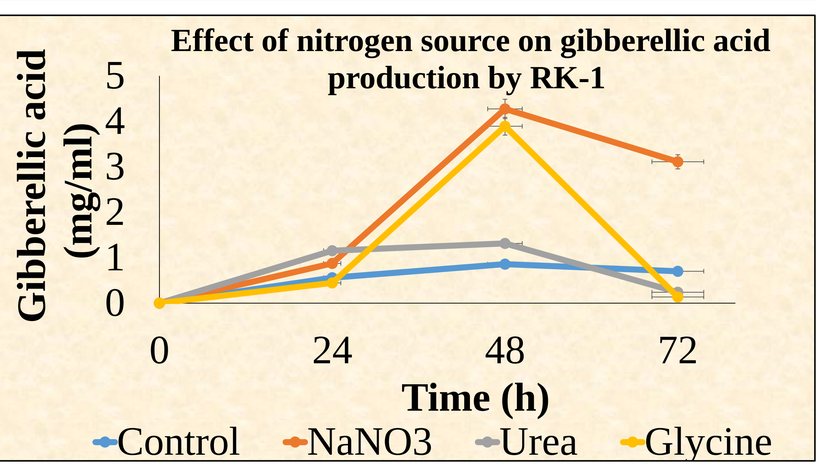
Phosphate solubilization by RK-7.

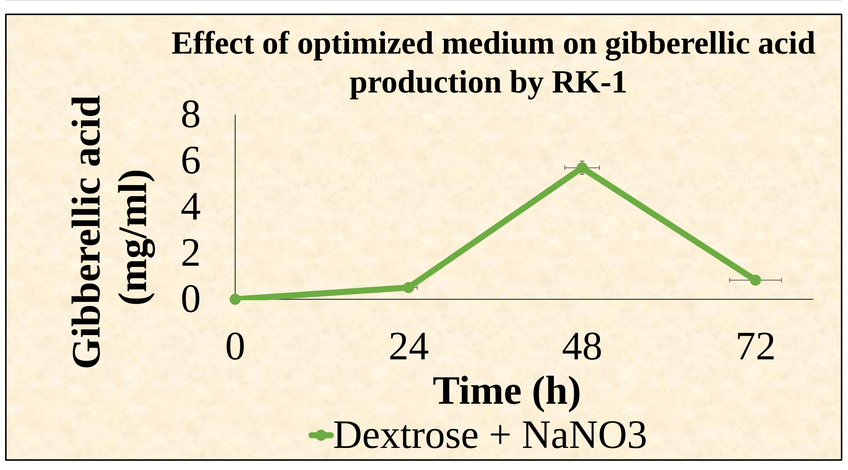
Solubilization of different phosphorus sources like Aluminum phosphate (AP), Zinc phosphate (ZP) and Tricalcium phosphate (TCP) was studied using Pikovskaya's broth. The phosphate solubilized was estimated by Stannous chloride method (King, 1932).

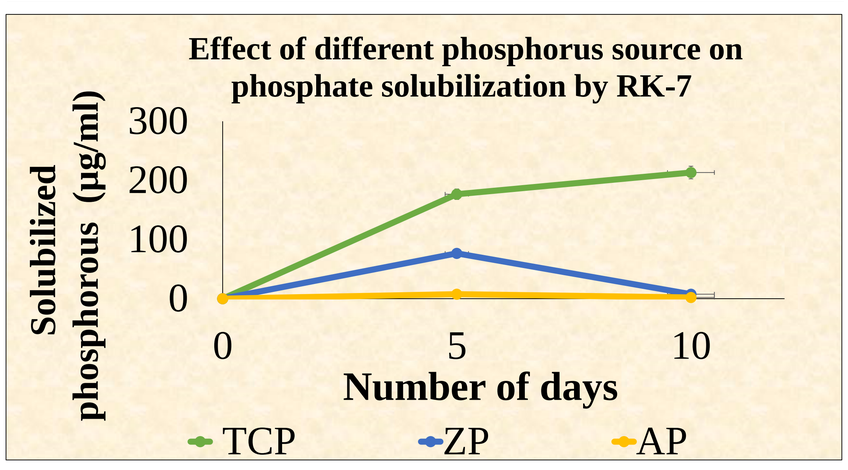












EFFECT OF RK-1, RK-7 & MIXED CULTURE ON *Vigna radiata* GROWTH

Vegetative parameters	Treatments							
	Control		RK-1		RK-7		Mixed culture	
Day	10	20	10	20	10	20	10	20
Fresh weight (g)	0.21	0.38	0.35	0.75	0.38	0.65	0.26	0.79
Dry weight (g)	0.03	0.06	0.08	0.12	0.06	0.10	0.05	0.13
Shoot length (cm)	9.5	10.0	13.0	14.5	12.0	13.0	13.0	15.0
Root length (cm)	1.9	6.0	5.0	6.2	1.0	6.5	2.4	8.5
Root number (No.s)	1	1	1	1	1	1	1	1
Root hair number (No.s)	0	2	1	12	2	9	2	15
Leaf number (No.s)	2	2	2	4	2	5	2	5
Leaf area (cm^2)	2.40	2.50	5.25	6.75	5.60	6.00	5.25	6.80

CONCLUSION

The present study demonstrates that fermented panchagavya contains a various group of potential microorganisms possessing plant growth promoting abilities viz. gibberellic acid production and phosphate solubilization. Application of pure cultures RK-1 and RK-7, and in mixed culture significantly increased *Vigna radiata* growth as compared to control. It showed considerable improvements in the fresh weight, shoot length, root length, root hair number, leaf number and leaf area in *Vigna radiata*. Since the most soils in the world are deficient in plant-available nutrients, the use of these bioinoculants would increase the nutrients availability in the soil, which will also help to minimize the use of chemical fertilizers, reduce atmosphere contamination and encourage sustainable agriculture.

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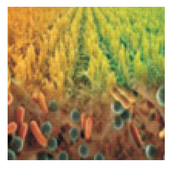
Graham, H. D., and Thomas, L. B. (1961). Rapid, Simple Colorimetric Method for the Determination of Micro Quantities of Gibberellic Acid. *Journal of pharmaceutical sciences*, *50*(1), 44-48.



Mining Barnyard millet rhizospheric microorganisms for functional plant health promoting traits

Registration No. 1.23

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Abstract
Barnyard millet (*Echinochloa spp.*) is one of the most under-researched crops grown in the submontane Himalayan region where this crop occupies a special place as food and fodder. In the present study, rhizospheric soil of barnyard millet was collected from four different districts of Uttarakhand namely, Chamoli, Pauri, Pithoragarh and Almora. Enumeration of microbial population was done on five different media (Pikovaskaya, Nutrient agar, Potato Dextrose Rose Bengal agar, king's B, Actinomycetes agar). Four out of 176 isolates were selected on the basis of growth ihnhibition of three fungal pathogens. Four bacterial isolates namely, AA17, AA12, MA13, and MN8 were further screened for biochemical properties including amylase, siderophore, chitin hydrolysis, xyalanase, ammonia production etc. All the isolates were positive for one or other properties. Effect of individual isolates and consortium was analyzed on germination and growth promotion in tomato. Significant effect was observed in consortium in comparison to individual bacterial isolates and control. Thus barnyard millet can be a potential source of plant growth ptromoting bacteria which can be used as a bioinoculant for economically important crops.

Introduction
Echinochloa sp. is an underutilized crop in terms of research and development despite of its nutritional and climate resilient properties. Rhizo-microbiome constitutes biologically diverse community of soil microorganisms that inhabit the plant rhizosphere influencing their growth and productivity by various beneficial biochemical and microbial interactions Due to vast untapped rhizospheric diversity of stress tolerant crops present study would be undertaken to find potential plant growth promoting agents from the rhizosphere of barnyard millet cultivated in hilly areas of Uttarakhand. Limited research is done on exploring rhizospheric microflora of such low input, stress tolerating crops which may be a rich source of beneficial microorganisms well adapted to such extreme conditions and may lead to development of potential bioinoculants for economically important crops.

Methodology
- Rhizospheric soil samples of baryard millet were collected from 4 districts of Utarakhand
- Enumeration was done on NA,RBA,AA,KB,Piko).
- 4 isolates selected on the basis of antagonistic traits against phytopathogens
- Functional characterization was performed for the four isolates
- Effect of all the four isolates and consortium on growth promotion in tomato seedlings.

Results
- Population count on different media was found to be maximum for bacterial population however fungal counts were lower than bacterial population.
- All the isolates were positive for one or more properties (Table 1)
- Considerable increase in germination, seedling length , fresh weight and dry weight was found.
- Sedling height was maximum for consortiium treated seeds. However all the treatments were better than control in all the three parameters**.**

Isolates	Siderophore production	Chitinase production	Ammonia production	Cellulase production	Amylase production	Voges proskauer	IAA Production
AA17	+	+	+	+	+	+	+
AA12	+	+	+	+	+	+	-
MA13	+	+	+	+	+	+	-
MN8	+	+	+	+	+	+	-

Table 1. Biochemical characterization of selected isolates

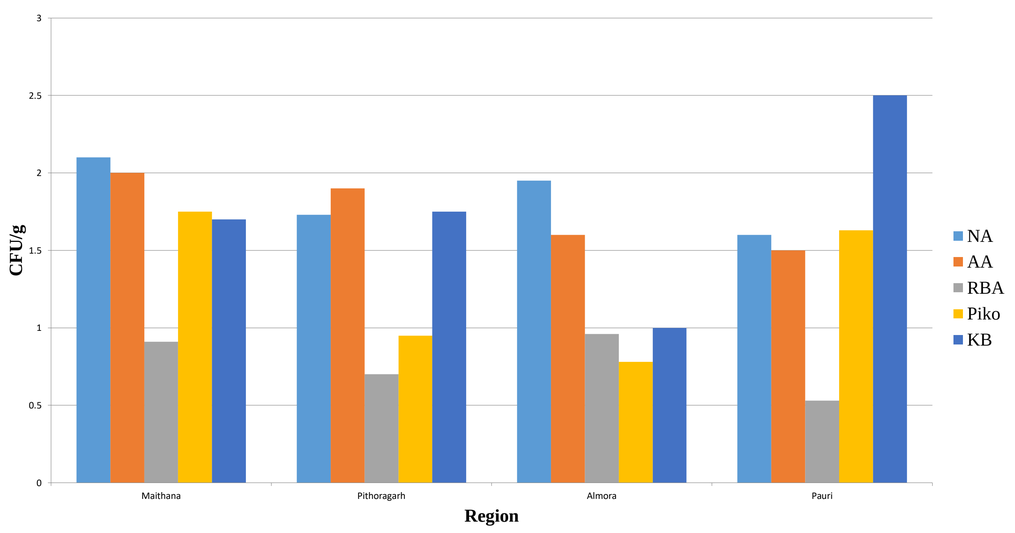
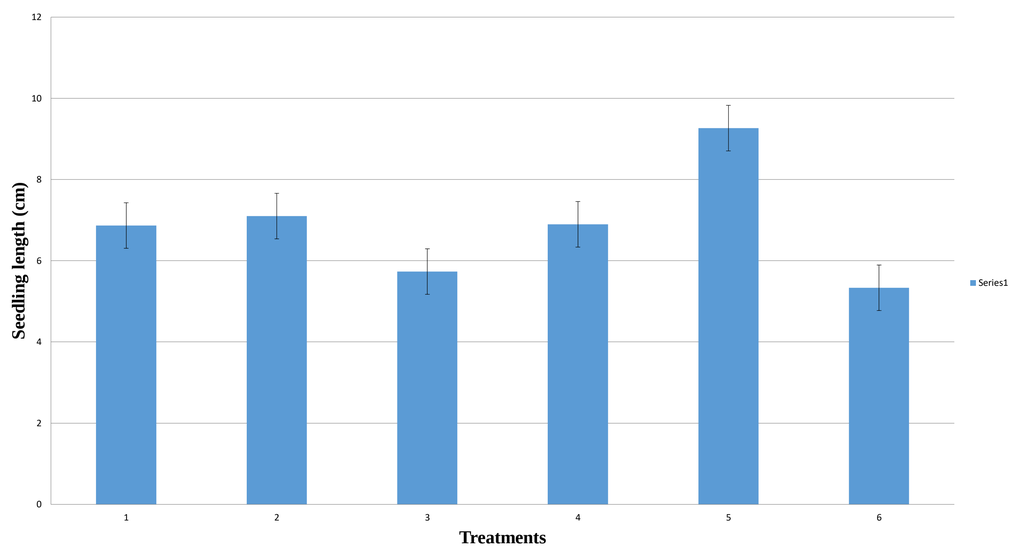


Figure1. Population count (cfu/g) on 5 different media)

Figure 2. Effect of four isolates and consortium on tomato seedlings



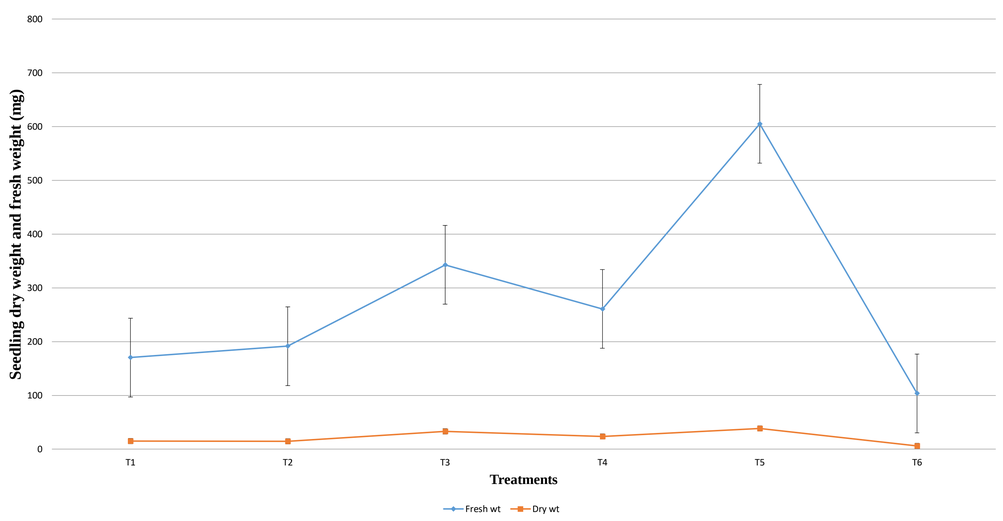


Figure3. Effect of seed biopriming on seedling length, fresh weight and dry weight

References:
Yagmur, B., Gunes, A. Evaluation of the Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Quality Parameters of Tomato Plants in Organic Agriculture by Principal Component Analysis (PCA). *Gesunde Pflanzen* **73,** 219–228 (2021).

Conclusion
Isolates recovered from rhizosphere of *Echinochloa* sp. show multiple plant health promoting properties and showed positive effect on germination and seedling growth parameters in tomato. These isolates can be used as promising bioinoculants in economically important crops other than tomato**.**

Acknowledgement: Authors are thankful to Director Experiment station and Department of microbiology, Pantnagar





INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA PROMOTES VIGOR IN TWO DIFFERENT WHEAT GENOTYPES BY STIMULATING SOIL ENZYME ACTIVITY

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Registration Number: 1.24

INTRODUCTION

After nitrogen (N), phosphorus (P) is second most important nutrient that plant needs at constant rate throughout early stages of development but majority of soil phosphorous is present in immobilized form. The phosphorous unavailability affects around two billion hectares of land worldwide. Considering this, the role of phosphate solubilizing bacteria (PSBs) in mitigating soil P unavailability becomes prominent. PSBs have the potential to solubilize immobilized form of phosphorous into most available form by promoting soil health. Thus to evaluate the role of 18 PSBs in phosphorous mobilization and in soil health we conducted pot trial with two superior wheat genotypes.

Objectives: i) checking the potential of 18 PSB qualitatively as well as quantitavely, ii) Pot trial to evaluate the potential of 18 PSB on two wheat genotypes, iii) Post inoculation soil enzyme analysis e.g. FDA, AP and urease and iv) Selection of PSB for field trial and development of consortia (L3 and P2)

METHODOLOGY

- Two superior wheat genotypes (UP262 and PBW 502) were taken.
- Seed bacterized with 18 PSBs (Sagevanshi et al 2012).
- Soil enzyme activities e.g. Alkaline phosphatase (AP), FDA (fluorscein di acetate hydrolysis) and urease.
- Phosphorous quantification

RESULTS:

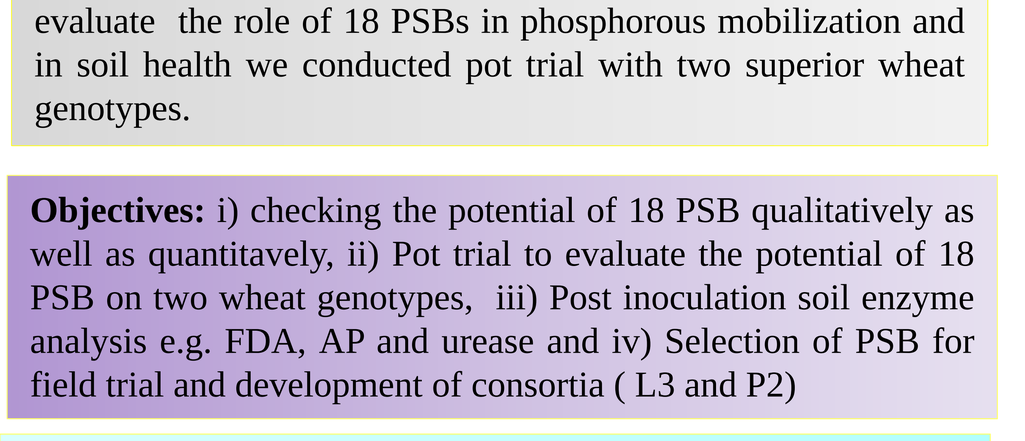

Fig.1. Figures depicting halo zone around bacterial colonies on Pikovskaya agar plate.

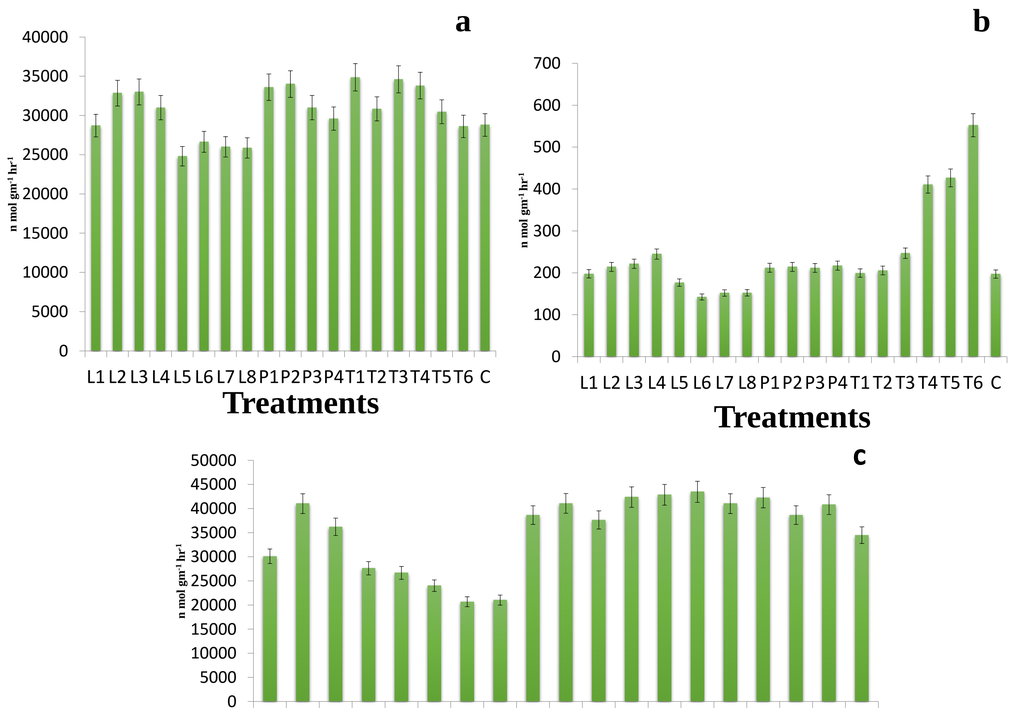

Fig 2. Response of PSB inoculation on soil enzyme activities a) Urease, b) FDA and c) AP (Alkaline phosphatase).

	PSB isolates	PHOSPHOROUS QUANTIFICATION (μgml^{-1})	ACCESSION NO.	UP 262		PBW 502	
				SL	RL	SL	RL
C	CONTROL	500±.83^{d}		17.66±.72^{ab}	12.33±.27^{a}	24.66±.47^{bc}	13.66±.54^{cde}
L1	*Pseudomonas simiae*	512±.23^{f}	MG966339	21.66±1.24^{cde}	114±.94^{ab}	29±.94^{a}	11±.47^{abcd}
L2	*Staphylococcus petrasii*	506.43±.2^{e}	MG966340	22±.94^{cdef}	16±.94^{abc}	28±.47^{b}	8±.94^{a}
L3	*Pseudomonas paralactis*	628.32±.17^{m}	MG966341	28±.72^{hi}	21±1.41^{defg}	36±1.8^{fghi}	15±.47de
L4	*Klebsiella variicola*	578.51±.07^{j}	MG966342	22.3±3.72^{defg}	24±1.88^{fg}	25±.47^{efgh}	10±.47^{abc}
L5	*Pseudomonas paralactis*	542.66±1.76^{g}	MG966343	22.66±.47^{defgh}	20±.47^{cdef}	28±.94^{defg}	11±.47^{abcd}
L6	*Streptomyces curacoi*	506±1.15^{e}	MG966344	20±1.24^{bc}	20±.94^{cdef}	25±1.88^{cdef}	12±.94^{abcde}
L7	*Streptomyces cellostaticus*	512.63±.12^{f}	MH031699	15±.72^{a}	19±1.4^{cde}	26±1.41^{bcde}	9±.94^{ab}
L8	*Pantoea conspicua*	510.63±.08^{f}	MG966345	20.33±.72^{bcd}	18±.47^{bcde}	26±.47^{bcd}	11±1.41^{abcd}
P1	*Pseudomonas hunanensis*	617.66±.88^{k}	MG966346	22.66±.47^{defgh}	22±.94^{efg}	30±.94^{efgh}	14±.94^{cde}
P2	*Pseudomonas aeruginosa*	811.32±.64^{l}	MG966347	27±.72^{i}	17±1.41^{bcd}	36±1.65^{j}	15.66±.72^{e}
P3	*Pseudomonas putida*	560.6±.19^{i}	MG966348	22.66±.98^{defgh}	20.66±1.65^{defg}	27.33±.94^{ij}	13±.47^{bcde}
P4	*Pseudomonas plecoglossicida*	476.33±4.25^{e}	MG966349	24.33±.72^{efghi}	25±.47^{g}	27±1.41^{cde}	20±2.3^{f}
T1	*Kitasatospora kifunensis*	542.65 ±1.6^{g}	MG966350	25.66±.98^{ghi}	24±1.41^{fg}	28±1.41^{ghij}	12±1.41^{abcde}
T2	*Klebsiella singaporensis*	543.1±.93^{g}	MG966351	25.33±.47^{fghi}	24±.94^{fg}	26±1.41^{efgh}	14±1.41^{cde}
T3	*Streptomyces antibioticus*	545.49±.65^{g}	MG966352	27±.94^{i}	24±1.41^{fg}	36±.94^{hij}	14±1.88^{cde}
T4	*Micrococcus yunnanensis*	412.34±.89^{a}	MG966353	26±1.18^{hi}	22±.47^{efg}	33±.72^{hij}	16±.94^{e}
T5	*Streptomyces griseoruber*	424.6±1.39^{b}	MG966354	21.66±.72^{cde}	22±.47^{efg}	32.33±.72^{def}	12±.47^{abcde}
T6	*Staphylococcus pasteuri*	553±1.15^{h}	MG966339	17.66±.72^{ghij}	19±.94^{cde}	29.33±.27^{bcde}	14±.94^{cde}

Table 1. Impact of PSB application on plant growth promotion of two different wheat genotypes e.g, UP262 and PBW 502 along with their P- quantification potential.

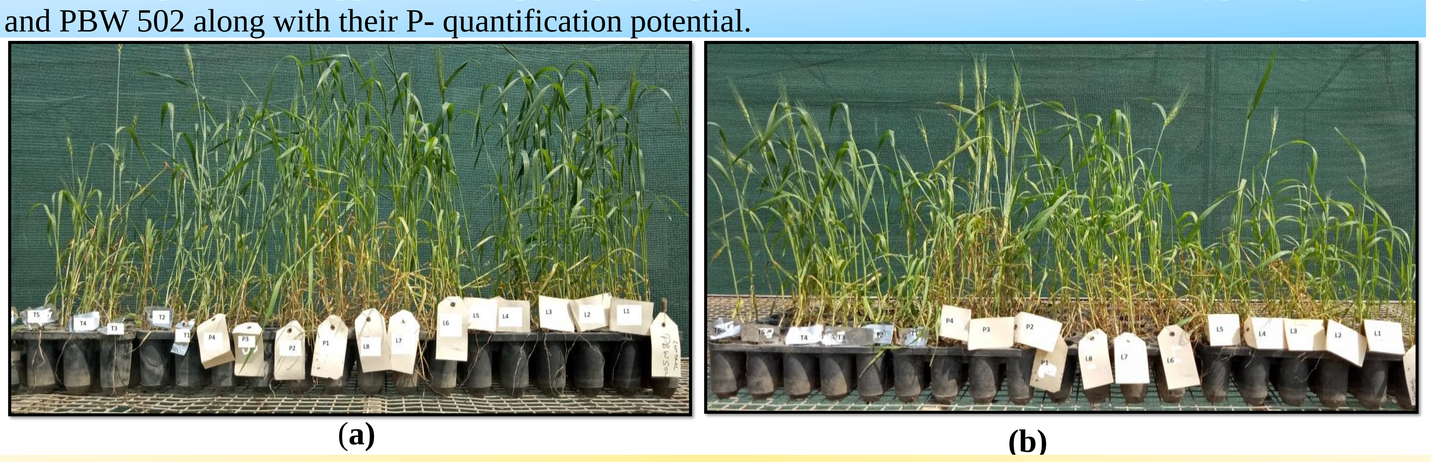

Fig3. Response of 18 PSB inoculations on two wheat genotypes e.g. **a)** CBW 38 and **b)** PBW 502.

Selection of strains
Development of consortia via compatibility test

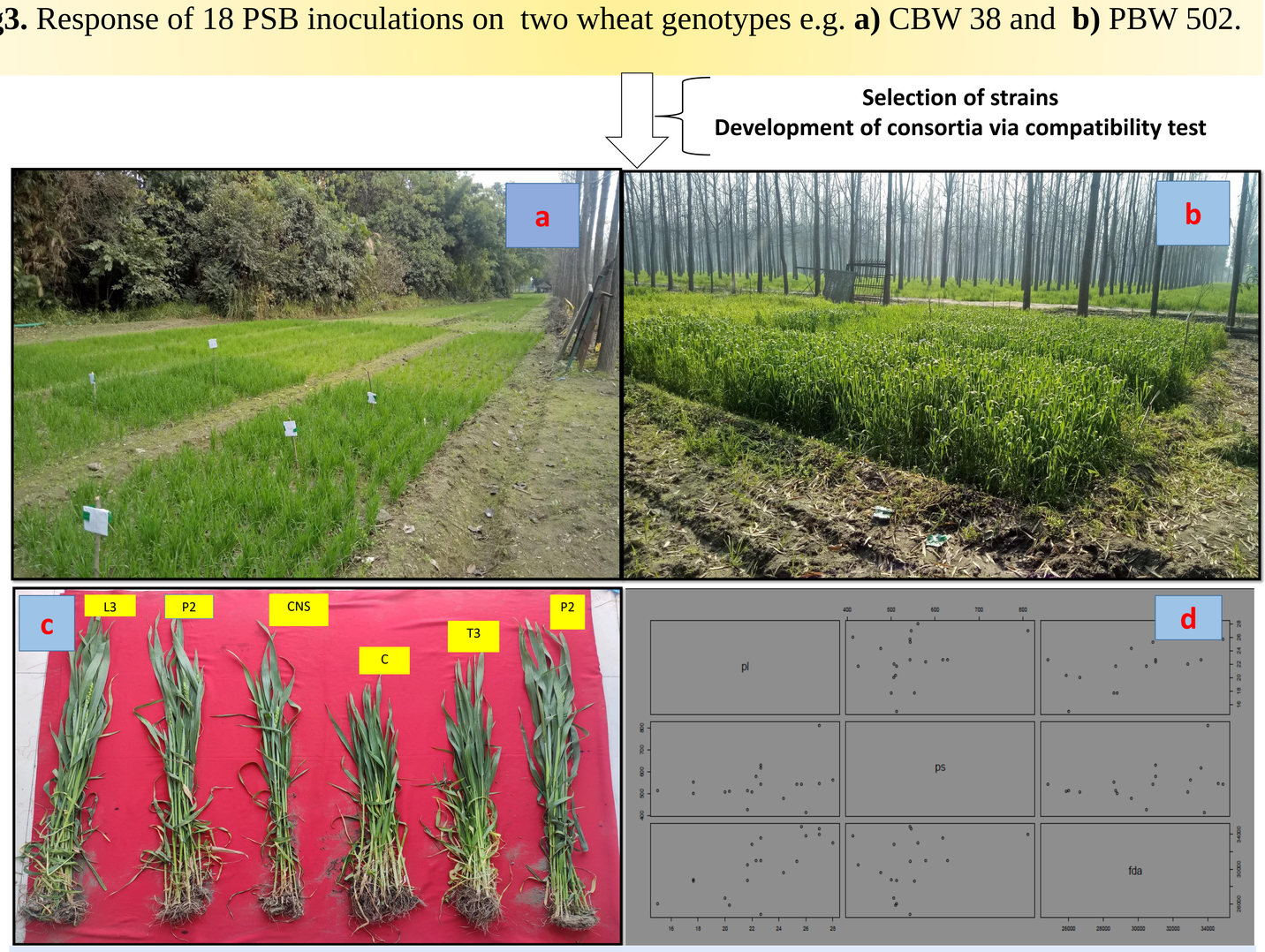

Fig 4. a) Field lay out at 30 days, **b)** Field lay out at 60 days, c) Significant difference in plant height treated with PSB with maximum response in CNS, **d)** Stastistical plot shows positive correlation (r=+0.8) plot between plant agronomic trait versus FDA.

CONCLUSION: Inoculation of 18 potential PSB promotes soil health via enhancing soil enzyme activity which further promotes plant vigor in two wheat genotypes.

REFRENCE: Sagervanshi A, Kumara P, Nagee A, Kumar A (2012) Isolation and characterization of phosphate solubilizing bacteria from anand agriculture soil. Int J life Sci Pharma Res 2:256–266.

Acknowledgement: Authors are thankful to A.F.R.C (Agroforestry Research Center) Pantnagar for providing land support





CHARACTERIZATION OF RHIZOSPHERE BORNE POTASH RELEASING BACTERIA AND THEIR REFLECTIONS IN QUALITY AND YIELD OF BANANA Cv. RASTHALI

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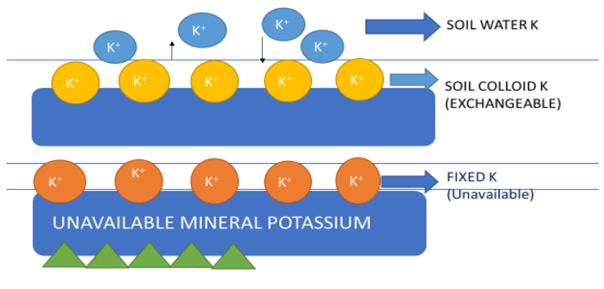
INTRODUCTION-WHY POTASH RELEASING MICROBES?



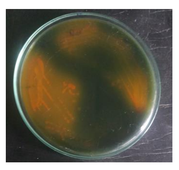
- **Direct uptake of K by plants is by solution K which is about 2% in Indian soils and the rest 98% are found in mineral form such as vermiculite, muscovite, feldspar, biotite and mica (Styriakova, 2003)- Unavailable to plants**
- **The potassium releasing microorganisms - use mechanisms such as an acidolysis, siderophore production, exchangeable reactions, chelation (complex formation) Si^{4+}, Al^{3+}, Fe^{2+} associated with K minerals, production of organic and inorganic acids, polysaccharide (extracellular and capsular) production, complex lysis or ligand formation and biofilm formation for the conversation of insoluble potassium to soluble potassium**

AIM OF THIS WORK

- **To characterize and identify potash releasing bacteria from banana rhizosphere**
- **To study its reflections on quality and yield of banana with levels of chemical fertilizers**

METHODOLOGIES FOLLOWED

- **Isolation, selection and identification of potash releasing bacterial isolates from banana rhizosphere**
- **Banana rhizosphere soil samples- Thoothukudi district of Tamil Nadu, India- Primary selection-50 isolates obtained based on clearing zone around the colonies**
- **Secondary selection based on (A) Potassium solubilization efficiency (KE)**



KE=Clearing zone diameter including colony diameter (cm)/Colony diameter (cm)

(B)Amount of K released- K released- Estimated on-3, 7, 12, 16 and 20 days of inoculation-Flame Photometer

(C) Polysaccharide production

- **Bio-chemical and molecular characterization of the isolates**- Gram staining, Carbohydrate utilization, MRVP, Casein hydrolysis, Citrate utilization, Urease test
- **Plant growth promotional traits of the KRB isolates-** IAA, Siderophore, Cellulase production; P,K,Zn,Si solubilization
- **16S r RNA sequencing-** 27 F 5'AGAGTTTGATCCTGGCTCAG 3' and reverse 1492 R 5' GGTTACCTTGTTACGACTT 3' primers – resulted 16S r DNA gene sequence was compared with the NCBI data using the BLAST search
- **Field experimental studies-** RBD, Banana Cv. Rasthali, Lateritic soil under garden land condition
- **Yield attributing parameters** *viz*., number of hands per bunch, number of fingers per hand, weight of fruits and yield; **Quality parameters** *viz*., pulp weight, length and girth of fruits, peel weight, pulp to peel ratio and TSS in pulp were recorded using standard protocols

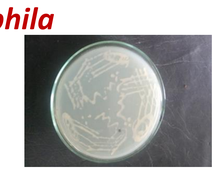
Sl.No	Bacterial isolates	Clearing zone diameter including colony diameter (cm)	Colony diameter (cm)	KE
1	KRB KKM1	1.7	0.5	3.4
2	KRB KKM2	1.8	0.4	4.5
3	KRB KKM3	1.0	0.3	3.3
4	Control-KRB TNAU	1.1	0.3	3.6

Sl.No	KRB isolates	Amount of inorganic potassium released (mgL^{-1}) Days of Inoculation				
		3	7	12	16	20
1	KRB KKM1	26.33	49.43	86.31	100.42	102.89
2	KRB KKM2	32.65	60.33	79.98	129.35	98.21
3	KRB KKM3	27.12	50.63	79.66	96.44	76.22
4.	TNAU-KRB	27.56	50.81	83.21	98.43	83.56

RESULTS

- The potash releasing bacterial isolates, KRB KKM1 and KRB KKM 2 were selected for further study based on KE, amount of K released, polysaccharide production- KRB KKM1 released 100.42 mgL^{-1} inorganic K on 16 days after incubation and that of KRB KKM 2 it was 129.35 mgL^{-1}
- Plant growth promotional traits of the KRB isolates
- Based on the nucleotide homology and phylogenetic analysis-KRB KKM1 ***Rhizobium pusense*** (99.86% identity) ; KRBKKM2 ***Stenotrophomonas maltophila*** (93.64% identity)

R.PUSENSE



S.MALTOPHILA

Sl.No	Characteristics studied	KRB KKM1	KRB KKM2
1	IAA production	+	+
2	Siderophore production	+	+
3	Cellulase production	-	-
4	Potash solubilization	+	+
5	Silicate solubilization	+	+
6	Phosphorus solubilization	-	-
7	Zinc solubilization	-	-

RESULTS

Sl.No	Isolates	Colony character	Gram reaction &Cell shape	G	F	S	L	M	C	Bio-chemical characteristics	
1	KRB KKM1	Medium, Flat, Creamish, margin irregular, light orange pigmentation	G -ve Rod	+	+	+	-	+	-	MR	Positive
										VP	Negative
										Urease test	Positive
										Casein Hydrolysis	Negative
										Citrate utilization	Positive
										Catalase	Positive
2	KRB KKM2	Medium size, Raised, White, spreading, Margin irregular, dark orange pigmentation	G –ve Rod	+	+	+	+	+	-	MR	Positive
										VP	Negative
										Urease test	Positive
										Casein Hydrolysis	Negative
										Citrate utilization	Positive
										Catalase	Positive

Sl.No	Treatments	Days for shooting (nos.)	Days to harvest after shooting (nos)	Number of hands per bunch	No. of fingers per hand	Fruit weight (g)	Weight of bunch (kg)	Yield t ha^{-1}
1	T1	280.78^{i}	111.44^{cegh}	8.56^{g}	9.22^{i}	81.24^{h}	8.17^{i}	18.46
2	T2	279.22^{i}	103.78^{a}	9.11^{efg}	9.44^{i}	83.23^{gh}	8.38^{hi}	18.94
3	T3	279.67^{hi}	105.11^{a}	9.11^{efg}	9.44^{i}	82.96^{gh}	8.60^{h}	19.45
4	T4	275.78^{defgh}	111.78^{cegh}	9.00^{fg}	9.67^{hi}	82.65^{gh}	8.75^{h}	19.78
5	T5	277.11^{ghi}	110.00^{cegh}	9.33^{dfe}	9.78^{hi}	82.38^{gh}	8.66^{h}	19.57
6	T6	278.11^{ghi}	110.78^{cegh}	9.11^{efg}	10.34^{h}	82.63^{gh}	8.80^{gh}	19.89
7	T7	277.67^{ghi}	103.56^{a}	9.56^{cdef}	10.33^{h}	83.97^{fg}	9.78^{fg}	22.10
8	T8	276.78^{cdefg}	114.78^{hi}	9.00^{fg}	11.56^{g}	83.70^{fg}	10.92^{ef}	24.68
9	T9	273.78^{abc}	108.33^{aceg}	9.45^{def}	12.00^{fg}	82.73^{gh}	10.93^{de}	24.70
10	T10	274.33^{bcde}	113.00^{gh}	9.56^{cdef}	12.44^{def}	82.53^{gh}	11.31^{d}	25.56
11	T11	273.56^{ab}	119.67^{i}	9.56^{cdef}	12.33^{ef}	84.07^{fg}	11.36^{d}	25.67
12	T12	272.44^{ab}	115.11^{hi}	10.1^{bc}	12.45^{def}	85.83^{def}	11.42^{c}	25.81
13	T13	273.11^{abc}	106.89^{ace}	9.78^{cd}	13.00^{cde}	88.76^{ab}	11.54^{bc}	26.08
14	T14	275.22^{bcde}	111.56^{cehg}	9.56^{cdef}	13.00^{cde}	85.84^{def}	11.86^{bc}	26.80
15	T15	273.89^{bcdef}	114.44^{hi}	9.56^{cdef}	13.11^{bcd}	86.96^{bcde}	12.13^{bc}	27.41
16	T16	275.89^{fghi}	111.22^{cegh}	9.44^{def}	13.67^{abc}	85.63^{ef}	12.28^{bc}	27.75
17	T17	278.44^{efh}	113.67^{gh}	9.47^{def}	13.67^{abc}	86.40^{cde}	12.72^{bc}	28.75
18	T18	273.44^{bcde}	111.11^{cefh}	9.89^{bcd}	13.78^{ab}	86.36^{cde}	12.79^{b}	28.91
19	T19	275.33^{bcdef}	111.67^{cdfh}	9.44^{def}	14.11^{a}	86.40^{cde}	13.1^{3bc}	29.67
20	T20	276.00^{bcde}	109.44^{acdfh}	9.67^{cde}	14.11^{a}	86.27^{cde}	13.14^{bc}	29.69
21	T21	274.00^{abcd}	112.11^{dfh}	9.89^{bcd}	14.33^{a}	88.00^{abdc}	13.12^{a}	29.65
22	T22	271.67^{a}	106.11^{abc}	10.33^{ab}	14.22^{a}	89.46^{a}	13.48^{a}	30.46
23	T23	272.56^{ab}	110.89^{bcdfh}	10.78^{a}	14.11^{a}	88.4c3^{ab}	13.42^{a}	30.32
SED		1.19	2.93	0.29	0.37	1.08	0.38	0.32
CD (0.05)		2.40	5.72	0.58	0.75	2.18	0.75	0.64

- Mean number of hands per bunch (10.33 vs. 9.78), number of fingers per hand (14.22vs13.00), fruit weight (89.46g vs. 88.76) and yield of 30.46 t ha^{-1} (26.08 t ha^{-1}) were higher in plants that received 75% of recommended NPK (110:35:330g /plant)+5 g *Azospirillum*+5g phosphorus solubilizer + 2 ml potash releasing bacteria (1ml each of KRB KKM 1&2 holding 10^{10} cells ml^{-1}) per pit at the time of planting and on 5th month of planting.
- A higher potassium content of 479.45 mg $100g^{-1}$ of fruit pulp compared to control (408.99 mg $100g^{-1}$) revealed the influence on potash releasers on quality of banana.

CONCLUSION

It is concluded that the potash releasing bacterial combination, *Rhizobium pusense* KRBKKM1 and *Stenotrophomonas maltophila* KRBKKM 2 along with *Azospirillum*, phosphobacteria and 75% RDF could be recommended as biofertilizer for banana.

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Meena, V. S., Maurya, B. R. and Bahadur, I., Potassium solubilization by bacterial strain in waste mica. *Bangladesh J. Bot*., 2014, **43**(2), 235-237.

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Investigating the applicability of selected plant growth promoting microbes in pesticide bioremediation by *in-silico* characterization, modelling, and docking (1.26)

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Introduction

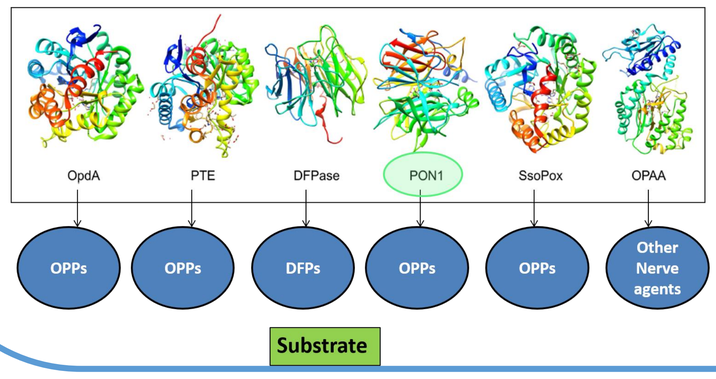
➢This study aims at identifying and comparing the pesticide-degrading potential of some selected plant growth promoting microorganisms using *in silico* approach.

➢ Molecular docking of pesticide hydrolyzing enzymes with harmful pesticides can identify the potential microbes that can degrade a specific pesticide and give a lead for their onsite bioremediation.

Table 1. Enzymes involved in pesticide degradation

Enzymes		Enzyme (kDa)	Gene	Organism	Reference
1.Alkaline phosphatase		**86 kDa**	Alpl/phoA	Bacteria, Fungi	
2. Carboxyl esterase		**56.5 kDa**	*CE/carE1*	Bacteria, Fungi	
3. OP hydrolase	**OPH**	**72 kDa**	*opd*	Bacteria	***NCBI UNIPROT PDB KEGG BRENDA***
	PTE	**19 kDa**	*hocA*	Bacteria	
	DFPase	**35.21 kDa**	dfpase	Sea squid	
	PON1/ TaPON1	**43kDa**	*Pon1/ tapon1*	*Rats Trichoderma*	
	SsoPox	**144kDa**	*php(S)*	*Archaea*	
	OPAA	**178.28 kDa**	*opaa*	*Alteromonas Proteobacteria*	
4.Monooxygenase		**41kDa**	*Cyp450*	Bacteria, Fungi	

Fig.1. 3 D structure of OP hydrolyzing enzymes



Methodology

Fig.2. Bioinformatic pipeline

Organophosphorus hydrolase – *A-OPH, P-OPH,* TaPON1, *opd, opd A, opd B, ophC2, opd D, mpd, aryl esterase; A-esterase, paraoxonases, aromatic esterase, Somanase, hocA, SsoPOX*

↓

Insilico characterization of OPD genes and enzymes, multiple alignment, phylogeny

↓

Protein homology modeling and model evaluation and validation

↓

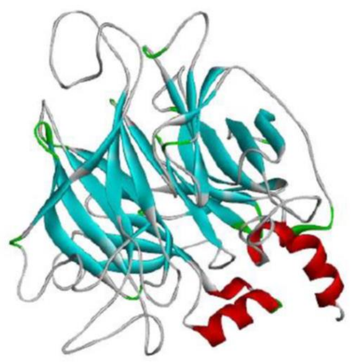
Molecular docking of modeled protein with hazardous pesticides

↓

Correlating *In-silico* and *In-vitro* results of pesticide degrading potential of the selected microbe

Experimental results

Fig3. ThPON1 protein modelling

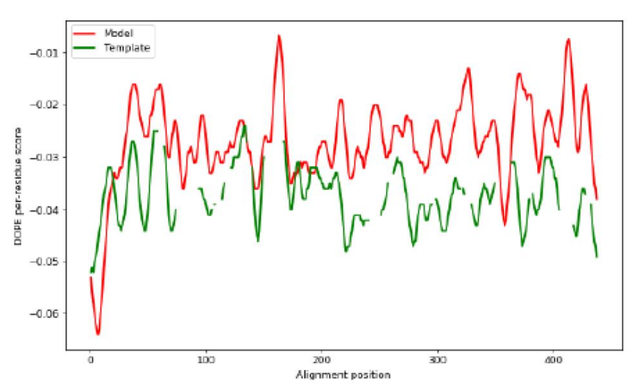


>PNP56599.1:1-437 hypothetical protein THARTR1_03295 [*Trichoderma harzianum*]

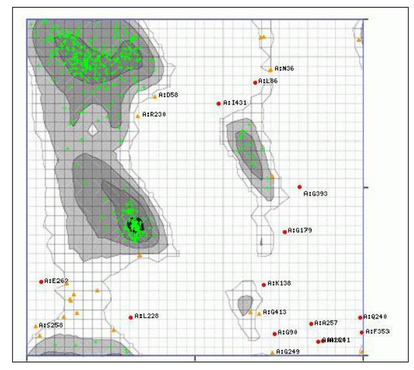
MAGRLSIAVVLLAVLLGYVYNFYVQRTVYVSGLFRNPEKTEITPEDYKVIENAINCEDLHHHEPSGLIFAACE
DSAGSRLAWFPPLEHFGNPSTERMKGKLQVIDPKTFKTEVLALEGFSGPFVTHGIDVIDDPDKPKGEAVYI
FAVNHKPNPDHYRENGDVNAPKSHSVVELFHHAIGSKTARHVRTIWHPLFVTP**NDLFAESPTSFFVTND**
HYYTEGFMRAVEDLLPRATWTNVLHVQLQEPESVDGGDSAGVHASIALENLHNLNGLCHGRAKDDIFA
NGCASGLLHVGKIRGDANKIIKVTETVELGSPIDNPSYFRDPYANSSFDASGIVSCGPTRGIDFFSNKGKEFV
LEPIMVWKASPKAGKREEGGAAINDGGNWDVNVIFQDDGHRIRAASISVLVAIDPKEEGGRRRAWLFV
SSYHASNAIAVKIDL

Aryl esterase domain NDLFAESPTSFFVTNDHYYTEGFMRAVEDLLPRATWTNVLH

Fig4. Protein model evaluation and validation

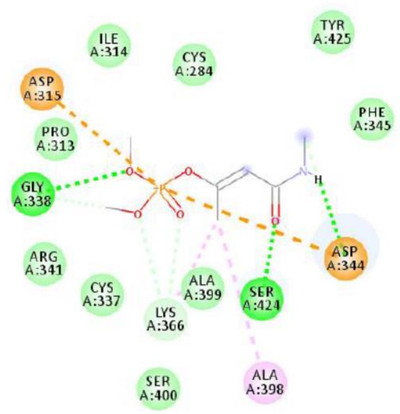


DOPE profile

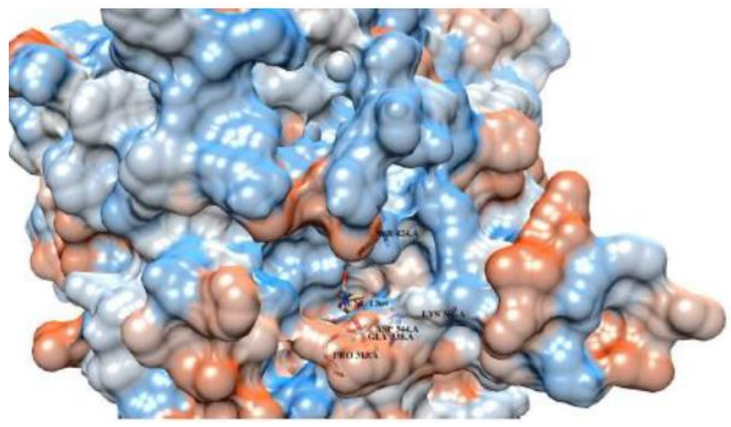


Ramachandran plot

Fig 5. Molecular docking of ThPON1 with monocrotophos pesticide



5a. 2D interaction of docked complex



5b. Docked complex in surface view

➢*In silico* analysis showed the binding of monocrotophos at the active site of the modeled protein which is a prerequisite for the decontamination of pesticides

➢In *In-vitro* studies also the *Trichoderma harzianum* showed very high tolerance to monocrotophos (LD_{50} value >1900 ppm).

Conclusion

Modeled proteins have been docked with hazardous organophosphate pesticides monocrotophos. Monocrotophos docked to the hypothetical protein with a reasonable score of -2.4 at its active site at serine 424. *Trichoderma harzianum* also showed very high tolerance to Monocrotophos in wet lab experiments.

Acknowledgment

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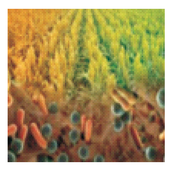
1.Singh et al., 2006, .2. Dutta *et at.*, 2013, 3. Modeller 10.1 4. Autodock, NCBI, UNIPROT, PDB, PUBCHEM




Role of rhizospheric pseudomonad BSP9 and its biosurfactant as a green approach to increase yield of *Brassica juncea*

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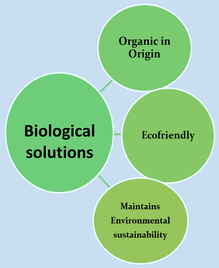
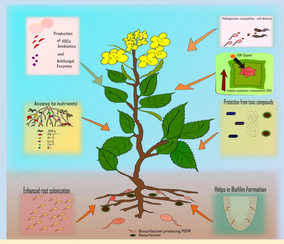
Introduction

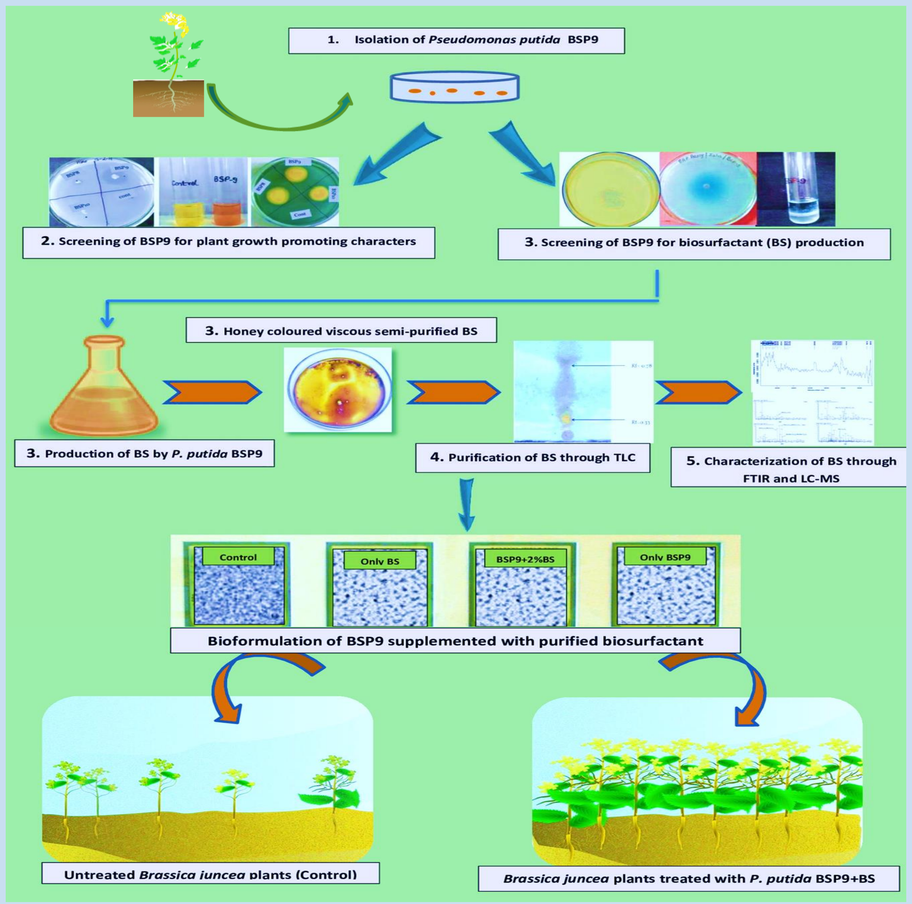
- Increasing demands of crop production with burgeoning population has become a global concern.
- In the race of enhancing crop productivity, humans are adversely impacting the environment by applying high amounts of agro-chemicals.
- These agrochemicals being recalcitrant or xenobiotic in nature severely impact the health of soil and are also harmful to beneficial micro-biomes leading to its deterioration
- To obviate this problem, sustainable agronomic methods like use of plant growth promoting rhizobacteria (PGPR) and their is the best possible substitute



Objectives: In the present study, novel bioformulations were developed using a rhizospheric pseudomonad BSP9 and its biosurfactant to check their impact on various growth promoting attributes of *Brassica juncea.*



Effect of biosurfactant producing PGPR on plant growth promotion



Methodology

Isolation of bacteria from rhizosphere of *B. juncea* and its molecular identification using 16s rRNA sequencing was done

Biosurfactant production tests of isolates:oil displacement test (Morikawa et al. 2000), blue agar plate (BAP) assay (Siegmund and Wagner, 1991) and emulsification activity (E24) (Cooper and Goldenberg, 1987)

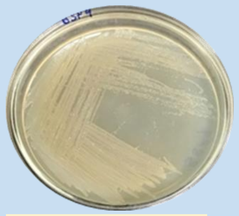
Plant growth promoting (PGP) potential of the isolate : phosphate (P) solubilization (Pikovskaya, 1948), and production of IAA (Ahmad et al. 2008) and siderophore (Schwyn and Neilands 1987; Arora and Verma, 2017).

Biosurfactant production at labscale, its extraction and purification (by TLC method) and its structural characterization (FTIR and LC-MS) was carried out.

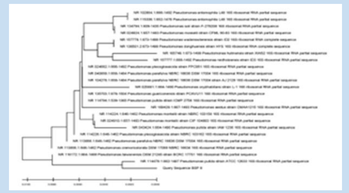
Preparation of talc based bioformulations according to Nandakumar et al. 2001) using BSP9 and its biosurfactant in various concentrations.

Field trial was conducted using *B. juncea* and plant growth parameters were recorded

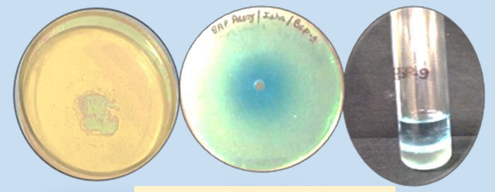
Results



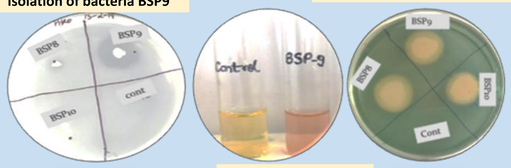
Isolation of bacteria BSP9



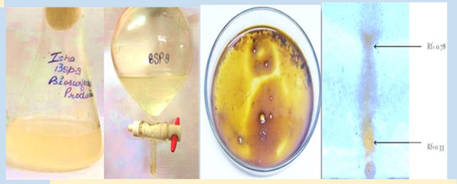
Molecular identification of bacteria BSP9



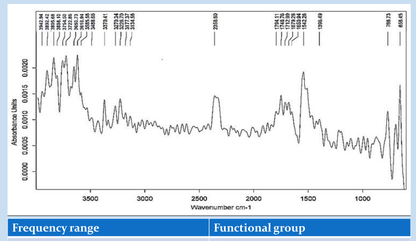
Screening of biosurfactant



PGP characterizaton

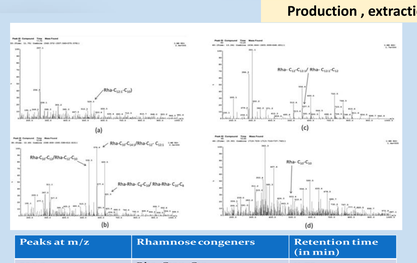


Production , extraction and purification of biosurfactant



Frequency range	Functional group
3650-3134 cm^{-1}	-OH group
1748 cm^{-1} and 1712 cm^{-1}	-C=O stretching
1396 cm^{-1}	C-H and OH deformation
2358 cm^{-1}	phosphines ($P-H_2$)
1000 cm^{-1}	C-H, C-O and CH_3 vibrations

FTIR ANALYSIS



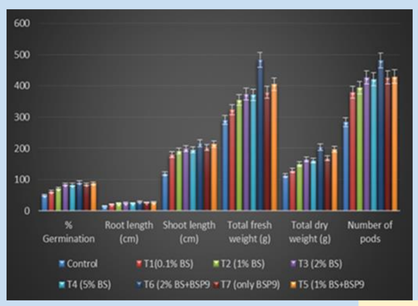
Peaks at m/z	Rhamnose congeners	Retention time (in min)
529.3	Rha-$C_{12:1}$-C_{10}	11.75
532.3 and 576.4	Rha-C_{10}-C_{12}/Rha-C_{12}-C_{10} and Rha-C_{10}-$C_{14:1}$/Rha-C_{12}-$C_{12:1}$, respectively	12.49
557.5	Rha- C_{10}-$C_{12:1}$/Rha- $C_{12:1}$-C_{12}	13.24
503.4	Rha-C_{10}-C_{10}	15.00
621.5	Rha-Rha-C_8-C_{10} or Rha-Rha-	12.49

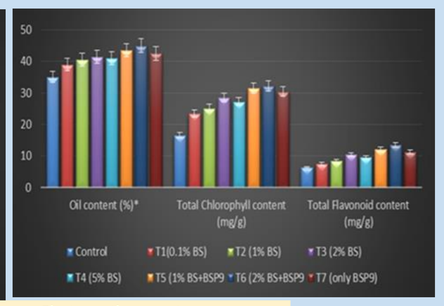
LC-MS ANALYSIS

Characterization of purified biosurfactant

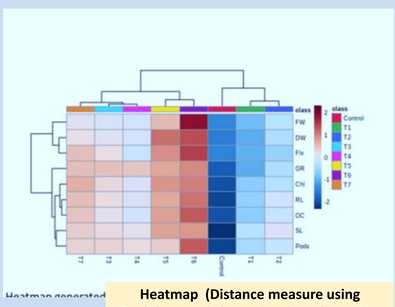
Preparation of bioformulations using BSP9 and biosurfactant

Field trial using *B. juncea* as test crop





Plant growth parameters



Heatmap (Distance measure using Euclidean and clustering algorithm using Ward's method)

Conclusion

- From the study, it can be concluded that use of BSP9 and its rhamnolipid biosurfactant is a novel technique for enhancing productivity of *B. juncea*.
- Owing to its multiple PGP properties, biosurfactant producing ability and non-pathogenic nature, it can serve as an eco-friendly and sustainable approach to increase crop productivit minimize our dependence on agro-chemicals.

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(3-4, September 2021)