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RESEARCH ARTICLE
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EFFECTS OF GROWTH CONDITIONS ON SIDEROPHORE PRODUCING BACTERIA AND SIDEROPHORE PRODUCTION ISOLATED FROM AGRONOMIC FIELD OF DIFFERENT ZONES OF BILASPUR DISTRICT OF CHHATISHGARH

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Abstarct- Siderophores are synthesized and secreted by many bacteria, yeasts, fungi, and plants for Fe (III) chelation. Eight microbial isolates were tested for their ability to produce siderophores, with Pseudomonas flurescens showing the highest production at 72%, followed by Azospirillum lipoferum at 63%. Microbial isolates producing siderophore significantly improve nodulation in green gram, offering ecological advantages and encouraging their use as inoculants with root nodule bacteria. The amount of total iron (10–17 M) in the soil is toolow to support microbial growth (10–7 M); siderophoresthat bind to Fe3+, transport it back to microbial cells and make it available for microbial growth (Meyer, 2000). Within the graminaceous plants, maize is also classified as strategy II plant that acquires iron by the release of phytosiderophores in the rhizosphere coupled to induction of high affinity system for iron-phytosiderophore complex that transports it across the plasma membrane of root cells (Marschner and Roemheld, 1994)

Introduction

Iron is necessary for practically all forms of life since it is involved in numerous metabolic processes in cells. Cofactor iron is involved in transcriptional control, biodegradation, cellular respiration, DNA synthesis and repair, and metabolic pathways. Iron is also essential for porphyrins to function as plants' photosynthetic apparatus. It has been observed that cells may be poisoned by free ferric iron. As a result of the Fe2+'s ability to engage in the "FentonHaber Weiss reaction," harmful free radicals are created. To avoid harmful effects, organisms including bacteria, plants, animals, and human bodies need to maintain a specific concentration of iron. However, iron shortage is a more frequent occurrence in plants. Because of its low solubility and restricted absorption, iron has a low bioavailability for plants. Because iron readily forms low solubility ferric hydroxides at neutral pH, iron's solubility and reactivity are primarily dependent on pH. Plants that are iron deficient will produce less chlorophyll and develop the plant disease chlorosis, which will reduce biomass and yield or perhaps kill the plant. Numerous earlier studies suggested that iron deficiency was a significant worldwide agricultural issue.

Bacterial Siderophore as Iron Chelator

Because there is less fert in the soil, nature has given bacteria a clear absorption strategy through the formation of siderophores. Greek for "iron carriers," siderophores are low molecular weight (500–100 dt), ferric ion-specific chelating agents that are produced by a variety of bacteria, including Rhizobium, Bacillus, Serratia, Azotobacter, Bacillus, and Enterobacter. In addition to obtaining iron from their surroundings to produce a mineral that is vital and available to microorganisms,

siderophores also combine with other metals in the environment to generate complexes that improve the availability of these elements to microbial cells, such as molybdenum, manganese, cobalt, and nickel.

Types of Siderophore

The three primary groups of siderophores that the oxygen ligands for Fe3+ organization distinguish between are hydroxamates, catecholates, and carboxylates.

1. Hydroxymate Type of Siderophore
2. Catecholate Type of Siderophore
3. Carboxylate Type of Siderophore

Mechanism of Siderophore Mediate Iron Transport

Many enzymes, including peroxidases, catalases, and superoxide dismutases (SOD), depend on iron as a critical component. Thus, a low iron level impairs not only the development and metabolism of cells but also weakens their defenses against oxidative damage. For the intake and storage of iron, all organisms need well-designed, controlled mechanisms. One element of a multi-component system that delivers ferric iron into a cell is the siderophore. When the siderophore is released into the environment, it first forms a strong bond with iron (Fe+3). Then, via certain siderophore receptors, the siderophore-iron complex is transported through the cell membrane and into the inside of the cell. distinct bacteria have distinct kinds of siderophore-specific receptors. Fec A and Fep A receptor proteins are found in the outer membrane, while the Ton B-Exb B-Exb D protein complex is found in the inner membrane.

Application of Siderophore-Producing Bacteria

Siderophore as a Plant Growth Promoter- Siderophores are a safer, more environmentally friendly substitute for chemical pesticides that are employed in several agricultural industries. According to Briat et al. (1995) and Schenk et al. (2012), iron is a micronutrient that is necessary for several critical physiological processes, including the biosynthesis of chlorophyll and redox reactions in plants. Thus, the quantity and quality of crop production are greatly reduced in conditions of iron deficiency. Nonetheless, a number of researchers have looked into the interactions between siderophores and microbes in the presence of iron deficiency.

Siderophore as Potential Biocontrol Agent- Bacteria that produce siderophores are crucial to the biological control of several phytopathogens. The siderophore that bacteria create binds strongly to iron, rendering it inaccessible to plant pathogens and preventing phytopathogen proliferation (Beneduzi et al. 2012; Ahmed and Holmstrom 2014). This is particularly true for common biocontrol agents as Bacillus species and Pseudomonas (Beneduzi et al. 2012).

Application of Siderophore as Bioremediator- Due to the fast buildup of heavy metals and metalloids from the petrochemical sector in soil, there has been a lot of research done on the possible application of siderophores in metal bioremediation over time.

Material and Method Microbial strains and culture conditions

Biofertilizers provided the laboratory stock cultures of Rhizobium phaseoli, Pseudomonas fluorescens, Pseudomonas striata, Bacillus subtilis, Bacillus polymyxa, Bacillus megaterium, Azotobacter chroococcum, Azospirillum lipoferum, and a few more species.

Detection of siderophore

The synthesis of siderophores by microorganisms that promote plant development was subjected to qualitative testing using both Chrome Azural S (CAS) liquid and plate assay methods. The strains were distributed over CAS agar plates and let to incubate at 28°C for 48 hours. Following incubation,

a thin layer of CAS reagent in 0.6% agar was applied to the bacterial growth, and plates were then incubated for a further 24 hours at 28ºC. The production of siderophores was indicated by the formation of a yellow-orange color zone around the colonies in the plate assay and a blue-to-orange color shift in the liquid assay (Schwyn and Neilands, 1987).

Estimation of siderophore

CAS-shuttle test was used to quantify the siderophore generated by various plant growthpromoting bacteria. Both strains were cultivated on CAS agar medium and incubated separately for 24–30 hours at 28 °C with continuous shaking at 120 rpm on a shaking incubator. Every 20 minutes throughout the incubation period, 5 ml broths were centrifuged at 10,000 rpm for 10 minutes at 4 oC in a cooling centrifuge, and the cell-free supernatant was combined with 0.5 ml of CAS solution. The resultant color was measured at 630 nm using a spectrophotometer with a reference that contained 0.5 ml of CAS solution and 0.5 ml of uninoculated succinate medium. Using the formula % Siderophore units = [(Ar - As)/Ar] x 100, where Ar is the absorbance at 630 nm of the reference (CAS assay solution + uninoculated media) and As is the absorbance at 630 nm of the sample (CAS assay solution + supernatant), the percentage of siderophore units was calculated as the proportion of CAS color shifted.

Field experiment

An experimental farm was the site of a field experiment. Ten treatments, three replications, and a randomized block design were used in the setup of the experiment.

Statistical analysis

Statistical Methods for Agricultural Workers, the data from the field experiment was collected using a completely randomized design. When needed, appropriate critical differences (C.D.) and standard errors (S.E.) at the 5% level were calculated and applied to the interpretation of the results. seven isolates tested positive for the disease (Table 1). On the CAS assay, it was evident that every positive isolate produced a siderophore. On CAS agar plates, Rhizobium phaseoli do not exhibit growth. The CAS agar test yielded positive results for Pseudomonas flurescens, Azotobacter chroococcum, Pseudomonas striata, Bacillus subtilis, Bacillus polymyxa, Bacillus megaterium, and Azospirillum lipoferum.CAS quantitative assay.

In quantitative CAS assay- The percentage of CAS color altered was estimated using percent siderophore units. The highest amount of siderophore was produced by Pseudomonas flurescens (72%), followed by Bacillus megaterium (39%), Bacillus subtilis (60%), Azospirillum lipoferum (63%), and Pseudomonas striata (53%). The same pattern was seen in the qualitative and quantitative identification of siderophores made by various bacteria that promote plant development.

Table 1: Siderophoregenesis by plant growth promoting organism

Sr. No	Microbial inoculants	CAS Agar Test	% Siderophore
1	Rhizobium phaseoli	-	0
2	zotobacter chroococcum	+	32
3	Pseudomonas striata	+	53
4	Bacillus subtilis	+	60
5	Bacillus polymyxa	+	8
6	Bacillus megaterium	+	39
7	Pseudomonas flurescens	+	72
8	Azospirillum lipoferum	+	63

When the percentage of CAS color shifted, the amount of siderophore produced by both Pseudomonas sps. was estimated as a percentage of siderophore units. Azospirillum lipoferum and Pseudomonas

fluorescens have demonstrated the highest siderophore yields by liquid CAS testing, with 72% and 63% siderophore units, respectively (Bholay et al. 2012). Sadat, Mansoureh, and others (2012). According to reports, Pseudomonas fluorescens is a significant component of rhizobacteria, which promote plant growth in a variety of ways. Twenty Pseudomonad strains were isolated from Malaysian paddy areas' rhizosphere soils for this study, and they were evaluated for their ability to promote plant growth. Out of the 20 antagonist bacterial strains examined, 15 strains (72%) produced the plant growth-promoting hormone IAA, while all 20 tested isolates of pseudomonads were positive for siderophores and HCN.

Nodule attributes

The data presented in Table 2 regarding the characteristics of green gram nodules show that siderophore-producing bacteria and Rhizobium phaseoli have a major impact on green gram nodulation.

Table 2: Effect of siderophore producing microorganisms on nodulation attributes in green gram

Sr. No	Treatments	No. of nodules plant^{-1}	Nodule fresh wt plant^{-1} (mg)	Nodule Dry wt plant^{-1} (mg)
T_1	Absolute control	12.63	18.00	7.57
T_2	Only RDF	19.63	33.00	12.00
T_3	RDF+Rhizobium phaseoli	24.00	46.33	20.00
T_4	T3 + Pseudomonas flurescens	36.00	73.67	35.33
T_5	T3+ Pseudomonas striata	29.00	40.59	19.57
T_6	T3+ Bacillus subtilis	21.31	50.57	18.23
T_7	T3+Bacillus polymyxa	24.66	50.55	29.00
T_8	T3+Bacillus megaterium	23.66	39.00	19.00
T_9	T3+ Azotobacter chroococcum	29.00	39.00	29.57
T_{10}	T3+ Azospirillum lipoferum	27.33	45.50	28.53
	S.Em.±	1.49	47.00	25.00
	C.D. at 5 %	3.79	6.74	4.17
	C.V. %	9.56	10.25	11.80

Microbial inoculants influenced the number of nodules which ranges from 12.63 to 29.00 per plant showing significantly higher number of nodules in RDF + Rhizobium phaseoli + Pseudomonas flurescens (T4) treated plots followed by RDF + Rhizobium phaseoli + Pseudomonas striata (T5) and RDF + Rhizobium phaseoli + Azotobacter chroococcum (T3). Whereas, significantly lower number of nodules per plot were noted in absolute control. Microbial inoculants influence the nodule fresh weight and nodule dry weight which sowing significantly higher fresh weight of nodules and nodule dry weight in RDF + Rhizobium phaseoli + Pseudomonas flurescens (T4) treated plots Whereas, significantly lower fresh weight of nodules per plant was noted in absolute control. The increase in number of nodules, fresh weight and dry weight of nodules per plant with siderophore producing microorganisms along with Rhizobium phaseoli might be a result of more iron availability in nodulating period of green gram which might have enhanced nodulation process. Earlier report shows that the treatment of Bradyrhizobium (mung bean) USDA 3447 + P. chrysogenium exhibited an increase in nodule number and nodules activity in mung bean as reported by Mahmoud and Abd-alla (2001)

Conclusion

Eight microbial isolates that promote plant growth were examined for their ability to produce siderophores. It was discovered that only seven produced more siderophores. On CAS agar plates, Rhizobium phaseoli do not exhibit growth. The highest percentage of siderophores were produced by Pseudomonas flurescens (72%), followed by Azospirillum lipoferum (63%). Therefore, Pseudomonas fluorescens was able to resolve the main issue in the current investigation, which was the negative impacts of chemical fertilizers on plant growth and productivity. Consequently, a biological platform was developed to address this issue. Extracellular water soluble is produced by Pseudomonas fluorescens. Siderophore, which has been shown to improve nodulation in green grams, both fresh and dry nodule weight

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