Isolation and Screening of Plant Growth-Promoting Rhizobacteria (PGPR) Isolates Across Different Seasons in South Gujarat, India

Abstract

Plant Growth Promoting Rhizobacteria (PGPR) a major constituent of Rhizobacteria, encourage the plant growth through their diverse mechanisms. In this investigation, 33 different Isolates were isolated from the rhizosphere soils of various research stations in south Gujarat were screened for their plant growth promoting activity. All the 33 tested isolates in pre monsoon and post monsoon were evaluated and documented various PGPR activities. Thirteen isolates of pre-monsoon season and twenty isolates of post monsoon season selected for further study. Based on colonial characteristics and morphology of isolates, 33 were selected for Siderophore, HCN, IAA production phosphate solubilizing and antagonistic activities. Among 33 isolates, 10 isolates showed phosphate solubilizing properties, 10 isolates hadSiderophore production, 6 isolates had IAA activity and no one organism produced HCN. All the isolates were found to have nitrogen-fixing potential. Among these, those isolated before the monsoon season have more number of Gram positive bacteria while those isolated post monsoon were Gram positive as well as Gram negative.

Keywords:PGPR, Siderophore, Nitrogen fixation, IAA

INTRODUCTION

Plant Growth Promoting Rhizobacteria (PGPR) have emerged as an essential component of sustainable agriculture, providing a natural and environmentally benign alternative to chemical fertilizers and pesticides. These beneficial bacteria colonize the rhizosphere and help plants grow through a variety of methods, including nitrogen fixation, phosphate solubilization, and phytohormone synthesis. Recent research has demonstrated the potential of PGPR as green bioinoculants, emphasising their significance in plant health and yield (Basu et al., 2023).

In recent years, there has been an increased emphasis on PGPR due to their capacity to boost plant growth and resistance, particularly in the context of climate change and environmental sustainability. Hasan et al. (2021) conducted a thorough analysis of the significance of PGPR in sustainable agriculture, highlighting their ability to improve soil fertility and plant health through a variety of biochemical interactions. Furthermore, Bhattacharyya and Jha's (2012) study has been essential in proving the appearance and relevance of PGPR in agriculture, paving the door

for further research and application of these beneficial bacteria. Microorganisms play an important role in agriculture by boosting the transfer of plant nutrients and decreasing the use of chemical fertilizers. Among microorganisms, PGPR has the capacity to promote plant growth (Suresh et al., 2010). Beneficial plant-microbe interactions in the rhizosphere can affect plant development and soil fertility. The positive benefits of PGPR can be either direct or indirect. PGPR directly promotes plant growth by producing metabolites such as auxins, cytokinins, gibberellins, and phosphate mineral solubilization (Hasan et al., 2021). Indirect growth promotion happens when pathogens are removed by the formation of secondary metabolites like hydrogen cyanide and siderophores (El-Hadad et al., 2010).

Seasonal fluctuations are critical to the success of PGPR, as varied environmental circumstances can alter the activity and efficacy of the bacteria. Gupta et al. (2024) examined the changes in root characteristics caused by PGPR that contribute to sustainable crop production. Understanding these seasonal fluctuations is critical for maximizing the usage of PGPR in agricultural techniques.

PGPR can modify auxin content and stimulate development in plants such as *Vigna radiata* (Ali et al., 2010). Microbial cyanide generation in the rhizosphere can affect plant growth and yield (Bakker & Schipper, 1987). *Serratia nematodiphia* has been proven to stimulate plant growth in crops such as black pepper (Dastager et al., 2011). The isolation and characterisation of PGPR from non-rhizospheric soil resulted in significant effects on cowpea seedling growth (Deepa et al. 2010).

An alluring substitute for chemical fertilizers that can also contribute to environmental pollution is the use of PGPR as crop inoculants for biofertilization, phytostimulation, and biocontrol (Grover et al., 2021). Numerous characteristics of *Bacillus* species, which are common in agricultural soils, make them suitable candidates for PGPR. They produce HCN, siderophores, protease, antimicrobials, and phosphate solubilizing enzymes, among other things that promote plant growth (Idris et al., 2010).

In the present study, we investigated the production of HCN, siderophores, nitrogen fixation, antimicrobials and phosphate solubilization by 17 isolates of PGPR isolated from soils of different localities in Gujarat. The objectives of study were, (1) Isolation of different free living nitrogen fixing bacteria with respect to different seasons (2) Biochemical Characterization of isolates and (3) Evaluation of PGP and antimicrobial activities.

MATERIALS AND METHODS

Site description of soil sampling

Soil samples from different sites were collected in sterile bag. Sampling was carried out before monsoons and post monsoonsseasons, Soil samples were collected from different depths (0-15 cm) of soil.

Enumeration of total bacterial population and free living nitrogen fixing bacteria

All collected soil samples were brought to laboratory and processed immediately for isolating PGPR organisms using Ashby'sMannitol agar medium.

Total viable count was carried out by serial dilution of 1gm soil and resulting two dilution were spread over the Nutrient agar plate. For the counting of free living nitrogen fixing bacteria serially dilute sample was spread over Ashby'smannitolagar plate. Their results are depicted in Table 1 and 2 respectively.

PGPR activities of different isolates

HCN activity was checked by method given by Bakker and Schipper (1987) (2).Phosphate solubilizing activity was checked on Pikovaskya′s agar medium having insoluble phosphate.Siderophore production was checked on Chrome Azurol-S (CAS) agar plates.IAA production was checked by Salkowsky method. Antagonistic activity of isolates were checked against *Fusarium* and *Sclerotium*and they were tested by dual culture technique. Bacterial isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA culture medium. 9 mm mycelial disc from seven days old PDA culture of *Fusarium* and *Sclerotium*were placed at the opposite side of Petri dishes perpendicular to the bacterial streak respectively and incubated at 27±2°C for 5-7 days. Petri dishes inoculated with fungal discs alone served as control.

Nitrogen estimation was carried out by Kjeldahl method in which nitrogen free medium was inoculated with active culture isolates (uninoculated flask also kept as blank) and incubated on rotary shaker at room temperature for 48 hrs. After incubation, broth is analyzed for estimating total nitrogen.

RESULTS AND DISCUSSION

Results showed that total number of viable count was high during the monsoon season compared to summer season (Table-1). Number of free living nitrogen fixing bacteria were also higher

during post monsoon season (Table-2). Isolation of free living nitrogen fixing bacteria was carried out on Ashby'sMannitol agar medium before and post monsoon seasons.Thirteen isolates of pre-monsoon season and twenty isolates of post monsoon season were selected for further study. Based on colonial characteristics and morphology of isolates(for identification purposes), 33 selected forSiderophore, HCN, IAA production, phosphate solubilizing and antagonistic activities (Table-3).Among 33 Screened,10 isolates showed phosphate solubilizing properties, 10 showed Siderophore production, 6 Isolates had IAA activity and no one organism produce HCN (Figure 1,2 and 3). It was found that the isolates of A19, A24 and A28 Isolates have multiple characteristics to produce Siderophore, phosphate solubilization and nitrogen fixing ability. A12 isolate has nitrogen fixing, Phosphate solubilising and IAA production potential while A21 isolate has Nitrogen fixing, siderophore and IAA production potential. Zone of solubilization of isolates were measured and result showed that isolates of A12 and A16 have highest Zone of utilization/colony ratio (Table-4). IAA quantitative analysis indicates that isolates A15 and A26 produce highest amount of IAA is 38.6ppm and 39.0ppm respectively (Table-5).Out of 33 nitrogen isolates, 13 organisms isolated premonsoon season and 20 organisms isolated postmonsoon seasons. Among these, those isolates isolated before the monsoon season have more number of Gram positive bacteria while those isolated post monsoon season Gram positive as well as Gram negative.No antagonistic effect was by isolates against test organisms. Findingsof biochemical characterization of selected isolates is shown inTable-6. Result of nitrogen estimation indicates that isolates of A15, A20 and A30 fix nitrogen in higher amount as compared to other isolates. However, all the isolates have potential to fix atmospheric nitrogen (Table-7).

Table-1: Total Microbial count in different soil samples collected during various seasons

Sr. No.	Location of soil sample	cfu/gm (preMonsoon season)	cfu/gm (postMonsoon season)
1.	Regional Cotton Research Station, Maktampur, Bharuch	$1.5x\ 10^8$	$1.85x\ 10^8$
2.	NARP, Bharuch	$1.6x\ 10^8$	$2.1x\ 10^8$

3.	Agricultural Research Centre, Tanchha	$1.2x\ 10^8$	$4.9x\ 10^8$
4.	Agricultural Research Station, Achhalia	$1.2x\ 10^8$	$3.6x\ 10^8$

Table-2: Total viable count of free living nitrogen fixing in soil samples collected during different seasons

Sr. No.	Location of soil sample	cfu/gm (premonsoon)	cfu/gm (postmonsoon)
1.	Regional Cotton Research Station, Maktampur, Bharuch	$2.6x\ 10^4$	$5.8x\ 10^5$
2.	NARP, Bharuch	$1.2x\ 10^4$	$2.1x\ 10^5$
3.	Agricultural Research Centre, Tanchha	$0.8x\ 10^4$	$2.9x\ 10^5$
4.	Agricultural Research Station, Achhalia	$1.9x\ 10^3$	$1.6x\ 10^4$

Table-3: Different properties of free living nitrogen fixing bacteria

Properties	Nitrogen Fixation	Phosphate solubilisation	HCN production	Siderophore production	IAA production
Isolates Isolated monsoon season					
A1	Positive	-	-	-	-
A2	Positive	-	-	-	Positive
A3	Positive	Positive	-	-	-
A4	Positive	-	-	Positive	-
A5	Positive	-	-	-	-
A6	Positive	Positive	-	-	-
A7	Positive	Positive	-	-	
A8	Positive	-	-	Positive	-
A9	Positive	-	-	-	-
A10	Positive	-	-	Positive	-
A11	Positive	-	-	Positive	-
A12	Positive	Positive	-	-	Positive
A13	Positive	-	-	-	-
Isolates isolated monsoon season					
A14	Positive	-	-	Positive	-
A15	Positive	-	-	-	Positive
A16	Positive	Positive	-	-	-
A17	Positive	-	-	-	Positive

A18	Positive	-		-	-
A19	Positive	Positive		Positive	-
A20	Positive	-		-	-
A21	Positive	-	-	Positive	Positive
A22	Positive	Positive	-	-	-
A23	Positive	-	-	-	-
A24	Positive	Positive	-	Positive	-
A25	Positive	-	-	-	-
A26	Positive	-	-	-	Positive
A27	Positive	-	-	-	-
A28	Positive	Positive	-	Positive	-
A29	Positive	-	-	-	-
A30	Positive	-	-	-	-
A31	Positive	-	-	Positive	-
A32	Positive	-	-	-	-
A33	Positive	Positive	-	-	-

Figure: 1.Siderophore productionby different isolates

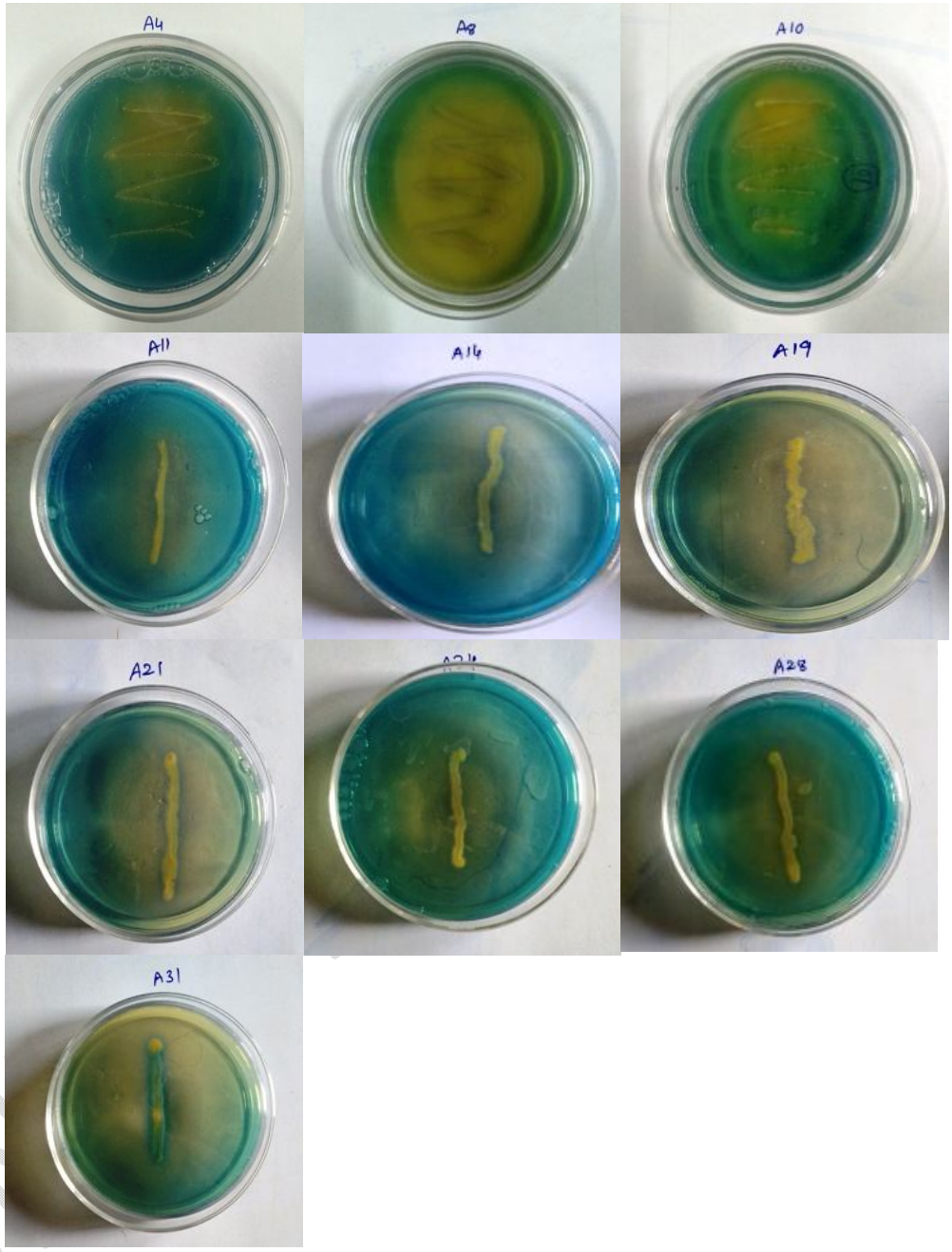

Figure: 2. Phosphate solubilization by different isolates

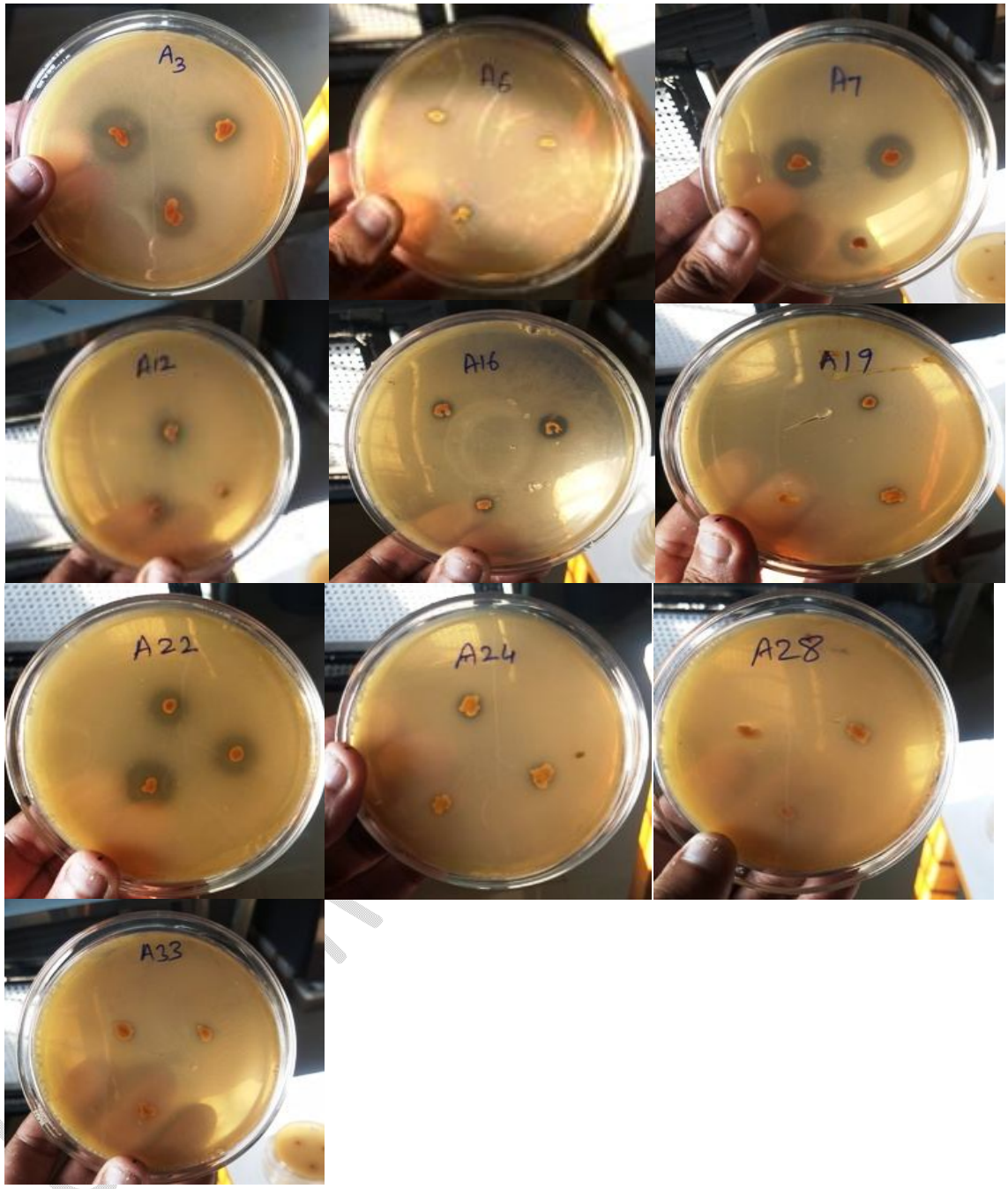

Figure: 3. IAA Production by different isolates

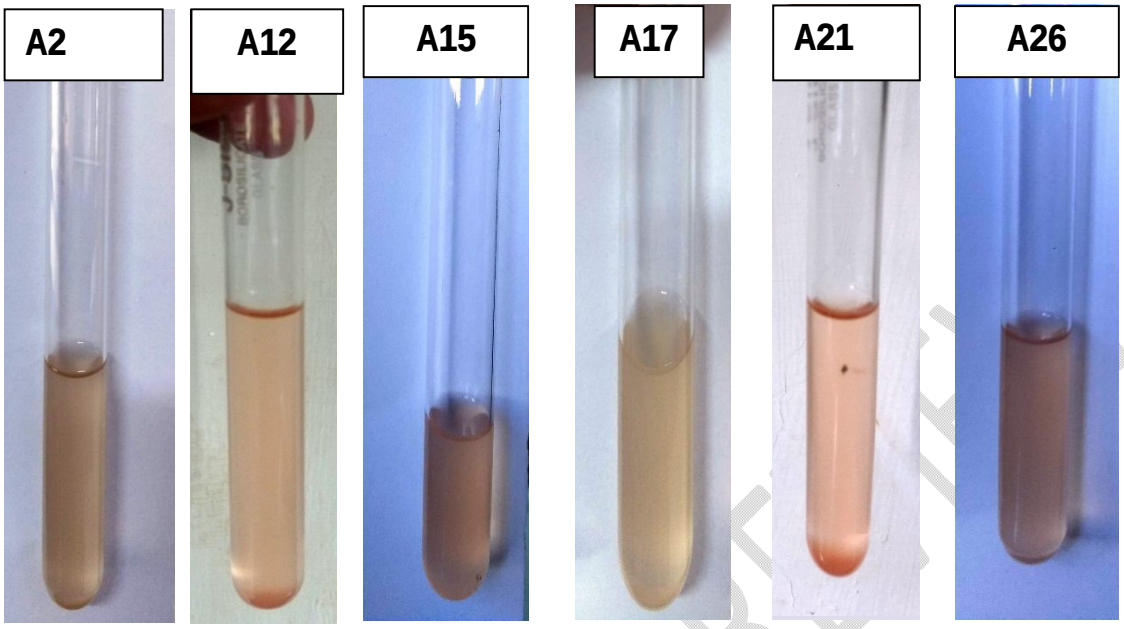

Table-4: Phosphate solubilizing activity on pikovasky medium (Zone of solubilization)

Isolates	Zone ratio	Isolates	Zone ratio
A3	4.00	A19	1.33
A6	1.25	A22	2.00
A7	2.50	A24	1.25
A12	1.20	A28	1.10
A16	1.25	A33	1.06

Table-5 : IAA production by different isolates

Isolates	IAA (ppm)
A2	23.5
A12	12.8
A15	38.6
A17	10.0
A21	18.5
A26	39.0

Table-6. Biochemical Characterization of different isolates

Biochemical testswerecarried out for selected isolates with different colonial characteristics and PGP activities.

Sr. No.	Test	A12	A19	A21	A24	A28
1	Lactose	Positive	-	-	-	-
2	Xylose	Positive	-	-	-	Positive
3	Maltose	Positive	-	-	-	Positive
4	Fructose	Positive	Positive	Positive	Positive	Positive
5	Dextrose	Positive	Positive	Positive	Positive	Positive
6	Galactose	Positive	-	-	-	-
7	Raffinose	Positive	-	-	-	Positive
8	Trehalose	Positive	Positive	Positive	-	Positive
9	Melibiose	Positive	-	-	-	Positive
10	Sucrose	Positive	Positive	Positive	Positive	Positive
11	L- arabinose	Positive	Positive	Positive	Positive	Positive
12	Mannose	Positive	-	Positive	-	-
13	Inulin	Positive	-	Positive	Positive	Positive
14	Na- Gluconate	-	-	Positive	-	Positive
15	Glycerol	Positive	Positive	Positive	Positive	Positive
16	Salicin	Positive	Positive	Positive	Positive	-
17	Dulcitol	-	-	-	Positive	-
18	Inositol	-	-	Positive	-	-
19	Sorbitol	-	-	Positive	Positive	-

20	Mannitol	Positive	-	Positive	Positive	Positive
21	Adonitol	Positive	-	-	-	-
22	Arabitol	Positive	-	-	-	-
23	Erythritol	Positive	-	-	-	--
24	α- methyl –D-glucoside	-	-	-	-	-
25	Rhamnose	-	-	-	Positive	-
26	Cellobiose	Positive	-	-	Positive	-
27	Melezitose	-	-	-	Positive	Positive
28	α- methyl –D-Manoside	-	-	-	Positive	-
29	Xylitol	-	-	-	Positive	-
30	ONPG	-	-	-	-	Positive
31	Esculin hydrolysis	Positive	Positive	Positive	Positive	Positive
32	D- arabinose	Positive	-	Positive	Positive	-
33	Citrate utilization	-	-	-	-	-
34	Malonate utilization	-	-	-	-	Positive
35	Sorbose	-	-	-	Positive	Positive
36	ONPG	-	Positive	-	-	Positive
37	Lysine Utilization	-	-	-	-	-
38	Ornithine Utilization	-	-	Positive	-	-
39	Urease	Positive	Positive	Positive	Positive	Positive
40	Phenylalanine deamination	-	-	-	-	-

41	Nitrate reduction	Positive	-	Positive	Positive	Positive
42	H_2S production	-	-	-	-	-
43	Citrate Utilization	-	-	-	-	-
44	Voges-Proskeaur	Positive	-	Positive	Positive	Positive
45	Methyl red	-	-	-	-	-
46	Indole	-	-	Positive	-	Positive

Table:7 Nitrogen estimation of isolates by kjeldahl

Isolates	% N	Isolates	% N
A1	0.00168	A18	0.00128
A2	0.00114	A19	0.00147
A3	0.00228	A20	0.00321
A4	0.00270	A21	0.00178
A5	0.00290	A22	0.00254
A6	0.00200	A23	0.00198
A7	0.00228	A24	0.00226
A8	0.00319	A25	0.00178
A9	0.00159	A26	0.00245
A10	0.00228	A27	0.00178
A11	0.00250	A28	0.00125
A12	0.00410	A29	0.00245
A13	0.00228	A30	0.00345
A14	0.00200	A31	0.00149
A15	0.00364	A32	0.00267
A16	0.00224	A33	0.00267
A17	0.00219		

CONCLUSION:

Microbial population along with free living nitrogen microbes is increased in post monsoon season. Pre monsoon season, number of Gram positive bacterial population is higher than the Gram negative. Isolates A19(*Streptomyces coelicolor)*, A24*(Enterobacter*sp.*)*, and A28(*Bacillus altitudinis)*have potential characteristics to produce Siderophore as well as nitrogen fixing and phosphate solubilizing properties. The isolate A12 has characteristic to produce IAA along with nitrogen fixation and phosphate solubilization Properties.

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REFERENCES

1. Ali, B., Sabri, A. N., and Hasnain, S. (2010). Rhizobacterial potential to alter auxin content and growth of Vigna radiata (L.). *World Journal of Microbiology and Biotechnology*, 26: 1379-1384.
2. Bakker, A. W., and Schipper, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated plant growth stimulation. *Soil Biology and Biochemistry*, 19: 451-457.
3. Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., and El Enshasy, H. (2023). Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. *Sustainability*, *13*(3), 1140.

4. Bhattacharyya, P. N., and Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 28(4): 1327-1350. doi:10.1007/s11274-011-0979-9.
5. Dastager, S. G., Deepa, C. K., and Pandey, A. (2011). Potential plant growth-promoting activity of Serratia nematodiphia NII-0928 on black pepper (Piper nigrum L.). *World Journal of Microbiology and Biotechnology*, 27: 259-265.
6. Deepa, C. K., Dastager, S. G., and Pandey, A. (2010). Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (Vigna unguiculata (L.) Walp.) seedling growth. *World Journal of Microbiology and Biotechnology*, 26: 1233-1240.
7. El-Hadad, M. E., Mustafa, M. I., Selim, S. M., Mahgoob, A. E., El-Tayeb, T. S., and Abdel Aziz, N. H. (2010). In vitro evaluation of some bacterial isolates as biofertilizers and biocontrol agents against the second stage juveniles of Meloidogyne incognita. *World Journal of Microbiology and Biotechnology*, 26: 2249-2256.
8. Grover, M., Bodhankar, S., Sharma, A., Sharma, P., Singh, J., and Nain, L. (2021). PGPR Mediated Alterations in Root Traits: Way Toward Sustainable Crop Production.*Frontiers in Sustainable Food Systems*, *4*, 618230.
9. Gupta, R., Khan, F., Alqahtani, F. M., Hashem, M., and Ahmad, F. (2024). Plant Growth-Promoting Rhizobacteria (PGPR) Assisted Bioremediation of Heavy Metal Toxicity. *Applied Biochemistry and Biotechnology*, 196(5): 2928-2956.
10. Hasan, A., Tabassum, B., Hashim, M., and Khan, N. (2021). Role of Plant Growth Promoting Rhizobacteria (PGPR) as a Plant Growth Enhancer for Sustainable Agriculture: A review. *Bacteria*, *3*(2), 59-75.
11. Idris, H. A., Labuschagne, N., and Korsten, L. (2010). Suppression of *Pythium ultimum* root rot of sorghum by rhizobacterial isolates from Ethiopia and South Africa. *Biological Control*, 45: 72-84.
12. Suresh, A., Pallavi, P., Srinivas, P., Kumar, V. P., Chandra, S. J., and Reddy, S. R. (2010). Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. *African Journal of Microbiology Research*, 4: 1491-1494.