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ISOLATION AND IDENTIFICATION OF PGPR TRAITS FROM SOIL SAMPLES OF THE SAURASHTRA REGION

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) is an eco-friendly and potent microorganism that can serve as both a nitrogen fixer and a biocontrol agent. Hence it can be used as a substitute for chemical fertilizers and pesticides. So, in the present investigation, total 9 rhizospheric soil samples (2 from Veraval, 1 sample from Khijadiya, 1 from Rajkot, and 3 samples from Morbi) from a different region of Saurashtra have been collected. Serial dilutions method was employed followed the by spread plate method for the isolation of rhizospheric bacterial strains. Total 41 bacterial strains were isolated from soil samples among which 4 bacterial strains potentially act as rhizobacteria. They were screened by various growth promotion tests such as the HCN test, Ammonia test, siderophore production, IAA production, and chitinase assay. KS2, KC8, KC9, and KC11 show the highest results for all these tests. So, these traits can be further used as potential biofertilizers to promote the growth of plants. According to the results of test these traits may belong to *Azatobacter* sp., *Bacillus* sp., and *Pseudomonas* sp.

KEYWORDS: PGPR, Rhizobacteria, Biofertilizer, Growth

Bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR) (Anelise Beneduzi *et al*; 2012). Plant growth-promoting rhizobacteria have the capability to colonize the root surface of the plant to enhance growth. The rhizosphere is the area around plants where microbes have the highest zone for microbial activity and a rich region in nutrient uptake. PGPR acts as a potent organism for sustainable development increasing crop production without the use of chemicals and pesticides and positive results for controlling plant diseases caused by harmful pathogens. Nowadays the use of PGPR adds environmentally effective yielding crop development by direct or indirect methods. The mechanism of PGPR includes the hormonal and nutritional balance which shows resistance towards plant pathogens and diseases causing pathogens. It solubilizes the nutrient uptake by plants for better growth yield. PGPR shows synergistic and antagonistic effects towards plant and pathogenic microorganisms like fungi and bacteria, respectively. PGPR is an important agent as a biocontrol as well as a biofertilizer. Variety of PGPR such as *Pseudomonas, Bacillus, Klebsiella, Azotobacter, and Enterobacter* influence the crop production. Based on interactions with plants PGPR are categorized as free-living rhizobacteria known as extracellular PGPR and symbiotic which are known as intracellular PGPR (Govind Gupta *et al*; 2015). The free-living rhizobacteria are those which live freely outside the plant cells whereas symbiotically associated rhizobacteria live inside the plants. The mechanism of PGPR works in both ways direct and indirect way. It increases the efficiency of nitrogen-fixing, phosphate solubilizing, and various hormone production for plant growth (Pravin *et al.*, 2016).

MATERIALS AND METHODS

Sample Collection

Soil sample has been collected from the agricultural field of 9 different areas of the Saurashtra region as listed below: Veraval (2 samples), Khijadiya (3 samples), Rajkot (1 sample), and Morbi (3 samples).

Isolation of Bacteria from Soil Sample

The serial dilution method (Aneja, 2005) was used for the isolation of bacteria. 1 g of soil was transferred to 10 ml of sterile distilled water in test tubes. Dilutions were made up to 10-8. Pipette out 0.1 mL suspension from 10^{-6}, 10^{-7}, and 10^{-8} dilutions and spread it on N- agar media which was priorly autoclaved at 121^{O}C and 15 lbs pressure. Incubate the plates at 37^{O}C for 24-48 hrs.

Physicochemical Analysis of Soil

The collected soil samples were evaluated for physical and chemical soil quality measures such as soil

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pH, electrical conductivity (EC), organic carbon (OC), organic nitrogen (N), phosphorus (P), potassium (K), phosphorus and micronutrients like Fe, Zn, Mn and Cu have been analysed from Gujarat State Fertilizer and Chemical Limited (Walkey and Black Method, 1979).

Identification of Bacterial Isolates

Identification of isolates was done on the basis of microscopic and morphological observations. Microscopic observation was done by performing Gram's staining. Colonies were observed for morphological identification which involves the following characteristics: size, shape, margin, elevation, colour, consistency, and opacity using Bergey's manual of systemics.

QUALITATIVE AS WELL AS QUANTITATIVE CHARACTERIZATION OF PGPR TRAITS OF THE ISOLATED RHIZOBACTERIA (PGPR)

HCN Production

Overnight incubated liquid cultures of each strain were used to determined concentration of HCN. Modified colorimetric methemoglobin method (von Rohr *et al.*, 2009), used for the quantitative analysis of HCN production. It was primarily described by Baumeister and Schievelbein (1971). Overnight bacterial cultures were prepared in Luria-Bertani broth (Miller) (LB) (Sigma-Aldrich, USA) supplied with 5 g L−1 glycine (Sigma-Aldrich, USA) (LBgly) and the pH was adjusted to 7.4. Maximum potential of the HCN production can be determined by using this medium. 0.34% (w/v) methemoglobin reagent was prepared in 4 mM $NaNO_2$ solution and incubated for 10 min, and mixed with phosphate buffer in 1:1 ratio and the solution has incubated for 30 min at 37^{O}C. A KCN solution is employed as the standard concentration. Optical density of all liquid samples was measured at 424 nm by using bottom 96 well micro assay plates (Von rohr *et al.* (2009).

IAA Production

Qualitative analysis of IAA production by different isolates was determined using Salkowski's reagent (Gordon and Weber, 1951). The freshly grown cultures of all the pure isolates were transferred into test tubes containing 5 mL Nutrient broth (LB) broth supplemented with 100 µg/mL L-tryptophan and incubated at 37°C for 2 days. The broth was then centrifuged at 10,000 rpm for 5 minutes. 1 ml of Supernatant was transferred to fresh test tubes and 2 mL of Salkowski's reagent was added to the tubes. The solutions were gently mixed and incubated at room temperature for 30 minutes. The development of pink color was recorded spectrophotometrically at 530 nm with uninoculated broth as control. • The standard curve was plotted with 5-100 mg/mL of IAA (Sigma Aldrich).

Chitinase Assay

Colloidal chitin was prepared according to the modified method described by (Mathivanan *et al.*, 2014). Chitinase assay was performed on a nutrient agar plate supplemented with 1% chitin by inoculating the freshly grown culture of all pure isolates. Plates were allowed to incubate at room temperature for 5 days, and the zone of clearance due to chitin hydrolysis was recorded as positive chitinase-producing bacteria.

Siderophore Production

Bacterial isolates were assayed for siderophore production on the Chrome azurol S agar medium (Sigma, Ltd.) described by Schwyn and Neilands (Schwyn and Neilands, 1987). Chrome azurol S agar plates were spot inoculated with the isolates and incubated at 37^{O}C for 48–72 hours. The development of yellow–orange halo around the growth was considered as positive for siderophore production.

RESULTS AND DISCUSSION

Isolated Rhizobacteria from Soil Sample and it's a Brief Overview

Soil is the reservoir of a variety of microflora that involves beneficial as well as harmful microorganisms. Environmental factors such as moisture present in the soil, pH, and temperature are essential in the distribution of microflora. Among these rhizobacterial isolates show a beneficial effect on plants. Total 41 bacterial isolates are obtained from different soil samples.

Characterization of Soil Samples

An important role of micro and macro elements present in soil sample for crop growth promotion is well known fact. The major elements of soil samples specially in rhizsphere region is dominating with carbon, phosphorus, nitrogen, potassium, and the micro elements in trace amounts for example zinc, iron, manganese etc. play a vital role. In the present study a consolidated data in form of tables 1 reflects the distribution of the different major and minor element with its quantitative aspects. These data are helpful for understanding the intensity of availability of different rhizobacterial isolates. (Figure 1)

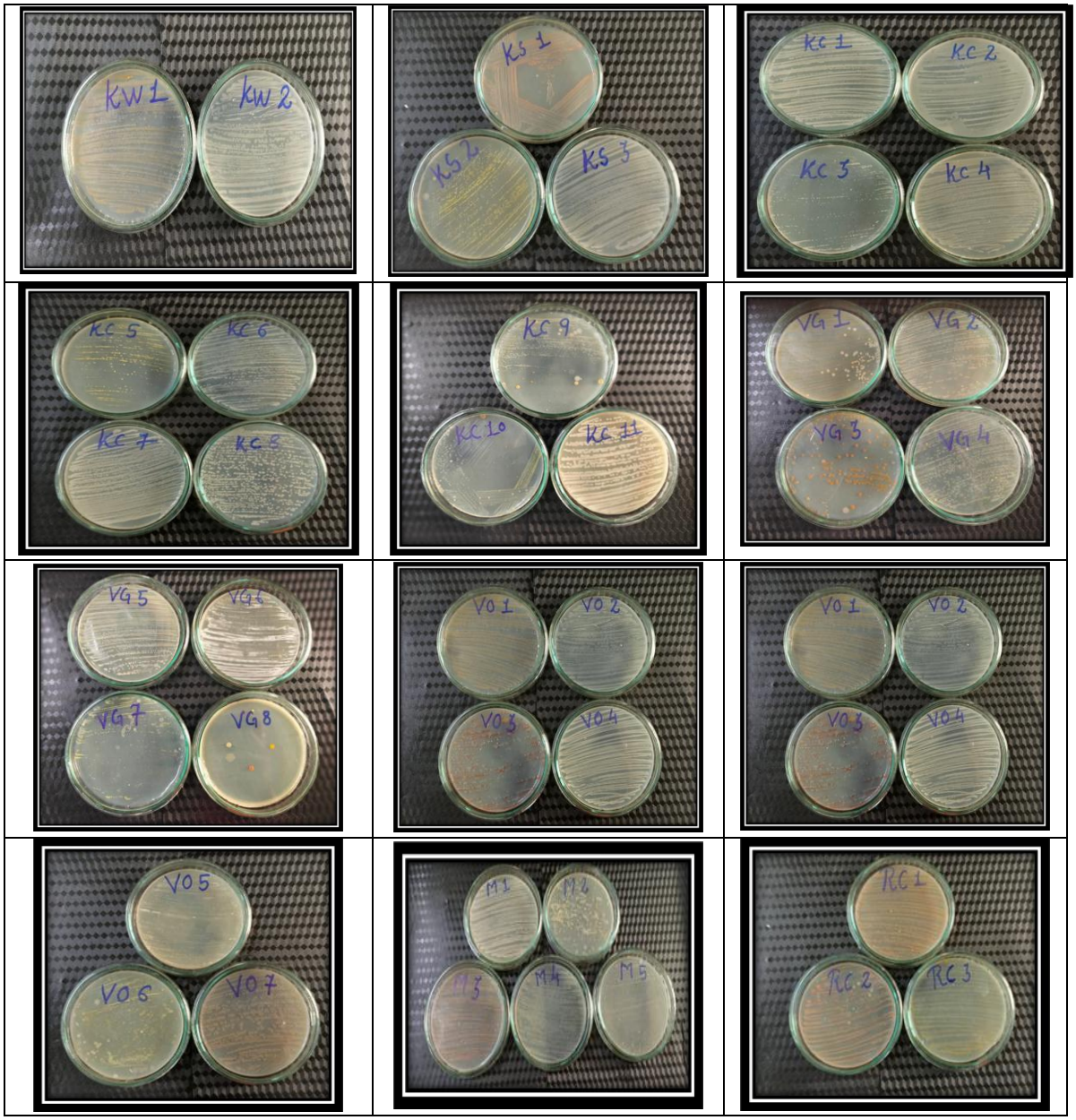

Figure 1: Representative Pure Cultures of Bacterial Isolates from Different Soil Samples

Table 1: Soil Sample Analysis of Various Regions of Saurashtra

Test Parameters	Quantity (ppm)			
	Rajkot	**Khijadiya**	**Veraval**	**Morbi**
Total nitrogen/ organic carbon (OC)%	0.51	0.60	0.49	0.36
P_2O_5 Kg/Ac	21.00	16.00	10.00	20.00
K_2O_7 Kg/Ac	34.00	34.00	71.00	317.00
pH	7.63	7.66	7.75	7.73
Electrical conductivity (EC)	2.10	1.03	0.54	1.50
S, ppm	10.20	9.80	10.20	12.50
Microelements				
Zn	0.41	1.49	0.59	1.15
Fe	0.85	1.49	0.54	0.65
Mn	12.16	14.37	9.14	8.32
Cu	0.33	1.71	0.72	3.19

Key attributes for characterization of soil includes the measurement of pH, soil electric conductivity, organic carbon and nitrogen, soil potassium, phosphorus, and estimation of micronutrients. Our study analyses pH content for PGPR isolates near about 7 pH which shows alkaline soil which would be beneficial for plant yield and soil fertility (Arshad *et al.*, 2009) availability of nutrients is greatly impacted by pH change. Thus change in pH content have both positive and negative effects on soil quality.

Organic matter improves soil quality by increasing the nutrient content and improves the fertility of soil. According to our results we found low content may decreases the soil quality.

Soil electrical conductivity measures the salt concentration of soil which increases the nutrient availability and water holding capacity affecting the crop yield and sustainability of soil. Excessive salt concentration and salt concentration effects the plant growth and water holding capacity. According to our results we found that the electrical conductivity is in normal range which affects the water holding capacity as well as plant growth.it may also increase the crop yield, irrigation and crop management (Arshad *et al.*, 2009).

Basic Identification of Collected Rhizobacterial Isolates

In present study rhizobacterial species identified on the basis of microscopic and morphological structure. As the result of Gram's staining about 70% species showed Gram's negative and rest of them showed Gram's positive result under 100X oil emulsion lens. Following tables show the result of morphology of bacterial colony. (Table 2 & Figure 2)

Table 2: Morphological Identification of Various Rhizobacterial Isolates from Different Soil Samples of the Saurashtra Region

Sample	Size	Shape	Color	Texture	Elevation	Margin	Pigmentation
KW1	Large	Circular	Black	Smooth	Flat	Entire	No
KW2	Large	Circular	Orange	Smooth	Flat	Entire	No
KW3	Large	Irregular	Cream	Dry	Umbonate	Filli form	No
KS1	Moderate	Circular	Orange	Dry	Convex	Entire	Yes
KS2	Moderate	Circular	Light yellow	Smooth	Convex	Entire	No
KS3	Large	Irregular	White	Dry	Flat	Entire	No
KS4	Large	Irregular	Cream	Dry	Umbonate	Filli form	No
KC1	Large	Irregular	White	Smooth	Flat	Entire	No
KC2	Large	Regular	White	Smooth	Flat	Umbonate	No
KC3	Large	Circular	White	Smooth	Convex	Entire	No
KC4	Small	Circular	Yellow	Smooth	Convex	Entire	No
KC5	Large	Irregular	Cream	Dry	Filli form	Umbonate	No
KC6	Large	Irregular	Orange	Smooth	Flat	Entire	Yes
KC7	Large	Circular	White	Dry	Flat	Entire	No
KC8	Small	Circular	White	Smooth	Convex	Entire	No
VO1	Small	Circular	Light yellow	Dry	Raised	Entire	No
VO2	Large	Irregular	Cream	Dry	Raised	Filli form	No
VO3	Moderate	Circular	White	Dry	Raised	Entire	No
VO4	Large	Circular	Brown	Smooth	Flat	Entire	No
VG1	Large	Circular	White	Smooth	Flat	Entire	No
VG2	Large	Irregular	Cream	Smooth	Raised	Filli form	No
VG3	Large	Circular	White	Dry	Flat	Entire	No
VG4	Small	Circular	Light yellow	Dry	Raised	Entire	No
MC1	Large	Irregular	Cream	Dry	Raised	Filli form	No
MC2	Moderate	Circular	Orange	Smooth	Flat	Entire	Yes
MC3	Large	Circular	White	Dry	Convex	Entire	No
MC4	Moderate	Circular	Brown	Smooth	Flat	Entire	No

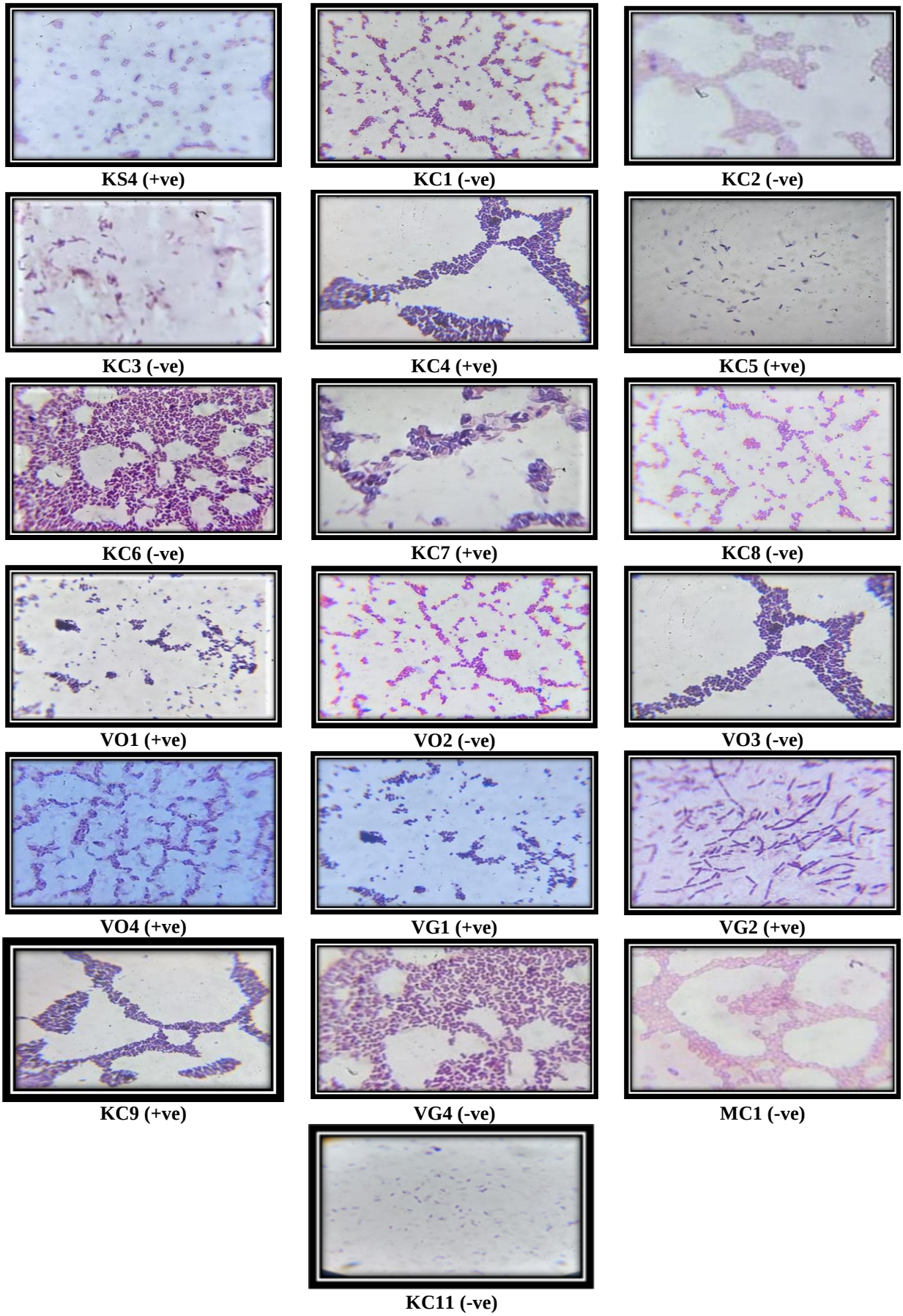

Figure 2: Illustrative representation of Gram's Staining Technique of Bacterial Isolates

From the above results of the identification method the bacterial traits are majorly belong to genus such as *Bacillus, Pseudomonas, Azotobacter*. Singh et al 2020 also reported that rhizospheric region soil has dominantly presence of these rhizobacterial traits.

Evaluation of Different Growth-Promoting Factors as PGPR Traits

For the identification of potent PGPR bacterial traits growth promotion biochemical parameters such as HCN, Siderophore, IAA and Ammonia production test have been employed using all the 41 bacterial isolates. The result revealed the following table 3 has reflected the consolidated overview of PGPR traits on determination of 4 different important attributes. Among the 41 isolates 4 isolates shows the maximum production of growth promotion parameters respectively (Figure 3 to 7).

Table 3: Summary of result of growth promoting tests for PGPR traits

Sr No.	Sample No.	HCN Production	Siderophore Production	IAA Production	Ammonia Production
1	KW1		+	+	+
2	KW2		+	+	+
3	KS1	+	+	+	+
4	KS2	+	+	+	+
5	KS3	+	+		+
6	KS4		+	+	+
7	KS5		+	+	+
8	KC1		+	+	+
9	KC2		+	+	+
10	KC3		+	+	+
11	KC4		+	+	+
12	KC5		+	+	+
13	KC6	+	+	+	+
14	KC7		+	+	+
15	KC8	+	+	+	+
16	KC9	+	+	+	+
17	KC10	+	+	+	+
18	KC11	+	+	+	+
19	VG1	+	+		+
20	VG2	+	+		+
21	VG3	+	+		+
22	VG4	+	+		+
23	VG5		+	+	+
24	VG6		+		+
25	VG7	+	+	+	+
26	VG8		+	+	+
27	VO1		+	+	+
28	VO2		+	+	+
29	VO3		+	+	+
30	VO4		+	+	+
31	VO5		+	+	+
32	VO6		+	+	+
33	VO7		+	+	+
34	M1		+	+	+
35	M2		+	+	+
36	M3		+	+	+
37	M4		+	+	+
38	M5		+	+	+
39	RC1		+	+	+
40	RC2		+	+	+
41	RC3		+	+	+

Note*: KW=Khijadiya wheat, KS=Khijadiya sorghum, KC= Khijadiya chickpea, VG= Veraval garlic, VO= Veraval onion, MC=Morbi corn, RC=Rajkot corn

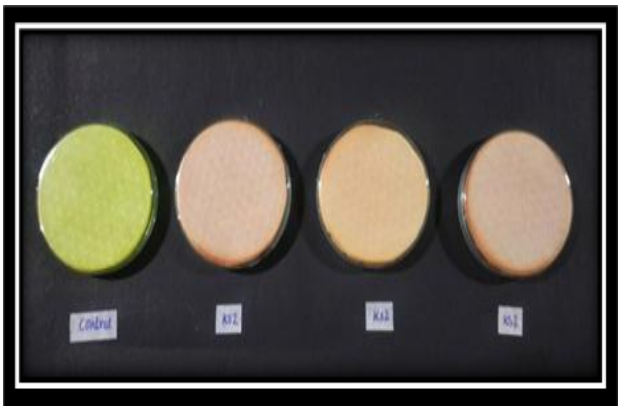

Figure 3: Representation of HCN production

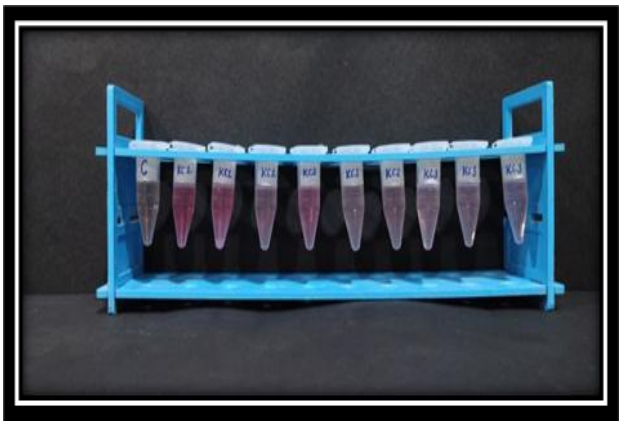

Figure 4: IAA Production of Bacterial Isolates

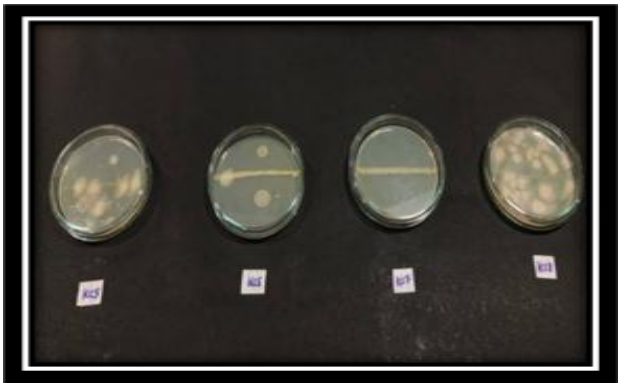

Figure 5: Chitinase Assay of Bacterial Isolates

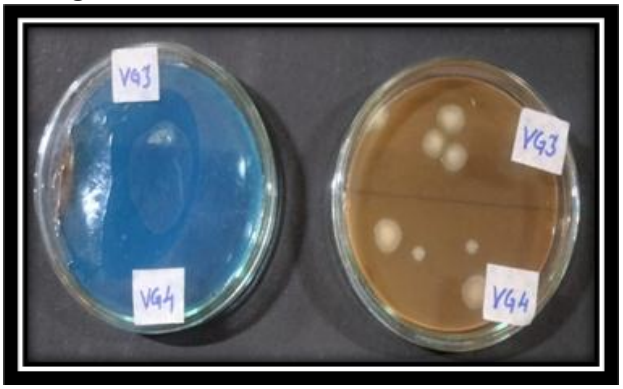

Figure 6: Siderophore Production

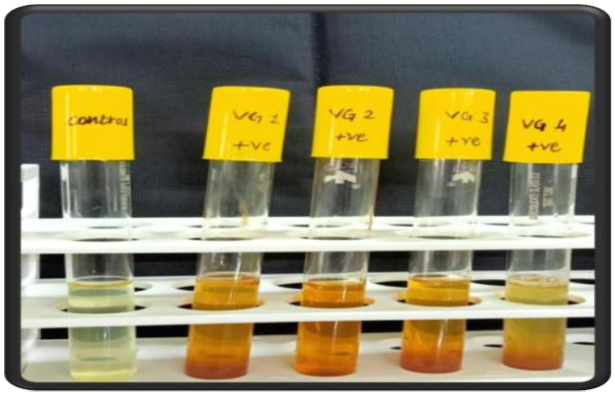

Figure 7: Ammonia Production Assay of Bacterial Isolates

Evaluation involves siderophore production, ammonia production, chitinase assay and IAA production. To screen out siderophore Chrome azurol sulfonate (CAS) agar solid medium is used (Alexander *et al.*, 1991). The results obtained as orange zone around the bacterial colony which shows the reduction of iron by CAS utilized by siderophore. PGPR isolates were found positive for the production of IAA without the addition of L- tryptophan in nutrient broth and ammonia production (Singh *et al.*, 2020). Among 41 isolates 4 were showed positive result for these tests.

CONCLUSION

In present investigation total 41 bacterial species were isolated from the soil sample of different regions of saurashtra area. Morphological and microscopical identification of all isolates was done by standard methods. As per the results obtained among the 41 isolates 4 isolates KS2, KC9, KC11, and KC8 have shown the higher efficiency of the nitrogen fixing ability by the production phosphate solubilization, production of siderophores and ammonia ability to the PGPR traits. So, we consider these selected bacterial species can employed as biofertilizer, nitrogen fixers and biocontrol agents.

Future Scope

Isolated PGPR traits can be employ as nitrogen fixing ability in plants as well as utilized as biocontrol agent to control the plant pathogen and disease management.

REFERENCES

Bashan Y., Holguin G. and Lifshitz R., 1993. Isolation and characterization of plant growth-promoting rhizobacteria. Methods in plant molecular biology and biotechnology, pp.331-345.

Bhatt S., Pandhi N. and Raghav R., 2022. Improved salt tolerance and growth parameters of groundnut (Arachis hypogaea L.) employing Halotolerant Bacillus cereus SVSCD1 isolated from Saurashtra Region, Gujarat.

Basu A., Prasad P., Das S.N., Kalam S., Sayyed R.Z., Reddy M.S. and El Enshasy H., 2021. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. Sustainability, **13**(3): 1140.

Frommel M.I., Nowak J. and Lazarovits G., 1991. Growth enhancement and developmental modifications of in vitro grown potato (Solanum tuberosum spp. tuberosum) as affected by a nonfluorescent Pseudomonas sp. Plant Physiology, **96**(3): 928-936.

Glick B.R., Holguin G., Patten C.L. and Penrose D.M., 1999. Biochemical and genetic mechanisms used by plant growth promoting bacteria. World Scientific.

Glick B.R., Karaturovíc D.M. and Newell P.C., 1995. A novel procedure for rapid isolation of plant growth promoting pseudomonads. Canadian journal of microbiology, **41**(6): 533-536.

Gupta G., Parihar S.S., Ahirwar N.K., Snehi S.K. and Singh V., 2015. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J. Microb Biochem Technol, **7**(2): 096-102.

Kloepper J.W., Hume D.J., Scher F.M., Singleton C., Tipping B., Laliberté M., Frauley K., Kutchaw T., Simonson C., Lifshitz R., Zaleska I. and Lee L., 1988. Plant growth-promoting rhizobacteria on canola (rapeseed). Plant Disease, **72**: 42- 45.

Modi K. and Jacob F., 2017. Isolation and characterization of phosphate solubilizing Bacillus consortia for plant growth promotion. J. Plant Protect Res., **5**: 278-283.

Modi K. and Patel P., 2017. Isolation and characterization of plant growth promoting rhizobacteria associated with Saccharum officinarum L. *Curr.* Synth. Syst. Biol., **5**: 2332-0737.

Sharma P. and Shrivastava D.K., 2017. Isolation and characterization of PGPR from rhizospheric soil. International Journal of Scientific & Engineering Research, **8**: 54-58.

Shaikh S.S. and Sayyed R.Z., 2015. Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In Plant microbes symbiosis: Applied facets (pp. 337-351). Springer, New Delhi.

Shiferaw B., Prasanna B., Hellin J. and Banziger M., 2011. Feeding a hungry world: past successes and future challenges to global food security in maize. Food Security, **3**: 307-327.

Charles Pravin J., Nirmal D., Prajoon P. and Ajayan J., 2016. Implementation of nanoscale circuits using dual metal gate engineered nanowire MOSFET with high-k dielectrics for low power applications. Physica E: Low-dimensional Systems and Nanostructures, **83**: 95-100. https://doi.org/10.1016/j.physe.2016.04.017

Gilbert U. Adie and Oladele Osibanjo, 2009. Assessment of soil-pollution by slag from an automobile battery manufacturing plant in Nigeria. African Journal of Environmental Science and Technology, **3**(9): 239-250, http://www.academicjournals.org/ajest ISSN 1991-637X © 2009 Academic Journals

Lindsay W.L. and Norvell W.A., 1978. Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. Soil Science Society of Americal Journal, **42**: 421-428 https://doi.org/10.2136/sssaj1978.03615995004200030009x

Solon A. Gordon and Robert P. Weber, 1951. Colorimetric Estimation of Indoleacetic Acid. Plant Physiol., **26**(1): 192-195. doi: 10.1104/pp.26.1.192

Ghosh S. and Basu P.S., 2006. Production and metabolism of indole acetic acid in roots and root nodules of *Phaseolus mungo.* Microbiological Research, **161**(4): 362-366: https://doi.org/10.1016/j.micres.2006.01.001

Matthias Rudolfvon Rohr, Gerhard Furer and Helmut Brandl, 2009. Effect of iron and phosphate on bacterial cyanide formation determined by methemoglobin in two-dimensional gradientmicro cultivationss. Journal of

Microbiological Methods, **79**(1): 71-75. https://doi.org/10.1016/j.mimet.2009.08.008

Mathivanan K. and Rajaram R., 2013. Anthropogenic influences on toxic metals in water and sediment samples collected from industrially polluted Cuddalore coast, Southeast coast of India. Environmental Earth Sciences, **72**: 997-1010.

Bernhard Schwyn and Neilands J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry, **160**(1): 47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Shomi F.Y., Uddin M.B. and Zerin T., 2021. Isolation and characterization of nitrogen-fixing bacteria from soil sample in Dhaka, Bangladesh. Stamford Journal of Microbiology, **11**. https://doi.org/10.3329/sjm.v11i1.57145

Soon Y.K., Haq A. and Arshad M.A., 2009. Carbon and nitrogen contents of different-sized light fraction organic matter as influenced by tillage and residue management. Canadian Journal of Soil Science, Volume **89**, https://doi.org/10.4141/CJSS08065

Anelise Beneduzi Adriana, Ambrosini Luciana and Passaglia M.P., 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genetics and Molecular Biology, **35**(4suppl 1) https://doi.org/10.1590/S1415-47572012000600020

Adriana Ambrosini, Anelise Beneduzi, Thais Stefanski, Felipe G. Pinheiro, Luciano K. Vargas and Luciane M.P. Passaglia, 2012. Screening of plant growth promoting Rhizobacterial isolated from sunflower (Helianthus annuus L.). Plant and Soil, **356**: 245-264.

Roberto Farina, Anelise Beneduzi, Adriana Ambrosini, Samanta B.de Campos, Bruno Brito Lisboa, Volker Wendisch, Luciano K. Vargas and Luciane M.P. Passaglia, 2012. Diversity of plant growth-promoting rhizobacteria communities associated with the stages of canola growth. Applied Soil Ecology, **55**: 44-52: https://doi.org/10.1016/j.apsoil.2011.12.011

Gupta G., Parihar S.S., Ahirwar N.K., Snehi S.K. and Singh V., 2015. Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. Gupta *et al.*, J. Microb. Biochem. Technol., **7**:2. http://dx.doi.org/10.4172/1948-5948.1000188

Goswami D., Dhandhukia P., Patel P. and Thakker J.N., 2014. Screening of PGPR from saline desert of Kutch: Growth promotion in Arachis hypogea by Bacillus licheniformis A2. Microbiological Research, **169**: 66–75.

Hasan1 N.A., and Zulkahar I.M., 2018. Isolation and identification of bacteria from spoiled fruits, https://doi.org/10.1063/1.50626999.

Lham Lamizadeh, Naeimeh Enayatizamir and Hossein Motamedi, 2016. Isolation and Identification of Plant Growth-Promoting Rhizobacteria (PGPR) from the Rhizosphere of Sugarcane in Saline and Non-Saline Soil, International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706, **5**(10): 1072-1083, doi: http://dx.doi.org/10.20546/ijcmas.2016.510.113

Singh T.B., Sahai V., Ali A., Prasad M., Yadav A., Shrivastav P., Goyal D. and Dantu P.K., 2020. Screening and evaluation of PGPR strains having multiple PGP traits from hilly terrain, Journal of Applied Biology & Biotechnology, **8**(04): 38-44, DOI: 10.7324/JABB.2020.80406