Pseudomonas aeruginosa Predominates as Multifaceted Rhizospheric Bacteria with Combined Abilities of P-solubilization and Biocontrol

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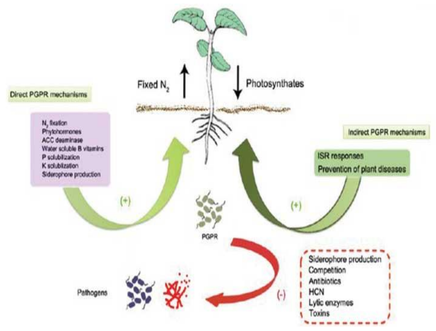
ABSTRACT

Use of multifunctional plant growth promoting rhizobacteria (PGPR) for managing plant growth and health could not only facilitate higher positive effects on plants but also enable their predominant rhizospheric prevalence. While multi-functional PGPR are common, those harbouring both direct and indirect traits of growth promotion are relatively fewer. The present work aimed at isolating and characterizing the otherwise unusual multipotential PGPR with P-solubilizing ability in combination with broad-spectrum biocontrol abilities from diverse soils and analysing their relative prevalence. Primary screening yielded 50 isolates with varying P-solubilizing potential; of which only 8 showed *In vitro* antibiosis of *E. coli*. Selected 14 isolates with varying degree of P-solubilizing and antibacterial potential were evaluated for siderophore, HCN and indole-3-acetic acid (IAA) production. While all selected isolates produced HCN, 13 of them produced IAA and 10 showed siderophore production, at varying levels. Biochemical characterization of these isolates indicated that siderophore production was maximum with fluorescent *Pseudomonas* isolates while isolates of *Enterobacteriaceae* family were best IAA producers. However, molecular characterization of isolates capable of efficient P-solubilisation along with strong ability to exhibit all the three biocontrol traits, identified them as *Pseudomonas* spp., typically *P. aeruginosa*. Overall, these results indicate that categorically *P. aeruginosa* species are likely to predominate as rhizobacteria with co-existence of discrete abilities to solubilize P as well as produce IAA, siderophore and HCN. The study also implies relatively higher metabolic versatility of *P. aeruginosa* species as compared to other members of fluorescent *Pseudomonas* family; thus, accounting for their rhizospheric abundance.

INTRODUCTION



Biocontrol	**Chitinase**	**Antifungal activity**
	Antimicrobial compounds	
	Hydrogen cyanide	Antibiosis
	Phloroglucinols	Antifungal activity, ISR
	Pyoluteorin and Pyrrolnitrin	Antibiosis
	Phenazines	Antibiosis, ISR
PGPR	Lipopeptides	Antibiosis
	Indole-3-acetic acid, cytokinin	Growth promotion, Alleviating salinity stress
	ACC deaminase	Alleviating salinity stress
	Organic acids	Phosphate solubilization
	Siderophores	Iron acquisition, nutrient competition, ISR

- Incorporation of beneficial rhizobacteria (PGPR) into agricultural systems has been an approach well explored to improve plant health and productivity in an eco-friendly manner. They promote growth by either or both direct and indirect mechanisms.
- An efficient PGPR should additionally possess high rhizospheric competence, excellent root colonizing ability and tolerance against prevailing edaphic factors.
- Multipotential PGPR harbouring and expressing both direct and indirect mechanisms simultaneously for plant growth promotion could act as an potential bioinoculant for sustainable agriculture.
- Considering P as one of the most essential yet unavailable nutrient, rhizobacteria with P-solubilizing ability along with efficient biocontrol abilities could make promising bio-inoculants with greater rhizospheric competence.
- The present study aims at screening and characterization of multipotential PGPR possessing strong P-solubilizing ability co-existing with selected biocontrol abilities (HCN, IAA and siderophores); with subsequent analysis of relative prevalence of such dual ability within the known rhizobacteria.

METHODOLOGY

Rhizospheric soil sampling and screening for P-solubilizing rhizobacteria

- About 5 g of rhizospheric soil was collected from different agricultural fields (sorghum, tobacco, paddy and maize) in the vicinity of Changa village, Dist. Anand, Gujarat (India).
- P-solubilizing rhizobacteria were screened and isolated on Pikovskaya agar medium according to the method described by Sharma et al., (2011) and were subsequently sub-cultured and maintained on nutrient agar medium.
- P-solubilizing ability of test isolates was further quantified and its PSI was calculated by the method given by Nosrati et al., (2014)

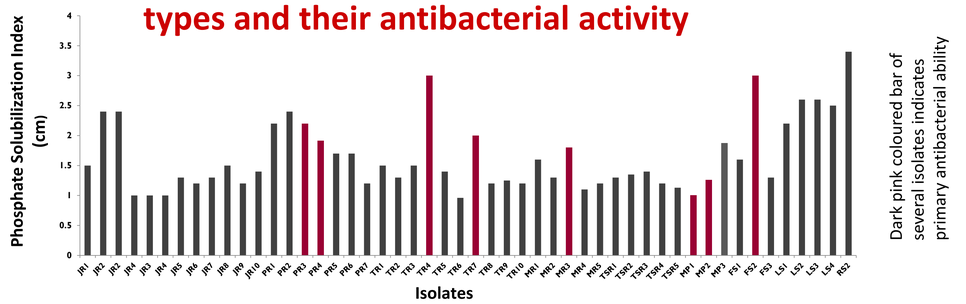
Invitro evaluation of selected plant growth promoting attributes of the bacterial isolates

Selected P-solubilizers

- Selected isolates were tested for ability to antagonise *E. coli* using soft agar overlay technique with slight modification (Hockett and Baltrus 2017). Antibacterial ability of all the test isolates was recorded qualitatively as positive or negative.
- Phytohormone IAA was quantitated and detected by method given by Ahmad et al. (2008). IAA was quantified using a standard curve prepared by similar spectrophotometric measurement of pure IAA in range of 10 to 100µg/ml.
- Siderophore production by test isolates was measured using CAS solution assay Christina Jenifer et al. 2015). For quantitation, % siderophore units were calculated by following formula [{Ar-As}/Ar]*100; where Ar = absorbance of reference and As = absorbance of sample.
- HCN production by test isolates was measured qualitatively as per method given by Reetha et al. (2014).
- **Biochemical characterization**: Test isolates were characterized on preliminary basis using selected microscopic and biochemical tests according to Bergey's Manual of Determinative Bacteriology.
- **Molecular characterization**: Identification of selected bacterial isolates was carried out on the basis of 16S rRNA gene sequencing. using universal primers 8F (5'-AGAGTTTGATCCTGGC-TCAG-3') and 1492R (5'-ACGGCTACCTTGTTAC-GACTT-3. PCR products of appropriate sizes were then partially sequenced in single pass reaction using 8F primer (services out sourced from 1st Base, Singapore). The sequences obtained were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov) to identify the isolates on the basis of sequence similarities. Additionally, these partial nucleotide sequences were deposited at GenBank and the accession numbers were obtained.
- **Statistical analysis** Data for quantitative experiments is represented as Mean ± S.D as indicated in the respective figure legends. Correlation analysis between P-solubilization and biocontrol traits was performed using Microsoft Excel, for which absolute values of PSI values were retained while siderophore, IAA and HCN producing abilities were rated on a relative scale of 0-3, based on quantitative and qualitative determinations as described earlier.

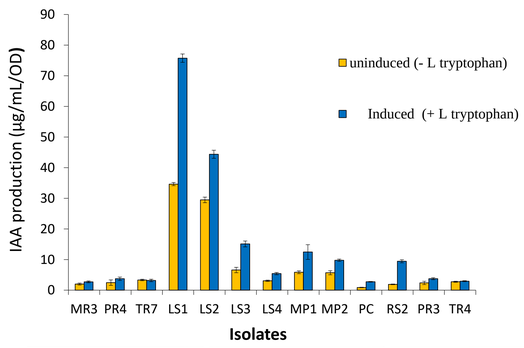
RESULTS

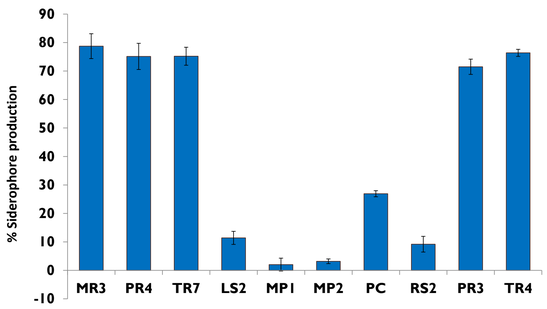
Isolation and screening of potential P-solubilizing bacteria from diverse soil types and their antibacterial activity

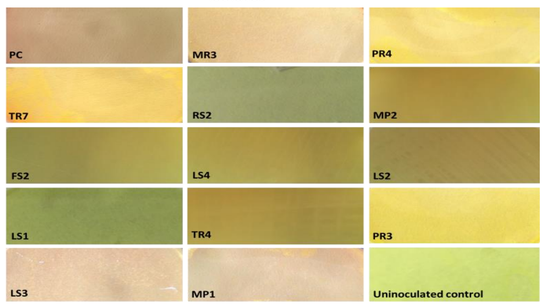


- 50 different P-solubilizers were isolated from diverse fields and there PSI ranged between 0.96-3.4; of which 14 were selected based in combination of P-solubilizing and antibacterial abilities.
- Selected 14/50 isolates (PR3, TR4, TR7, FS2, PR4, MR3, MP1, MP2, LS1, LS2, LS3, LS4, RS2 and PC) were characterized biochemically and subsequently for abilities to produce multiple biocontrol metabolites (IAA, siderophores, and HCN).

Characterization of biocontrol traits in selected P-solubilizing isolates







Isolate	Siderophore	IAA	HCN	PSI
MR3	3	1	2	1.8
PR4	3	1	3	1.91
PR3	3	1	2	2.2
TR4	3	1	3	3
TR7	3	1	3	2
LS1	0	3	2	2.2
LS2	2	3	2	2.6
LS3	0	2	1	2.6
LS4	0	1	2	2.5
MP1	1	2	1	1
MP2	1	2	3	1.26
RS2	1	1	1	3.4
PC	2	1	2	0
FS2	0	0	2	3

Correlation analysis

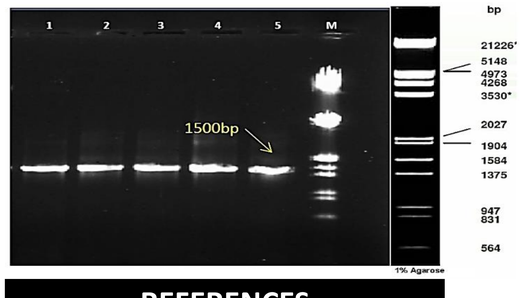
	Siderophore	IAA	HCN	PSI
Siderophore	1.00			
IAA	-0.24	1.00		
HCN	**0.53**	-0.18	1.00	
PSI	-0.18	-0.12	-0.11	1.00

Results are expressed as % siderophore production and values are represented Mean ± SD of 3 independent observations

Microscopic and biochemical characterization of selected isolates

Isolates	Gram Staining	Catalase test	Indole test	Nitrate reductase	Voges Proskauer test	Methyl Red test	Oxidase test	Pigment (fluore-scent)	Predicted family/genus
MR3	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	*Pseudomonas*
PR4	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	*Pseudomonas*
PR3	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	*Pseudomonas*
TR4	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	*Pseudomonas*
TR7	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	*Pseudomonas*
LS1	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Entero-bacteriaceae
LS2	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Identification unclear
LS3	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Identification unclear
LS4	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Entero-bacteriaceae
MP1	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Identification unclear
MP2	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Entero-bacteriaceae
RS2	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Entero-bacteriaceae
PC	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Negative	*Bacillus* related
FS2	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Entero-bacteriaceae

Molecular characterization of five selected isolates



S.No.	Isolate	Query Length (bp)	Query Coverage (%)	Homology (%)	Identified species	GenBank Accession Numbers
1	TR7	1004	98	98	*Pseudomonas aeruginosa*	MK372993
2	MR3	1086	95	95	*Pseudomonas aeruginosa*	MK372994
3	PR4	970	99	98	*Pseudomonas aeruginosa*	MK372995
4	FS2	1337	99	97	*Cronobacter sakazakii*	MK372996
5	RS2	1270	97	99	*Rosenbergiella sp.*	MK372997

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