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SCREENING OF RHIZOMICROFLORA FROM THE RHIZOSPHERE OF *CURCUMA LONGA L* FOR THEIR PLANT GROWTH PROMOTING ACTIVITIES

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ABSTRACT

Plant growth promoting rhizobacteria commonly inhabit rhizosphere of plants and enhance plant growth by exerting beneficial effects through production and release of metabolites. *Curcuma longa L* is one of important medicinal plant. Useful in boils, bruises, chronic bronchitis, cold cough and coryza, eosinophilia, liver and skin diseases, swellings and wounds of all kinds and dental problems. It Contains curcumin, an alkaloid and an essential oil. The rhizome underground portion of *Curcuma longa L* is traditionally used as antibacterial substance. Antibacterial substances from plant sources diffuse into surrounding soil area of the plant and inhibit growth of some microorganisms. Among these some microorganisms may produce plant growth promoting substances. Present study is intended to isolate microorganisms from rhizosphere of *Curcuma longa L.* In the present study total 10 bacterial and 11 actinomycetes isolates were obtained from the rhizosphere soil of *Curcuma longa L* from Solapur region. These isolates were identified on the basis of morphological, cultural and biochemical characters. All the isolates were screened for their enzymatic potential and plant growth promoting activities (PGP) viz. NH_3, HCN, siderophore and IAA production and PO_4 solubilization. The results showed that not all isolates possessed all 5 PGP activities. The percentage of NH_3, HCN, siderophore and IAA production and PO_4 solubilization for bacterial isolates were 100, 70, 30, 100 and 70% respectively and that for actinomycetes isolates were 63.3, 36.36, 36.3, 98.9 and 36.3% respectively. Hence these isolates can be used as sources for plant growth promoting substances and hence are agriculturally important.

KEYWORDS

PGP, Rhizosphere, *Curcuma longa L* and Medicinal plants.

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INTRODUCTON

Plant rhizosphere soil is a unique biological niche with a diverse microflora of bacteria, fungi, protozoa and algae and these microorganisms have played significant role in nutrition[1]. India is a natural, invaluable storehouse of medicinal plant diversity of great importance for human beings[2].

Plant growth promoting rhizomicroflora commonly inhabit rhizosphere of plants and enhance plant growth by exerting beneficial effects through production and release of metabolites[3] (Malleswari *et al*, 2010). Many different traits of these bacteria are responsible for growth promotion activities. It includes ability to produce plant hormones like IAA, gibberellic acid, cytokinins. Dissolve phosphates and other nutrients and also suppress growth of deleterious microorganisms by production of siderophore, chitinase, antibiotics and cyanide.

Curcuma longa L is one of important medicinal plant. It contains curcumin, an alkaloid and an essential oil. It is useful in boils, bruises, chronic bronchitis, cold cough and coryza, eosinophilia, liver and skin diseases, swellings and wounds of all kinds and dental problems. The rhizome underground portion of *Curcuma longa L* is traditionally used as antibacterial substance. Antibacterial substances from plant sources diffuse into surrounding soil area of the plant and inhibit growth of some microorganisms. Among these some microorganisms may produce plant growth promoting substances. The microorganism which are resistant to these substances only grow in this area[4].

In the present study bacterial and actinomycetes isolates were obtained from the rhizosphere of *Curcuma longa* L from Solapur region and all isolates were screened for their enzymatic potential andplant growth promoting activities viz. NH_3, HCN, siderophore and IAA production and Po_4 solubilization.

MATERIAL AND METHODS

A total of 10 bacterial and 11 actinomycetes isolates were obtained from the rhizospheric soil samples of *Curcuma longa L* from different places of Solapur region State Maharashtra.

The bacterial isolates were characterized and identified on the basis of their morphological, cultural and biochemical characteristics. All bacterial isolates were screened for their variousenzymatic activities viz. Amylase, caseinase, catalase, oxidase and urease.

Actinomycete isolates were identified on the basis of morphological, cultural and biochemical studies. The isolates were identified by using Bergey's manual of systematic Bacteriology Vol-4, Micro-IS software[5]. In order to study different enzymatic activities, the actinomycete isolates were spot or streak inoculated on suitable solid nutrient media. Enzymatic activities selected for the present study were amylase, caseinase, catalase, lipase, lecithinase, gelatinase, nitrate reductase, tyrosinase, urease and chitinase activity. All bacterial and actinomycetes isolates were tested for various plant growth promoting (PGP) activities viz. NH_3 production, HCN production, siderophore and IAA production and phosphate solubilization.

NH_3 production

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10ml peptone water in each tube and incubated for 48-72 hours at 28 ± 2^{o}C. Nesseler's reagent (0.5ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production. (Cappuccino and Sharman, 1992)[6].

HCN production

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lock (1948)[7]. Nutrient agar was amended with 4.4gm glycine per liter and bacteria were streaked on modified agar plate. A Whatman filter paper No.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of the plate and plates were sealed with paraffin wax and incubated at 28± 2^{0}Cfor 4 days. Development of orange to red colour indicated HCN production.

IAA Production

Each isolate was inoculated to 5ml. of nutrient broth containing 1mg/ml concentration of tryptophan and incubated for 72 hrs at 28 ± 2^{o}C. After 3 days the broth was centrifuged at 3000rpm for 30 minutes. The cell free supernatant was collected and used for detection of IAA production.

One ml supernatant was mixed with 2ml of Salkowski reagent (2ml of 0.5M $FeCl_3$ + 98ml 35% $HClO_4$) and tubes were incubated. Development of pink colour indicated IAA production.

Phosphate Solubilization

The isolates were streaked on Pikovskaya's agar plates individually to examine their ability to solubilize Tri calcium phosphate. The isolates showing clear zone of solubilization around growth indicated PO_4 solubilization.

RESULTS AND DISCUSSION

The bacterial isolates were identified by using PIBWin software (Bryant, 2004)[5]. Some bacterial isolates were identified on the basis of morphological, cultural and biochemical characteristics. Bergey's manual of determinative bacteriology was used as a reference to identify the isolates.

The actinomycetes isolates were identified based on morphological, cultural, biochemical characteristics and spore chain morphology. The isolates were further identified by using PIBWin software (Bryant, 2004). Bergey's manual of determinative bacteriology was used as a reference to identify the isolates[8].

Enzymatic activity

Total 10 bacterial isolates obtained from rhizospheric soil were tested for their enzymatic potential. Results of enzymatic activity shows thatthe percentage of isolates showing activity of enzymes viz. amylase, caseinase, catalase, oxidase and urease were reported as 100, 70, 80, 90 and 100% respectively. Figure No.1.

The actinomycetes isolates obtained from the rhizosphere of *Curcuma longa* were also tested for their enzymatic potential. Percentage of isolates showing activity of enzymes viz. amylase, caseinase, chitinase, urease, lipase, lecithinase, nitrate reductase and tyrosinase were reported as 45.4, 45.5, 36.36, 81.8, 45.45, 100 and 27.27% respectively Figure No.2.

Plant Growth Promoting Rhizomicroflora

All the bacterial and actinomycete isolates were tested for various plant growth-promoting activities like NH_3, HCN, siderophore and IAA production and phosphate solubilization.

The results showed different levels of plant growth promoting activities. All the 10 bacterial isolates except CL 2, CL6 and CL7 did not show all 5 plant growth promoting activities. The percentage of NH_3, HCN, siderophore and IAA production and PO_4 solubilization for bacterial isolates were 100, 70, 30, 100 and 70% respectively (Figure No.3). The percentage of NH_3, HCN, siderophore and IAA production and PO_4 solubilization for actinomycetes isolates were 63.3, 36.36, 36.3, 98.9 and 36.3% respectively (Figure No.4)

Quantification of IAA produced was also carried out colorimetrically. The results are presented in Table No.3 and amount of IAA produced was calculated from standard graph. The range of IAA produced by the bacterial isolates was 10.42-35.83µg /ml and that of actinomycetes was 2.978-35.837µg/ml.

Sutthinan Khamna (2010)[9] isolated Streptomyces from the rhizosphere of 14 different medicinal plants and found to produce IAA.

Ahmad E. A[10] obtained bacterial and actinomycetes isolates from 11 medicinal plants and all the isolates were screened for plant growth promoting traits like IAA. The collected isolates possessed multiple PGP activities. Among the 112 bacterial isolates 36 (32.14%) showed IAA production. Among all the collected isolates only eleven isolates were selected for IAA production and showed positive results ranging from 0.1 to 17µg/100ml.

Malleswari *et al*, (2010) isolated bacteria from rhizosphere of different medicinal plants and studied their PGP activity. Damam Malleswari *et al*, (2016)[11] isolated 62 actinomycetes from the soil samples collected from different medicinal plants rhizosphere and studied IAA, HCN, $NH_3$and siderophore production and PO_4 solubilization abilities and The results showed that, some strains showed high PGP activities. Among the 62 isolates, 47(75%) isolates were showing ammonia production and 15(24%) strains failed to show ammonia production.

Comparative of above studies we found that rhizosphere of medicinal plants like *Curcuma longa* harbor bacteria showing higher PGP activities. Thus these isolates can be used as sources of plant growth promoting substances and further tested for their ability to inhibit growth of plant pathogens.

Table No.1: Identification of bacterial isolates and their Id score by using PIBW in MICRO IS software

S.No	Isolate No	Id Score	Bacterial isolate identified as
1	CL 1	**	*Bacillus*
2	CL 2	0.99201	*Klebsiella oxytoca*
3	CL 3	**	*Planococcus*
4	CL 4		*Bacillus cereus*
5	CL 5	**	*Klebsiella*
6	CL 6	0.98986	*Klebsiella oxytoca*
7	CL 7	**	*Bacillus*
8	CL 8	**	*Bacillus*
9	CL 9	**	*Planococcus*
10	CL 10	0.91344	*Klebsiella pneumoniae sub. sp aerogenes*

Table No.2: Identification of actinomycetes isolates and their Id score identified by using PIBW in MICRO IS software

S.No	Isolate No	Identification score (Id core)	Matrix	Actinomycetes isolate identified as
1	CL-2-1	0.9999	Minor	*Streptomyces longisporoflavus*
2	CL- 2-2	0.99268	Major	*Streptomyces fulvissimus*
3	CL-2-11			Unidentified
4	CL-3-6	0.97132	Major	*Streptomyces cyaneus*
5	CL -3-7	0.97103	Minor	*Streptomyces longisporoflavus*
6	CL- 7-6	0.95627	Minor	*Streptomyces longisporoflavus*
7	CL-7-12	0.99409	Minor	*Streptomyces flaveolus*
8	CL-8-2	0.97932	Minor	*Streptomyces longisporoflavus*
9	CL-8-5	0.95900	Major	*Streptomyces phaeochromogenes*
10	CL-14-1	0.97036	Major	*Streptomyces fulvissimus*
11	CL-21-2	0.99994	Major	*Streptomyces pactum*

Table No.3: Isolate wise amount of IAA (µg /ml) produced by rhizobacteria

S.No	Isolate No	IAA(µg /ml)
1	CL 1	11.48±0.37
2	CL 2	17.87±0.22
3	CL 3	0
4	CL 4	23.19±0.31
5	CL 5	12.55±0.17
6	CL 6	18.93±0.08
7	CL 7	20±0.10
8	CL 8	0
9	CL 9	16.8±00.032
10	CL 10	38.08±0.23

Table No.4: Isolate wise amount of IAA (µg /ml) produced by rhizoactinomycetes

S.No	Isolate No	IAA(µg /ml)
1	CL 2-1	2.97±0.20
2	CL 2-2	2.97±0.18
3	CL 2-11	10.43±0.15
4	CL 3-6	11.48±0.23
5	CL 3-7	16.80±0.17
6	CL 7-6	25.31±0.61
7	CL 7-12	12.55±0.40
8	CL 8-2	20±0.27
9	CL 8-5	10.4±20.17
10	CL 14-11	25.31±0.61
11	CL 21-2	14.68±0.19

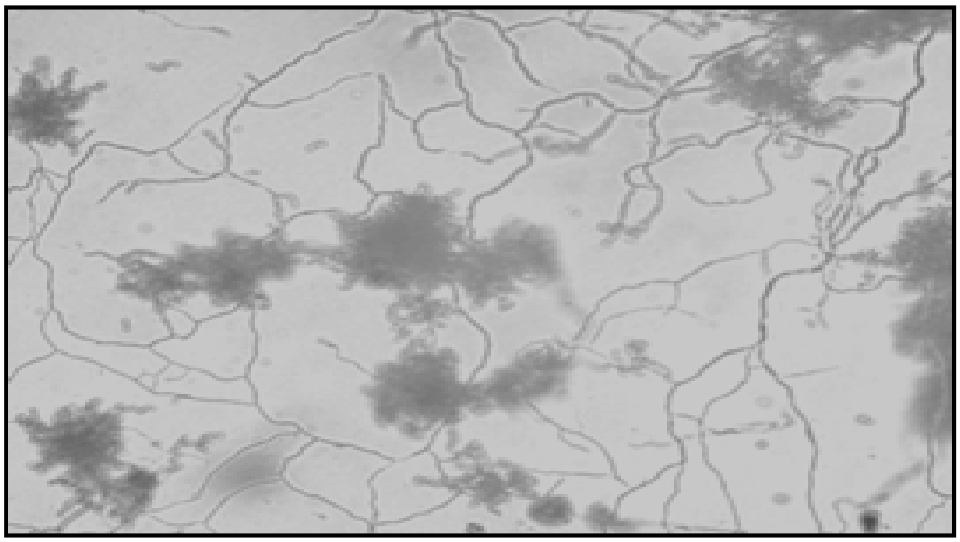

Photo plate - Spore chain morphology of actinomycete isolate Streptomyces *cyaneus* (CL-3-6)

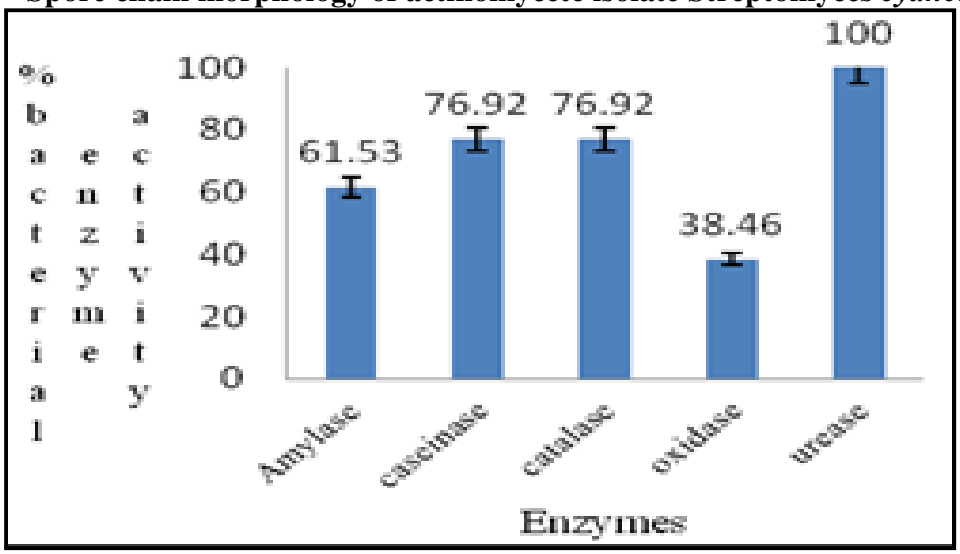

Figure No.1: Percentages of enzyme activity

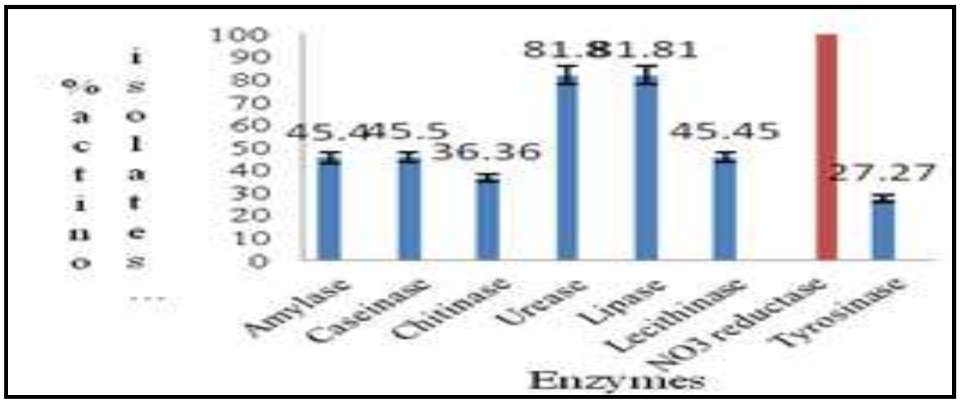

Figure No.2: Percentages of enzyme activity of bacterial isolatesof actinomycetes isolates

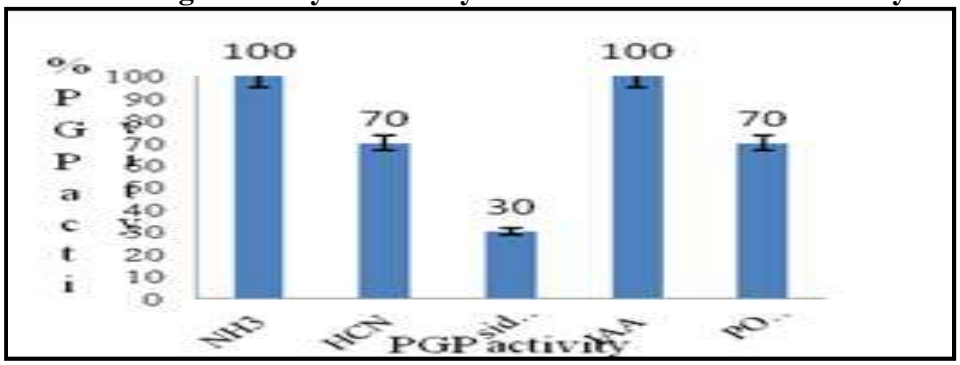

Figure No.3: Percent PGP activity

Figure No.4: Percent PGP activity of rhizobacteria of rhizoactinomycetes

Graphical presentation of Indole Acetic Acid (IAA) µg/ml production by bacterial and actinomycetes isolates

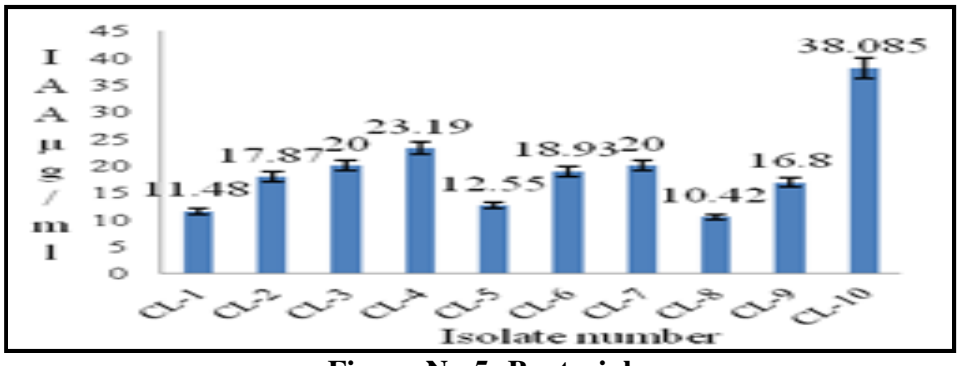

Figure No.5: Bacterial

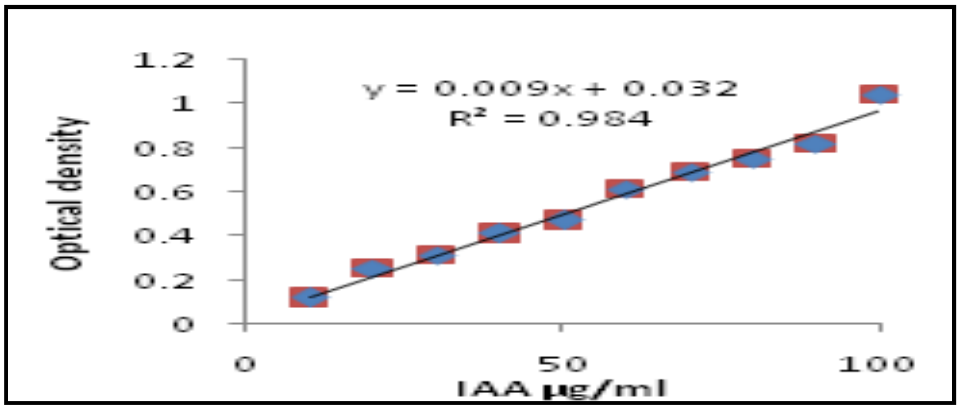

Figure No.6: Standard graph of IAA

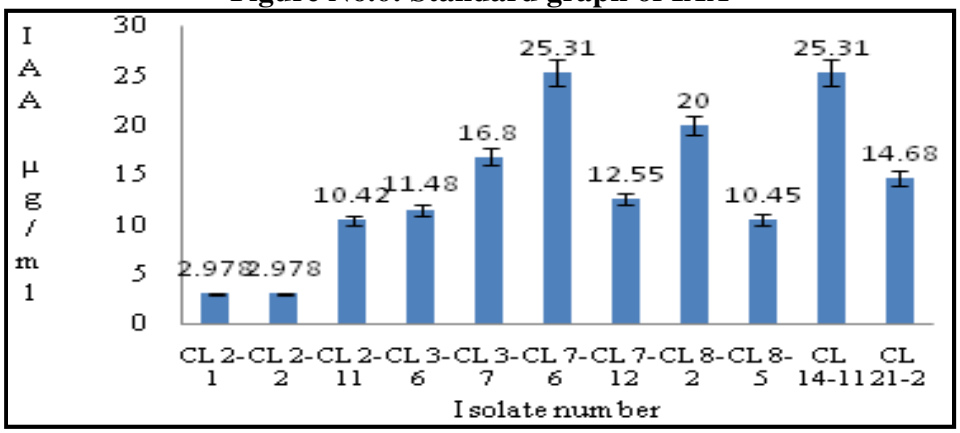

Figure No.7: Actinomycetes

CONCLUSION

Many bacterial and actinomycetes isolates from rhizosphere soil of *Curcuma longa* showed significant results for plant growth promoting activities like IAA. Many actinomycetes belong to genus *Streptomyces* and have potential IAA production ability. These potential isolates can be used as biofertilizer to improve plant growth and bio controlling agents.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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