Eco. Env. & Cons. 29 (May Suppl. Issue) : 2023; pp. (S191-S196)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i03s.037

Exploration of Saurashtra Soil PGPR Strain and its Attributes in Crop Productivity by Pot Plant Study

Shivani Patel[1], Neepa Pandhi[2] and Chitra Bhattacharya[1*]

[1]Department of Microbiology, Atmiya University, Rajkot, Gujarat, India
[2]Department of Microbiology, Shree M. & N. Virani Science College, Rajkot, Gujarat, India

(Received 11 December, 2022; Accepted 6 February, 2023)

ABSTRACT

The impact of rhizobacteria with PGPR trait in current agriculture practices is expanding its horizons at large in research and practices. The urge of improvised crop productivity and disease management for economically important myriad crops like cereal plant, and angiosperms, monocots, dicots need to address in versatile manner with integrated approach. The need of a potent trait of PGPR rhizobacteria and its real time application in field trials is more challenging and to address the same, the present research study has been carried out. The present study encompassing the one phase of the project is to isolate the Rhizobial trait from the soil sample followed by the screening of the potent traits. Total 4 potent rhizobacterial isolates have been screened among 41 bacterial isolates from different sites of the Saurashtra region. Qualitative and quantitative characterization of growth promotion factors such as HCN production, ammonia production, IAA production, and siderophore production of the isolates (KS2, KC8, KC9, and KC11 showed) were carried out. Among 21 isolates the 4 isolates KS2, KC8, KC9, and KC11showed the highest growth parameter production and have been stated as PGPR traits. Employing One way ANOVA a statistical design, the shoot and root length effect has been studied in detail with the individual strains KS2, KC8, KC9, and KC11 along with control. The study is quite encouraging with improved root length 10.54±0.33 cm and shoot length 27.20 ± 0.8 cm respectively. The future prospect of this study can be highlighted in long-term with further analysis on consortial development and its field trial applications.

Key words : *PGPR, Pot analysis, Biochemical Charaterization, ANOVA*

Introduction

Cumin (*Cuminum cyminum* L.) is an annual herb belonging to the family Apiaceae. Cumin is one of the initial known minor spices used by mankind. The common Indian name of cumin is Jira and it is produced by growing in most hot regions like India as in Gujarat. Cumin is one of the most broadly utilized seed spices by worldwide. It is the second most popular spice in the world after black pepper (Lal *et al.,* 2014). Gujarat has highly fertile land thus it gives the best place for crop production. During - the 19th centuryagricultural practice is followed by using chemicals like fertilizers and pesticides. Over usages of chemical fertilizers cause acidification of soil and crust which may lead to decrement in organic matters, humus content of soil varying other beneficial organisms enhancing the fertility and crop yield, also varies pH of soil. Even leads to increment of greenhouse gases that may lead to pollution and effect human health. As the soil converted to acidic nature it diminishes mineral intake and increase the concentration of harmful ions in soil also reduces the nutrient holding capacity as it reduces the humic content of soil. Hence loose soil fertility and crop yield. The term PGPR plant growth promoting

rhizobacteria as beneficial microbes was introduced by Becky *et al.* (2022). The term pant growth promoting rhizobacteria means that colonizing the root the surface of plant for easy plant development. Rhizosphere is the plant root area where microbes have the highest zone for microbial activity and rich region in nutrients uptake. PGPR plant growth promoting rhizobacteria acting as potent organisms for sustainable development increasing the crop production without the use of chemicals and pesticides and positive results for controlling the plant diseases caused by harmful pathogens.

Materials and Method

Isolation of Bacterial Strains from Rhizospheres Soil

Isolation of bacterial strains from the rhizospheric soil samples. 10-10 g of soil samples were collected from the rhizospheric soil of the Saurashtra region mixed with 100 ml of sterile water and stirred for 10 mins. Serial dilution method has been performed to maintain the 10^{-6} dilution and inoculated 0.1 ml of final dilution into nutrient agar plates and incubate the plates at 37 ± 2 °C for 24 hrs segregate the colonies and examined by employing microscopic and morphological characterization. Then purified isolates are inoculated onto a nutrient agar slant and preserved it for further study in a refrigerator at 4 °C (Mostafa *et al*., 2021).

Characterization of the Most Potent Rhizospheric Bacterial Strains as Plant Growth-Promotion

Phosphate Solubilization

Pikovskaya agar media was employed for the study of the efficacy of bacterial strains for the primary screening of phosphate solubilizing assay. Bacterial cultures were inoculated center of the PVK agar medium and incubated at 35 ± 2 °C for 48 hrs. Examine the results by observing the diameter of the clear zone formed around the bacterial cultures (Jasim *et al.,* 2014).

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).

Siderophore Production

king's B agar medium containing chrome azurol S as an indicator dye, Fe3+ solution, and hexadecyltrimethylammonium bromide (HDTMA) was used for the secretion of siderophore from the bacterial strains. The fresh bacterial culture was inoculated onto the agar plate of king's B medium and incubated at 30 °C for 72 hrs. The development of a yellow an orange halo around the growth was considered as positive for siderophore production (Kotasthane *et al.,* 2017).

Ammonia production

Isolated freshly grown cultures were inoculated in 5.0ml peptone water tubes to test for ammonia production. All the tubes were incubated at 37 °C for 72-96 hours. After 96 hours of incubation add 0.5 ml of freshly prepared Nessler's reagent. The positive result indicates the color intensity of yellow to the brown colour formation (Farzana *et al.,* 2021).

HCN production

Bacterial cultures were inoculated in the King's B medium amended with glycine for the determination of HCN production. 2% sodium carbonate mixed with 0.05% picric acid solutionwas soaked by using Whatman No. 1 filter and placed on the lid of inoculated kings's B medium Petri plate sealed with parafilm and incubated at 30 °C for 48 hrs. Observed the colour change of the filter paper from deep yellow to reddish-brown color (Geetha *et al*., 2014).

Evaluation Study of PGPR Trait by Individual Pot Study

The mature culture isolates were inoculated in 100 ml of nutrient broth and kept inan orbital incubator shaker at 37 °C for 7 days. After the completion of the incubation period, the 5.0 ml culture inoculum has been introduced into every pot and cumin seed. 10-10 seeds of cumin have been taken for every pot

experiment and were soaked for over-night. Then prepared for individual pot culture experiment (A. Karthick *et al.,* 2008).

a. Control Simple soil + Cumin seeds
b. Soil + KS2 + Cumin seeds
c. Soil + KC8 + Cumin seeds
d. Soil + KC9 + Cumin seeds
e. Soil + KC11 + Cumin seeds

Statistical Approach

The pot experiments were organized by one-way ANOVA at 5% level of significancewith three replicates of treatment.

Results and Discussion

Isolation and Identification of PGPR traits

In all total from four regions of Rajkot, different sampling sites of the Saurashtra Region were chosen for soil sample collection. A total of 21 bacterial isolates were obtained out of 21 only 4 bacterial traits (KC9, KC11, KC8, and KS2) are shown as PGPR activity by the microscopic identification and biochemical characterization such as HCN production, Ammonia, Siderophore, Phosphate solubilization, and chitinase activity of the most potent rhizospheric bacterial strains as plant growth-promotion.

HCN production

Microbial cyanides seem to play a role in the suppression of many plant diseases. A positive correlation between the production of HCN and suppression of root rot by bacterial isolates has been reported (Defago *et al.,* 1990; Rajni Devi and Richa Thakur, 2018). In the present study, Sucrose agar medium is used to detect the production of HCN. 4 to 5 drops of picric acid added into the inoculated plate have been changed from yellow to orange-red. Also in the present study, 13 isolates were tested for qualitative HCN production on nutrient agar plates supplemented with 2% glycine and 0.5% picric acid. 31.70% out of 21 isolates. KC9 and KC11 showed maximum HCN production. Several factors have been reported to influence the rate of HCN production. Glycine has been found to be the direct precursor of microbial cyanide production (Knowles, 1976, Voisard *et al.,* 1989; Rajni Devi and Richa Thakur 2018) and it has been found in root exudates (Bakker *et al.,* 1989; Curl *et al.,* 1986). The induction of plant resistance may be involved by HCN production which was reported by Rajni Devi and Richa Thakur (2018).

IAA production

The result revealed that indole salkowski reagent has been added to the culture supernatant after the 30 mins pink color was obtained. This indicates the production of IAA that stimulates and facilitates plant growth promotion. In the report of Rani *et al.* (2012) seven isolates of PGPR bacteria had shown the IAA production ability.

All 4isolates give production of IAA. L-Tryptophan is generally considered as an IAA precursor; because its addition to IAA-producing bacterial culture enhances IAA biosynthesis (IAA production

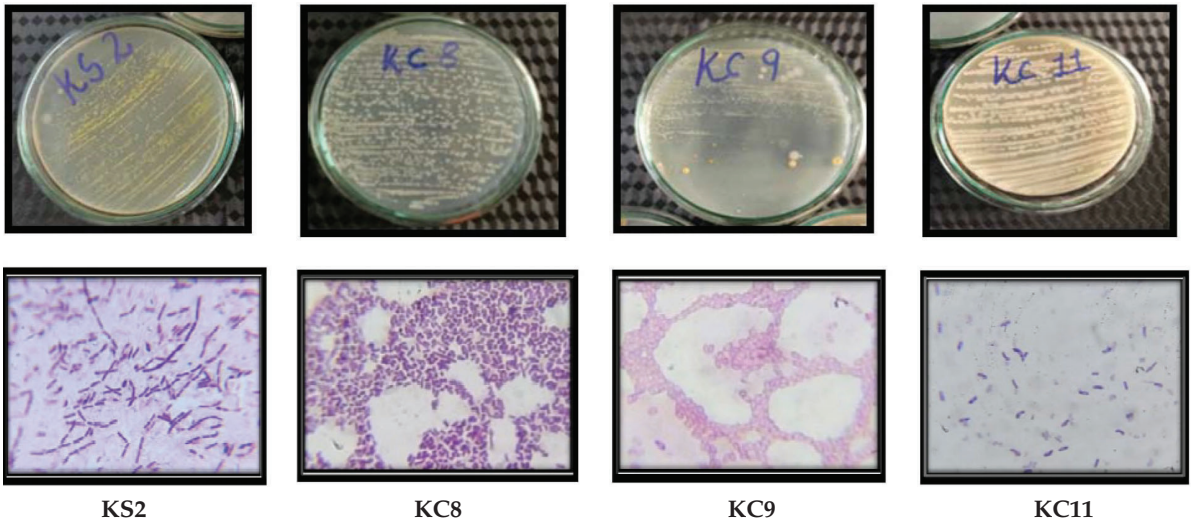

Fig. 1. Pure Culture Plates and Gram's Staining Representation of Potent PGPR Traits

was found in the medium amended with 0.1% tryptophan for all isolates Costacurta and Venderleyden, 1995; Mohite, 2013). All 4 isolates preferred Tryptophan for IAA production. Multifarious factors are responsible for the plant growth promotion employing PGPR and among them, one such important contributing factor is IAA. The IAA production is dependent on the growth stage of microbes and the availability of substrates, and also reports state the high intensity of IAA production in rhizosphere soils too (Rani *et al.*, 2012). In the report of Rani *et al.* (2012) seven isolates of PGPR bacteria had shown the IAA production ability.

Phosphate solubilization

Production of phosphate enzyme by the bacterial isolates. Agar well diffusion method is performed for the detection of phosphate solubilization assay. In the PVK medium bacterial suspension was inoculated in between the well and incubated at 37 °C for 24 hours only few cultures zoned the zone of hydrolysis.Among macronutrients required by a high amount for enhancement of plant growth is phosphorus ion, which exists in most cases as an insoluble form. Rhizospheric bacteria have the ability to convert phosphorus from insoluble to soluble forms through different mechanisms such as the production of enzymes and /or organic acids (Paul and Sinha, 2017). In the current study, the appearance of a clear zone around the bacterial growth on Pikovskaya agar media indicates their success in phosphate solubilizing. The clear zone was observed in KS2, KC8, KC9, and KC11which correlated with the amount of liberated phosphate. The results showed that phosphate solubilizing was achieved by bacteria. The production of low molecular weight organic acid is considered the main mechanism for phosphate solubilization by different bacterial species (Khan *et al.*, 2019).

Siderophore production

Siderophore facilitates iron, which is a cofactor for nitrogenase enzyme essential for atmospheric nitrogen fixation by plants. Four isolates from Rajkot – KS2, KC8, KC9, and KC11 displayed siderophore production.Siderophore production is an important attribute of PGPR trait as secondary metabolites, the iron fulfillment was mitigated by siderophore production by PGPR also protection from phytopathogens provided by siderophore-producing isolates. The heavy metal contamination can also bereduced by siderophore producers (Arora and Verma, 2017).

Ammonia production

All 4 bacterialisolates (KS2, KC8, KC9, and KC11) tested positive for nitrogen fixation, which facilitates the enhanced amount of available nitrogen in the form of ammonia, nitrates, or nitrites in the rhizosphere. Ammonia as an important chemical component exertsa phytopathogenic effect as reported by Fahsi *et al.* (2021). This paper also highlightsan intensified production of ammoniafrom 0.23 to 0.33 $\mu mol \cdot ml^{-1}$. As quoted by Tsegaye *et al.* (2019).

Evaluation study of PGPR trait by individual pot study

An evaluation study at the individual pot plant ex-

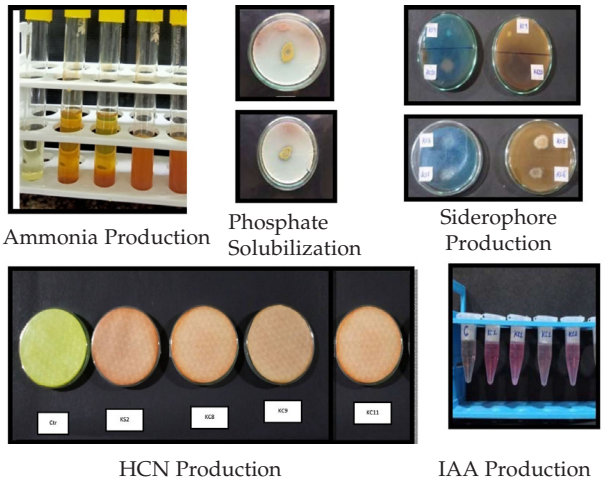

Fig. 2. Biochemical Characterization of Potent Bacterial PGPR Isolates

Table 1. A summary of observation of PGPR traits with selected isolates of Rhizobacteria

Sr No.	Sample No.	HCN Production	Siderophore Production	IAA Production	Ammonia Production	Phosphate solubilization
1	KS2	+	+	+	+	+
2	KC8	+	+	+	+	+
3	KC9	+	+	+	+	+
4	KC11	+	+	+	+	+

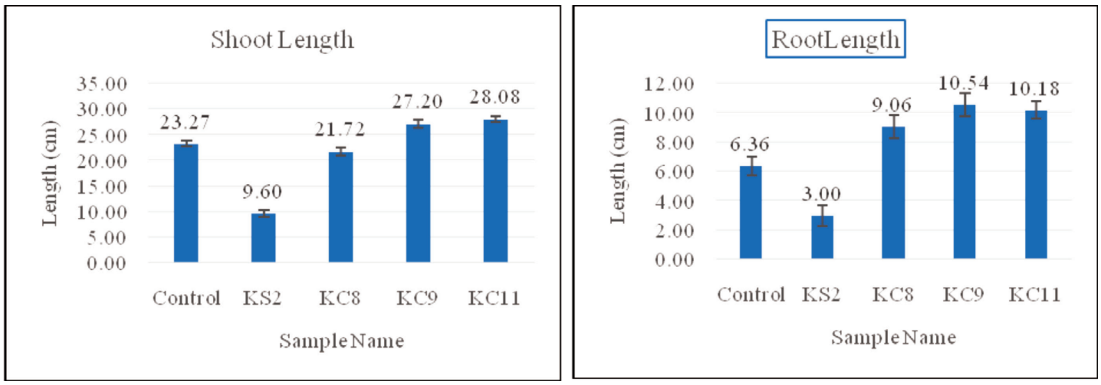

Fig. 3. Evaluation of study of root and shoot length of cumin plant employing potent rhizobacteria inoculum

Fig. 4. Plant growth promotion study at the individual level by the potent screened PGPR strains of Rhizobacteria

periment level has successfully revealed the determination of the PGPR trait of the four selected isolates reflected. Individual-level pot plant experiment with 4 potent PGPR bacteria with control has shown the growth effect as represented. The root and shoot growth with control has clearly been viewed in the figure showing the potential effect of PGPR traits whose detailed representation had been recorded. Similar reports on the effect of PGPR trait crop growth development have been recorded by Yadav and Yadav (2022). In this report four efficient phosphate solubilizing strains isolated from the rhizosphere has found to reflect the crop growth and yield of 0.19 g. In the present study, a series of individual sets of samples and control being tested from PGPR trait has found to record the highest root length (10.54± 0.33 cm) and shoot length (27.20± 0.8cm) respectively of KC9 (Fig. 3 and 4).

Conclusion

The overall study of a potent PGPR strain and its field application for crop productivity and management has been studied in detail in the following research. Out of 21 isolates, 4 potent PGPR strains followed by standard screening procedure found to show effective attributes of PGPR trait like HCN production, IAA, phosphate solubilization, siderophore production and ammonia synthesis. Further, in pot plant experiment the validation of PGPR trait has been confirmed by statistical one-way ANOVA design. KC9 has been found to reflect the highest root and shoot growth employing cumin seeds. The future aspect of the study is quite encouraging in terms of a potent consortial development.

References

Arora, N. and Verma, M. 2017. Modified microplate method for rapid and efficient estimation of siderophore produced by bacteria. *Biology, Medicine. 3 Biotech*, pp. 1-23.

Bahiru Tsegaye, Chandrajit Balomajumder and Partha Roy, 2019. Optimization of microwave and NaOH pretreatments of wheat straw for enhancing biofuel yield. *Energy Conversion and Management*. 186: 82-92

Bakker, A.W. and Bakker, P. and Schippers, B. 1989. Deleterious cyanide producing rhizosphere Pseudomonads as a factor limiting potato root growth and tuber yield in high frequency potato cropping soil. In: *Effects of Crop Rotation on Potato Production in the Temperate Zones*. Kluwer Academic

Publishers, Dodrecht, p. 153-62.
Becky, N. Aloo, Vishal Tripathi, Billy, A. Makumba and Ernest R. Mbega, 2022. Plant growth-promoting rhizobacterial biofertilizers for crop production: The past, present, and future. *Frontier of Plant Sciences.* 1-15.
Curl Elroy, A., Elroy, A. and Bryan Truelove, 1986. The Rhizosphere. Advanced Series in Agricultural Sciences. *Agricultural.* 15: 167-190.
Defago, G., Berling, C.H., Burger, U., Haas, D., Kahr, G. and Keel, C. 1990. Suppression of black root rot of tobacco by a Pseudomonas strain; potential application and mechanisms. In: *Biological Control of Soil Borne Plant Pathogen*. Oxon: CAB International, p. 93-08.
Fahsi, N., Mahdi, I., Mesfioui, A., Biskri, L. and Allaoui, A. 2021. Plant Growth-Promoting Rhizobacteria Isolated from the Jujube (*Ziziphus lotus*) Plant Enhance Wheat Growth, Zn Uptake, and Heavy Metal Tolerance. *Agriculture.* 11: 316. https://doi.org/10.3390/agriculture11040316.
Farzana Yasmin Shomi, Md. Borhan Uddin and Tamanna Zerin, 2021. Isolation and characterization of nitrogen-fixing bacteria from soil sample in Dhaka, Bangladesh. *Stamford Journal of Microbiology*. 11 (1) : 11-13.
Geetha, K., Enkatesham, E.V., Amballa, H. and Hadraiah, B.B. Solation, 2014. Screening and characterization of plant growth promoting bacteria and their effect on *Vigna radita* (L.) R. Wilczek. *Int J Curr Microbiol Appl Sci*. 3: 799–809.
Gordon, S.A. and Weber, R.P. 1951. Colorimetric Estimation of Indoleacetic Acid. *Plant Physiology.* 26: 192-195. https://doi.org/10.1104/pp.26.1.192.
Jasim, B., John Jimtha, C., Jyothis, M. and Radhakrishnan, E.K. 2014. Plant growth promoting potential of endophytic bacteria isolated from *Piper nigrum*. *Plant Growth Regul*. 71(1) : 1–11.
Karthik, A., Subramanian, G., Mallikarjuna Rao, C., Krishnamurthy Bhat, Ranjithkumar A., Musmade, P., Surulivelrajan, M., Karthikeyan, K. and Udupa, N. 2008. Simultaneous Determination of Pioglitazone and Glimepiride in Bulk Drug and Pharmaceutical Dosage form by Rp-Hplc Method. *Pak. J. Pharm. Sci.* 21 (4) : 421-425.
Khan, M.A., Asaf, S., Khan, A.L., Ullah, I., Ali, S. and Kang, S.M. 2019. Alleviation of salt stress response in soybean plants with the endophytic bacterial isolate *Curtobacterium* sp. SAK1. *Ann Microbiol.* 69(8): 797–808.
Knowles, C. J., 1976. Microorganisms and cyanide. *Bacteriol. Rev.* 40: 652.
Kotasthane, A.S., Agrawal, T., Zaidi, N.W. and Singh, U.S. 2017. Identification of siderophore producing and cynogenic *Fluorescent pseudomonas* and a simple confrontation assay to identify potential bio-control agent for collar rot of chickpea. *Biotech.* 7(2): 137.
Lal, G. Saran, P.L., Ganga Devi, Deepak and Rishi Raj, 2014. Production Technology of Cumin (*Cuminum cyminum* L.). *Advances in Vegetable Agronomy*. 223-231.
Mohite, B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J Soil Sci Plant Nutr*. 13(3): Epub 27.
Mostafa Mohamed, El-Sersawy, Saad El-Din Hassan, Abbas, A. El-Ghamry, Amr Mahmoud Abd El-Gwad, Amr Fouda, 2021. Implication of plant growth-promoting rhizobacteria of *Bacillus* spp. as biocontrol agents against wilt disease caused by Fusarium oxysporum Schlecht. in *Vicia faba* L. *Bio Mol Concepts.* 12: 197–214.
Paul, D. and Sinha, S.N. 2017. Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. *Ann Agrar Sci*. 15(1): 130–136.
Rajni Devi and Richa Thakur, 2018. Screening and identification of bacteria for plant growth promoting traits from termite mound soil. *Journal of Pharmacognosy and Phytochemistry*. 7(2): 1681-1686.
Rani, U. M., Arundhati and Reddy, G. 2012. Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. *African Journal of Biotechnology*. 11 (32): 8085-8091.
Venderleyden and Costacurta, 1995. Synthesis of phytohormones by plant associated bacteria. *Crit Rev Microbiol*. 21: 1-18.
Voisard, C., Keel, C., Haas, D. and De'fago, G. 1989 Cyanide production by Pseudomonas fluorescens suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J.* 8 : 351-358.
Yadav, A. and Yadav, K. 2022. Comparative growth analysis of cumin plants (*Cuminum cyminum* L.) treated with glycerol and talc based phosphate. *Journal of Applied and Natural Science.* 14(4): 1374 - 1386. https:// doi.org/10.31018/jans.v14i4.3859