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Original Research Paper

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SCREENING AND CHARACTERISATION OF SIDEROPHORE PRODUCING RHIZOBACTERIA FROM PADDY FIELD OF POONDI

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ABSTRACT To enhance the crop production and to manage plant diseases, many chemicals are being used. The use of such agrochemicals is hazardous to environment. To identify a viable alternate, biocontrol agent is necessary. Keeping this in point, the present investigation was undertaken to isolate PGPR bacteria from paddy rhizospheric soil. A total of 100 bacteria were isolated. On the basis of morphological, physiological and biochemical characterisation most of the isolates belong to Pseudomonas aeruginosa. The isolates were further screened for siderophores production by CAS method. In this 10 isolates showed positive results in siderophore production. The produced siderophore were further characterised based on their classification such as hydroxylate, catecholate and carboxamate depending on the iron ligating group. In the present study, the effect of culture media components in the yield of siderophore was studied. Among the 10 isolates, 3 of were produced catecholate type of siderophore efficiently in succinate medium at neutral pH in low iron concentration. Potential siderophore producing isolates was further applied on Vigna radiata (Green gram). There was a significant increase in the shoot and root length by roll towel method and Pot assay method.

KEYWORDS : siderophore, iron, CAS assay, *Pseudomonas*, succinate, catecholate.

INTRODUCTION

The variety of microbes that inhabit the earth's surroundings is unparalleled. It is estimated that microbes comprise 50% of the biological carbon and 90% of the biological nitrogen on Earth, respectively, in terms of sheer number and mass (Gupta, *et al*., 2016). It would appear that soil microbial communities are more diverse than those in most freshwater and marine environments. The rhizosphere, which is a very important and active area for root activity and metabolism, surrounds plant roots (Barra Caracciolo and Terenzi, 2021).

The group of bacteria known as *Pseudomonas* is the most diverse and significant to the environment on the planet. The *Pseudomonas aeruginosa* is gram-negative, rod-shaped, chemoheterotrophic bacteria that are distinguished by their yellow-green pigments and polar flagella (Diggle, & Whiteley, 2020). *Pseudomonas aeruginosa* is well-known for its functions as biocontrol agents against numerous soil-borne plant pathogens, phosphate solubilizers, and plant growth promoters (Chopra, *et al*., 2020).

The strains have been classified as rhizobacteria that promote plant growth based on three distinct characteristics. The capacity to produce Sidrophores and auxin are these characteristics. Phosphate solubilisation occurs in the medium (Vejan, *et al*., 2016). Plant Growth Promoting Rhizobacteria (PGPR), which were secrete a variety of plant growth promoting substances and biofertilizers to boost of the plant growth and crop yield. PGPRs either induce systemic resistance against pathogens throughout the plant's lifespan or have antagonistic effects on soil-borne pathogens (Bhattacharyya, and Jha, 2012).

Siderophores are chelating agents that are ferric ion specific and have a relatively low molecular weight. They are made by bacteria, actinomycetes, fungi, and some algae that grow under low ionic stress (Ahmed, and Holmstrom, 2014). There are three primary chemical categories that have been proposed for the iron ligation groups: catecholate, hydroxamate, and hydroxycarboxylates. The type of siderophore that bacteria produce is influenced by the availability and quantity of nutrients, and it may differ in culture-rich conditions from natural habitat (Duncan, *et al*., 2021).

Pseudomonas fluorescence and *Pseudomonas putida* produce pyochelin and pyoverdin, two types of siderophores (Hartney, *et al*., 2013). Pyoverdin are pigments that fluoresce in ultraviolet light and dissolve in water. The ability to produce siderophores not only enhances producer strain rhizosphere colonization but also plays an important role in plant iron nutrition, according to extensive research on siderophores produced by rhizosphere inhabitants (Tank, *et al*., 2012).

MATERIALS AND METHODOLOGY

Sample Collection And Isolation Of Rhizobacteria:-

Collection of soil sample was done from the rhizosphere region of paddy plant located in different places of Poondi, Thiruvallur district of Tamilnadu. Rhizospheric soil sample was collected carefully in sterile condition and then it was stored in ziplock bags for further process. The soil sample was air dried and 1 gm of soil was suspended in 99 ml of sterile distilled water and vortexed. By the method of Serial dilution, 0.1 ml diluted aliquots was spread on the nutrient agar plates as per microbiological methods. After 24 hours incubation, well isolated colonies were aseptically transferred to nutrient medium slant and maintained at 4^{0}C for future use (Majeed *et al*. 2015).

Siderophore Production:-

The 6 μL (10^{6}cells/mL) of each isolate was spotted onto the centre of each chrome azurol sulphonate (CAS) agar plate for the purpose of producing siderophore. Plates were examined for the formation of an orange or yellow halo zone around the colonies 48 hours after the incubation at 30 °C (Kotasthane *et al*., 2017).

Quantification of Siderophore Production

The isolate was grown for 48 hours in a succinate medium at 28 ± 3°C for quantitative estimation of siderophore production. The next step was 15 minutes of centrifugation at 10,000 rpm. Without cell supernatant was tested for how much siderophore as per CAS examine. The following formula was used to calculate the percentage siderophore unit (PSU) (Ghazy and El-Nahrawy 2021). After screening, the potential isolates were characterized by biochemical test

Chemical Characterization of Siderophore

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Various tests were carried out to determine the chemical nature of the iron ligation group of the siderophore produced by the selective bacterial isolates. All tests were carried out using the cell free supernatant of the cultures. The following tests were carried out namely Tetrazolium test (Dave and Dube, 2000), hydroxamate nature (Díaz de Villegas. 2002), Catecholate nature, and Catecholate siderophore (Takase *et al*., 2000; Manwar *et al*., 2004).

Effect of Various Media And Iron Concentration On Siderophore Production

The submerged fermentation was carried out using different media; different iron concentration, different pH, different T^{0} and different amino acids were utilized for optimization process. After incubation period, based on the Louden, *et al*., (2011); Ganesapillai *et al*., (2016) methods were utilized for the siderophore quantification.

Plant Study

Wet Paper Towel Germination Test

Among the isolates potential isolate of *Pseudomonas spp* (SP40) was utilized for the germination test and pot culture test for determination of the effect of isolate on plant growth. Ten selected seeds of *Vigna radiata* (green grams) were taken for Germination test and Pot culture study (Wuytack *et al*., 2003). The potential isolate of cell free supernatant was utilized for the above mention tests. After growth period, observed the length of the root and shoots.

RESULTS

Isolation And Screening Of Siderophore Producer

A total of 10 bacterial isolates were isolated from 2 different areas of paddy rhizosphere soils from Thiruvallur district (Poondi). These isolates were evaluated for their plant growth promoting trait by chromoazurol sulphonate assay plate method. Among the 10 isolates, 3 of were showed positive for the production of siderophore.

Morphological & Biochemical Characteristic Observations Of Pgpr Isolates

The morphological characteristics of PGPR isolates (SP34, SP40, and SP42) varied widely. All the isolates produced large, opaque, flat colonies with irregular edges. Another type of isolate shows smooth shiny surface with greyish-white moist colonies. Microscopic observation was performed to detect the characteristics of potential isolates such as shape, gram staining and motility. All potential isolates contain gram negative cell wall in nature with rod shaped structure. In hanging drop motility test also showed motile organism by the 3 isolates. The bacterial isolates were characterized by morphological & biochemical attributes which is identified as *Pseudomonas aeruginosa* (n=2) & *Escherichia coli* (n=1).

Quantitative Cas Assay

In quantitative CAS assay, produced siderophore units were calculated by the proportion of CAS colour shifted. It was found that 55% of production was showed by *P.aeruginosa* 1, 68% was by *P.aeruginosa* 2 and 40% was showed by *E.coli*.

Characterizations of Siderophore

In this study, all three isolates were subjected to determination for what types of siderophore was produced. Presently all isolates were showed positive for the production of catecholate type of siderophore. The change in the colour from yellow to red on addition of NaOH indicates as positive.

Optimization Process Siderophore Production

Among the 2 media tested, succinate medium was showed better result for the production of siderophore while using *Pseudomonas aeruginosa*-SP40 isolate. While using various concentration of iron tested, siderophore was production with inversely proportional to the concentration of iron while no siderophoregenesis was reported at iron concentration above 25μM by the selected isolate.

In case of different pH tested, better result was observed while using pH-7, while increase or decrease of pH value, siderophore yield was declined. Similarly, 30°C was better for the production of siderophore. While tested the various amino acids, threonine showed better production, especially *Pseudomonas aeruginosa* (SP-40) produced highest production (Fig.1).

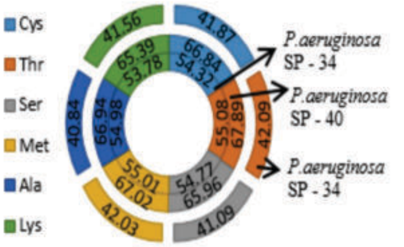

Figure 1: Effect of various amino acids on siderphore production

Plant study method

Wet paper method

From the optimization process, best parameter of pH, T^{0}, and amino acids were selected and utilized for this study, while using *Pseudomonas spp* (SP-40) better results of germination (*Vigna radiata*) was appeared than control seeds. The 21.8cm length of *Vigna radiata*'s shoot and 6.2cm were observed while using *P.aeruginosa* (SP-40). Same time 15.6cm and 3.5cm of shoot and root were observed while using control sample respectively. In case of percentage wise, 70% of seed was germinated with potential isolate of *Pseudomonas spp* (SP-40) and 40% of were observed when using without seed treated sample.

Figure 2: Efficacy of *P.aeruginosa* (SP-40) on seed germination

Pot Culture Method

The siderophore produced by *P. aeruginosa* (SP-40) was used as a growth-promoting agent on *Vigna radiata* is shown in Fig. 3 along with their controls. The treated seeds with and without siderophore were used for the study and observed for the growth of shoot length and root length in centimetres. The plants showed good efficient growth with seeds treated with siderophore produced by *P. aeruginosa* (SP-40) while compared with their corresponding controls. The 22.2cm of shoot and 5.9 cm of shoot and root where observed respectively.

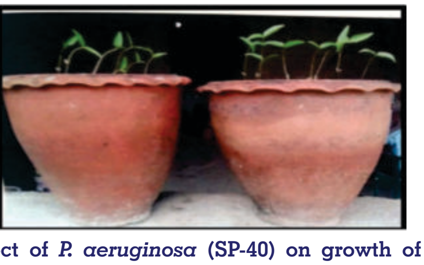

Fig.3 Effect of *P. aeruginosa* (SP-40) on growth of *Vigna radiata* by Pot Culture Method

DISCUSSION

Various groups of microorganisms produce beneficial characters that encourage plant growth. These rhizosphere microorganisms are soil occupant, nonpathogenic ability for plant development advancement. They are utilized in agriculture instead of chemical application due to their ability to improve crops. Plant growth promoting rhizobacteria are free-living bacteria that are isolated from the rhizosphere of plants and interact with microbes in a beneficial way (Saeed, Q *et al*., 2021).

The *Pseudomonas*, in particular, a rhizosphere microorganism, has an exceptional capacity to promote the growth of the host plant through a variety of mechanisms that suppress plant disease, including the production of potent siderophores. The Siderophores can also slow down the breakdown of IAA and reduce oxidative stress by inhibiting the formation of free radicals (Huo *et al*., 2021).

Certain strains of rhizosphere colonizing *Pseudomonas sp*. have gained worldwide attention in recent years due to their ability to promote plant growth effectively. Therefore, in this study, we further explore the existence of indigenous *Pseudomonas sp*. from Poondi, that were subjected to investigate for the plant growth capability.

Pseudomonas aeruginosa and *E.coli* were observed as the isolated bacterium in this study, which were isolated from agricultural soil based on biochemical and subjected to plant-growth-promoting characteristics. From the tested isolates, *P.aeruginosa* and *E.coli* species were showed positive for siderophore. The plant growth-promoting (PGP) traits like hydrogen cyanide (HCN), ammonia, and indole acetic acid (IAA), as well as solubilised phosphate and molecular characterization, have previously been used to document the isolation of *Pseudomonas aeruginosa* from rhizosphere soil from India (Andleeb, S *et al*., 2022). In this study, both isolates were belongs to catecholates types. Many workers around the world have reported many plant growth promoting abilities of rhizobacteria produce catecholates and hydroxamate type, siderophore (Pahari and Mishra, 2017; Patel *et al*., 2017).

Pseudomonas aeruginosa can produce higher yields of siderophores under iron stress conditions, as previously reported, and the qualitative assessment of siderophores production from isolated *Pseudomonas aeruginosa* under culture medium with iron limiting stress and neutral pH was demonstrated as the next step. The release of iron in soil in a bioavailable form that can be readily taken up by plants depends on the redox potential and soil pH. In soils, an oxidizing environment with high pH predominates, allowing the synthesis of Fe oxides and reducing its bioavailability. The effect of pH on siderophore production in strains SID 30 and SX9 was recently investigated by Wang *et al*. (2021) and Reddy Kiran Kalyan *et al*. (2022), and pH values of 7–8 were determined to be optimal for siderophore formation.

In the present study, potential isolate of *Pseudomonas aeruginosa* SP40 was utilized for the determination of efficacy on plant growth. The seeds treated with and without siderophore were used for the study, and shoot length and root length growth in centimetres were observed and seed germination rate was recorded in percentage. Sahu and Sindhu (2011) also reported similar results of enhanced siderophore production when supplemented with succinate. Patel *et al*., 2017, also observed the better growth of *Vigna radiate* with siderophore producing Pseudomonas sp.

CONCLUSIONS

The present investigation findings point to SP40 potential as a PGPR for enhancing *Vigna radiate* growth parameters and soil nutrients. For *Vigna radiate* farming, this PGPR strain can be used as effective bioinoculants. To conclude this study, the siderophore producing *Pseudomonas aeruginosa* (SP40) was used as a bioformulation treatment for seeds before was sowed were found to be very effective in plant growth promotion and seed germination of *Vigna radiate*. However, in order to guarantee the isolate's bioefficacy, field trials in various agroclimatic zones are required.

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