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ISOLATION AND SCREENING OF PHYTOHARMONE PRODUCING PLANT GROWTH PROMOTING RHIZOBACTERIA FROM THE TURMERIC (*CURCUMA LONGA*) PLANT.

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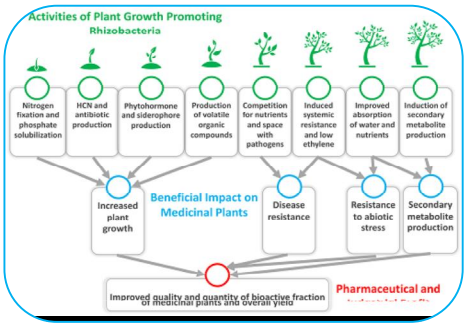
ABSTRACT



Plant growth promoting rhizobacteria are bacteria colonizes rhizome and roots of turmeric plants were isolated and screened for the phytoharmone production. In the present study rhizobacteria were isolated from the four different farms surrounding Sengaon tehsil Sengaon dist. Hingoli Maharashtra. The isolation of the rhizobacteria from turmeric region were done by using Luria Bertani (LB agar) and Nutrient agar. The total 39 isolates were isolated on the basis of colony morphology and characteristics. The all 39 isolates were screened for the production of Indole acetic acid (IAA), phosphate solubilization and siderophore production. For IAA production the isolates were grown in nutrient broth with 1% L-tryptophan, and tested by using Salkowskis reagent, the phosphate solubilization were done on Pikovaskayas agar medium and siderophore on CAS agar plates. Out of total 39 isolates, 13 isolates were shows positive for the IAA production, 9 isolates for the phosphate solubilization and 4 isolates for the siderophore production. Among them the isolates number TS2 shows highest number of IAA producer, TS10 shows highest phosphate solubilization and TS4 shows highest Siderophore production. Hence due to these results it was concluded that the PGPR are responsible for the growth and promotion of the turmeric crop.

KEYWORDS : *plant growth Promoting Bacteria, Indole acetic acid, Siderophore, phosphate solubilizing bacteria.*

INTRODUCTION:

India contributed 90 % of the total global production of turmeric (Curcuma Longa L.) and shares around 180 thousand hectares of turmeric cultivation with a total production of 702 thousand tones and gradually increasing. It also occurs in China, Iran, Sri Lanka Peru, and Pakistan. Among India, Maharashtra state is the sixth rank in an area under turmeric cultivation. Turmeric is a rhizomatous, perennial herbaceous plant extensively used in the Ayurveda, Unani, and Siddha. There are lots of medicinal uses of turmeric routinely practiced to cure the diseases like asthma, bronchial hyperactivity, rheumatism, diabetic wounds, sinusitis, smallpox, skin cancer, urinary tract infection, jaundice, menstrual problems liver ailments(A. Kumar et al., 2017).

Curcumin ((1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also called diferuloylmethane) is the main ingredient in turmeric which is the polyphenol which has a target for multiple signaling molecules. It has antioxidant and anti-inflammatory properties, used for arthritis also.(Hewlings & Kalman, 2017). Although there are lots of benefits to cultivating turmeric like commercial and health effects, the use of extensive chemicals to increase productivity, farmers are deteriorating the soil quality, groundwater quality, and air and human and animal health and hence the sustained health of the soil is important.

Plant growth promoting rhizobacteria are bacteria colonize roots and overcome the soil borne pathogens at the root surface.(Dasgupta, Ghati, Sarkar, Sengupta, & Paul, 2015). Rhizobacteria responsible for the plant growth and promotion is known as plant growth promoting rhizobacteria (PGPR), helps in the plant growth regulator production, nutrient fixation by production of harmones like siderophhore, abiotic stress tolerance, production of volatile substance, antifungal compounds protect from the plant fungal pathogens. Plant growth harmones include Auxin, cytokinin, siderophores, Gibbrellic acid, phosphate solubiization, HCN production etc. (V. Kumar & Sharma, 2017). Hence overdependence of the chemical fertilizers leads to encouragement of chemical fertilizer industry but it is harmfull to the soil as well as human health, and need to find alternative sources i.e. biofertilizers. Microbial inoculum or biofertilizers acted as promising biofertilizers.(Fasusi, Cruz, & Babalola, 2021).

. The phytoharmone produced by PGPR are responsible for the plant growth and promotion and significantly increases number of leaves, stem height, stem and rhizome weight of *curcuma longa L.* (A. Kumar et al., 2016). Due to increase in demand for the turmeric crops farmers uses excessive chemical fertilizers which leads to soil deterioration and human health affect and nutritional stress(Buckner et al., 2016). Hence there is need to sustainable agricultural practices by using organic farming or by using organic fertilizers along with microbial inoculation, because plant growth promoting rhizobacteria have a potential to increase biomass production of plants by secreting an growth hormone and provide accumulation of essential nutrients(Ashraf, Asif, Zaheer, Malik, & Ali, 2013).

MATERIALS AND METHODS:

Isolation of PGPR from rhizospheric soil.

The isolation of plant growth promoting rhizobacteria were done in two phases first by collecting soil sample and another is the isolation of PGPR from turmeric plants.

Collection of soil sample from the area around the Sengaon region:

Turmeric plants rhizospheric soil sample were collected from areas around the Sengaon region from four different villages(Tiwari, Devi, Singh, & Sharma, 2016). Sample collected according to standard protocol, the selected farm were marked in four region, from each region of farm one sample were collected. The rhizomes along with soil were collected by removing bulk soil above the surface and digging up to 0- 15 cm depth with the help of augur (spade) and rod (Vinayarani & Prakash, 2018). The extra soil was removed by shaking and collected in sterile polythene bags labeled properly.

a. Isolation of bacteria species:

The rhizospheric bacteria were isolated from rhizospheric soil by inoculating 10 gm of soil in 100 ml of sterile saline water and shaken for ten minutes. The isolation was done by using serial dilution method. 1 ml of this soil-saline suspension sample were transferred into 9 ml of sterile saline test tubes, and serial diluted up to 10^{-9}(A. Kumar et al., 2016). About 0.1 ml of each dilution from each tube were spread on nutrient agar and Luria Bertani agar plates supplemented with 50 µg/ml of Nystatin and 50 µg/ml cycloheximide and incubated for 72 hrs, at 27$\pm$ 1^0c (Damam, Moinuddin, & Kausar, 2016).

After incubation plates were observed for the growth and number of bacterial colonies. The percentage of Occurrence calculated for each isolate(Richard, 2020). The bacterial colonies which shows different morphological characteristics on agar plates were selected and maintained on nutrient agar slants.

1) Screening of isolates for the phytoharmone Production:

i. Indole acetic acid Production (IAA):

All isolates were screened for Indole acetic acid production by using salkowskis reagent method. The isolates were grown in 50 ml nutrient broth supplemented with 0.1 % L tryptophan for 24-48 hrs at 300C, the uninoculated sample kept as blank for reference.(Albdaiwi, Khyami-Horani, Ayad, Alananbeh, & Al-Sayaydeh, 2020) (Tiwari et al., 2016). After incubation the cultures were centrifuged at 10000 rpm for 10 min. the estimation of Indole acetic acid production were done using Salkowskis reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) in this 1 ml of supernatant is mixed with 2 ml of salkowskis reagent and incubated at room temperature. Pink color formation were selected as positive and quantification of production were determined by using an absorbance or optical density at 530 nm in UV/Vis spectrophotometer the estimation were done by plotting on standard graph (Anwar, Ali, & Sajid, 2016).

ii. Phosphate solubilization test:

The isolates were screened for the phosphate solubilization by using pikovaskayas medium(Tiwari et al., 2016). The loopfull of colonies or isolates were spot inoculated in the centre of pikovaskaya agar plates (PKV) containing with insoluble tricalcium phosphate and the incubated for 7 days at 280C. The occurrence of clear halo zone around the colony after incubation indicates positive for the phosphate solubilization (Lelapalli, Baskar, Jacob, & Paranthaman, 2021). The phosphate solubilization index (PSI) were calculated as per below

$PSI = (A+B)/B$

Where A= total diameter of the colony with halo zone.

B = Diameter of the colony(Wang et al., 2022).

iii. Siderophore Production:

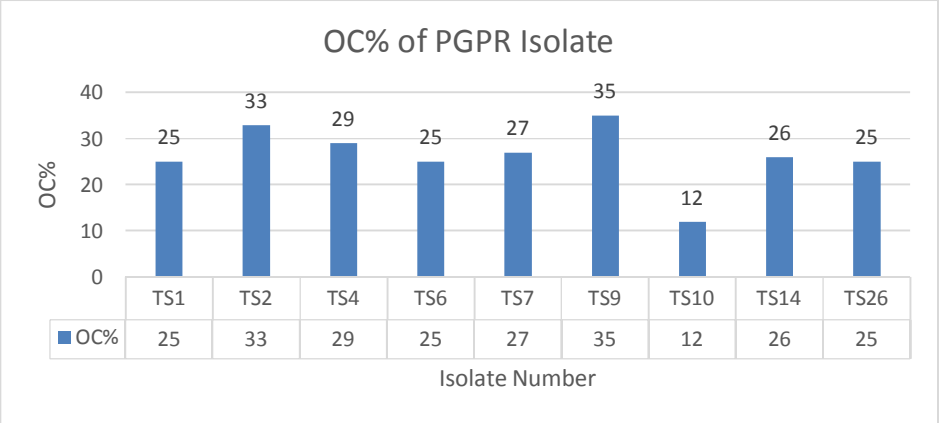
Siderophore production screening takes place by using Chrome Azural Sulfonate test. The isolates were spotted on the CAS agar plates and incubated for 48h. Observe for the color change. The CAS agar were prepared by the method given by Schwyn And Nielands(Srimathi & Suji, 2019). Aseptically the media was poured into plates(Srimathi & Suji, 2019). Spot inoculated the isolates into plates of CAS Agar and incubated for 48 hr. The color changing from blue to halo orange was considered as positive for the Siderophore production.

RESULTS AND OBSERVATION:

In Present study PGPR were isolated from the farms around the Sengaon region from turmeric rhizome and illustrated for the phytoharmone production.

Calculations of Percentage of Occurrence:

Total 39 isolates were isolated from four region of Sengaon and Designated as TS1, TS2,TS3, TS4,….to TS39 out of this isolate number TS1 to 15 having good percentage of occurrence (OC%). Ranging from 4% to 35%. Out of the total bacterial isolate number TS1, TS2 and TS4, TS5, TS6, TS7, TS09, TS26, TS14, having high OC% percentage. The occurrence percentage graphically presented as below.

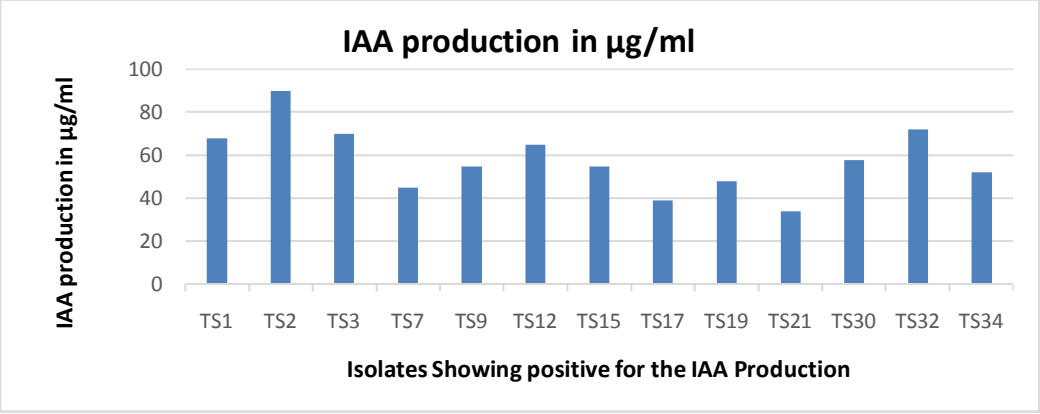


Graph1 shows the percentage of Occurrence of isolates, the percentage of isolates. Out of the total bacterial isolate number TS1, TS2 and TS4, TS5, TS6, TS7, TS09, TS26, TS14, having high OC% percentage.

The occurrence percentage were calculated as given below:
Occurrence percentage (OC %) were calculated as per
Occurrence percentage OC% = $Fc \times \frac{100}{N}$
Where Fc = frequency of occurrence of each isolate
N = total number of isolates.

1. **Screening for phytoharmone production:** All the 39 isolates were screened for the presence of phytoharmone production like Indole acetic acid, phosphate solubilization and Siderophore production.

i) **IAA production:** Indole acetic acid is a phytoharmone crucial for the plant growth and promotion. IAA majorly reported that it is productive phytoharmone produced by plant growth promoting rhizobacteria associated with plant roots. In the present study total 13 isolates shows positive results for the production of IAA in response to 0.1% L-Tryptophan. The overnight culture production of IAA is among the range of 34 to 90 µg/ ml. out of these TS2 shows maximum production and TS 21 shows lowest production. The below table shows the positive isolate with production concentration of IAA in µg/ml. The isolate number TS2 showing highest IAA production while TS21 showing lowest level of IAA production.

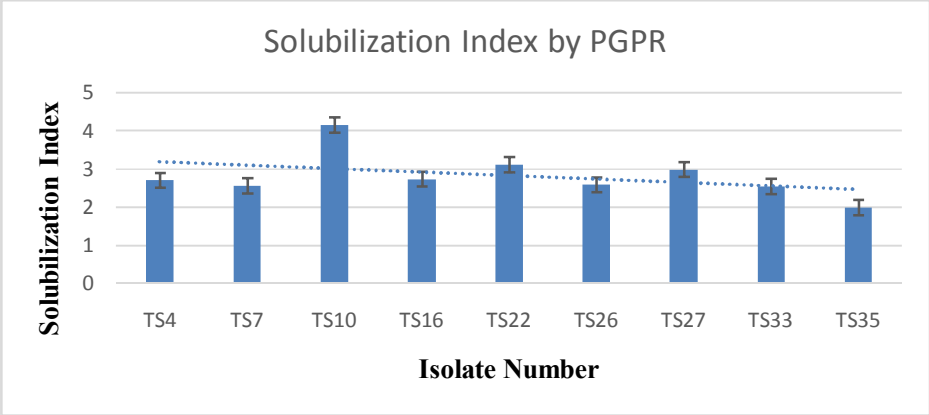


Graph 2 the graph Shows Production of IAA by PGPR Isolates.

The isolates Number TS2 shows the maximum level of IAA Production

The above chart showing the production of the production of IAA by different PGPR isolates. It present the isolate number TS2 showing highest number of IAA production and Isolate TS21 shows lowest amount of IAA production.

ii) Phosphate Solubilization: All isolates were examined for the phosphate solubilization ability by spot inoculating on pikovaskaya (PKV) agar Plates. The clear zone around the colony were indicates a positive for the solubilization of tricalcium phosphate in the PKV medium. Out of all 39 isolates, 9 isolates shows positive for the solubilization of phosphate. Potency of isolate for the solubilization for every positive isolate were determined using solubilization index in which diameter of zone of clearance minus diameter of colony measured. The graphical representation of Phosphate solubilization were presented as per below,



Graph 3: Shows the Phosphate solubilization Index.

The isolate number TS10 shows the maximum level of Phosphate solubilization

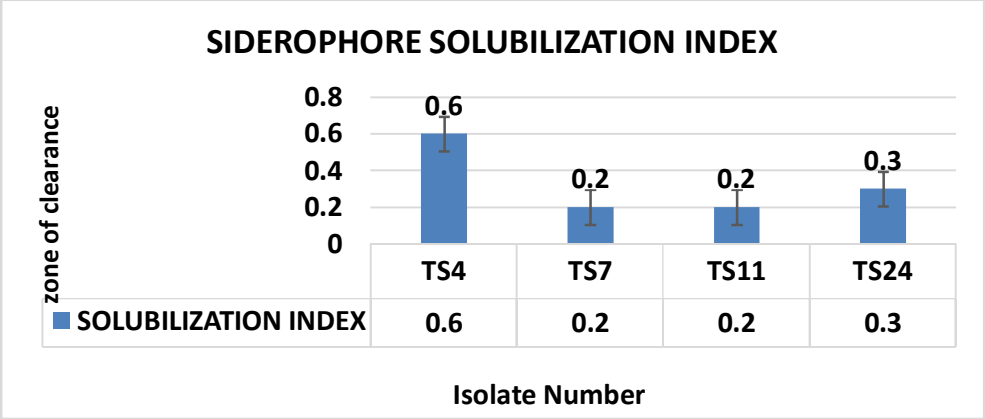
The graph shows the maximum solubilization of Phosphate was done by the isolate number TS10 which gives a solubilization index of approximately 4.15 mm.

iii) Siderophore production:

Siderophore having low molecular weight chelates the iron to avail it for the plants absorption. The isolates were screened for the Siderophore production using the CAS reagents, and found four isolates showing better halo zone of clearance. The solubilization index was calculated by:

Solubilization Index: A-B
Where,
A = zone clearance with Colony diameter in (mm),
B = Colony Diameter in (mm)

There zone of clearance size is given as below, the isolate number TS4 shows the better activity for the production of siderophore. Graphical representation of siderophore production by PGPR isolates.



Graph 4: The graph shows that the Isolate number TS4 shows the better

Activity For the production of Siderophore.

From the above graph it is clear that the isolate TS4 is good siderophore producers than other positive isolates.

DISCUSSION:

Turmeric (C*urcuma longa*) is the most ancient rhizomatous herb of family zingeberaceae spice commonly used in the household kitchen, medicine as anti-inflammatory, antimicrobial and anti-cancerous. The turmeric plant and microbes in the soil interacts and forms a symbiotic mutual association between them PGPR produces plant growth promoting phytoharmone like, Indole acetic acid, gibberellins, ammonia production, Hydrogen cyanide production, phosphate solubilization activity, siderophore production activity, nitrogen fixing ability. In this study total four sample were collected under the study from four region different region of the Sengaon District Hingoli. The soil sample were used for the isolation of the rhizobacteria and were identified on the base of colony morphology and characters.

The total 39 isolates was assessed for the production of phytoharmone and it was found that the 13 isolates shows positive for Indole acetic acid, 9 isolates shows phosphate solubilization and 4 isolates shows the positive for the siderophore production. All these positive isolates were examined for the efficiency for production of phytoharmone. Study shows that the isolates number TS2 shows the higher production of IAA i.e. 90 µg/ml, isolate TS10 shows the maximum number of phosphate solubilization activity 4.15mm in size, and TS4 shows the higher siderophore production through solubilization index i.e. 0.6 cm. It is also found that the other isolates also produces same hormone but in nearby quantity. Hence the PGPR are responsible for the plant growth and promotion. From observation and results it is concluded that the plant growth promoting rhizobacteria are responsible for the production of phytoharmone and plant growth and promotion.

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