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ISOLATION, SCREENING AND EVALUATION OF INDOLE ACETIC ACID PRODUCTION BY RHIZOSPHERIC AND ENDOPHYTIC MICROBIAL COMMUNITY

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ABSTRACT :

Microbial community is highly associated with plants in the form of mutual relationship in the region rhizosphere or endophytic. All endophytic or rhizospheric organisms including bacteria, fungi, and actinomycetes, are producers of phytoharmones i.e. indole acetic acid(auxin), gibberellic acid, cytokinin, siderophores, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatile compounds, antioxidants, up and down-regulation of stress-responsive genes. In the present study, all 54 isolates were isolated from the rhizospheric and endophytic region including rhizome, stem, and leaves of Curcuma longa L (Turmeric) for the evaluation of plant growth promoting activity. Isolates belonged from all three types i.e. bacteria, actinomycetes, and fungi. Isolation of bacteria was done on the nutrient agar, fungi on the potato dextrose agar and actinomycetes on the actinomycetes isolation agar. Screening of all these was done for the production of indole acetic acid using an salkowski reagent among them 14 isolates showing the highest activity for the production. Among them, isolate number 23 shows the highest indole acetic acid production (74 µg/ml) which is identified as fungi. These isolated species of microbial community could be used as a preparation of biofertilizer.

KEYWORDS : *Endophyte, Rhizosphere, Curcuma longa, Indole acetic acid,*

INTRODUCTION

Turmeric, Curcuma longa L., which is a member in Zingiberaceae, was originated from South-east Asia. Turmeric herb was used extensively as medication from the past until now as a prescription to cure dizziness and very popular among various country including in Asia, Europe, and the USA. (Kulpapangkorn & Mai-Leang, 2012).turmeric is acting as an anti-inflammatory, antioxidant, antiviral, antibacterial, antifungal, anticancerous, activity and also useful in diabetes, allergies arthritis, and in Alzheimer's disease also. (Aggrawal, B.B., Sundaram, C., Malani, N., Ichkawa, 2007).

Rhizome part of turmeric plant contains curcumin which shows a wide application of turmeric.(A. Kumar, Singh, Giri, Singh, & Pandey, 2014). Soil contains microorganisms near to the vicinity of plants. Microbes surrounded the plant's roots in the narrow range are known as rhizospheric organisms and bacteria are known as the rhizobacteria(Ahemad & Kibret, 2014). Rhizobacteria which helps the growth and development in the plants are known to be plant growth promoting rhizobacteria (PGPR). Plants secrete root exudates and design its microflora in the rhizosphere(Ahemad & Kibret, 2014). PGPR help to boost draught resistance in plants by secreting Exopolysaccharide, phytohormone, and 1-aminocyclopropane-1-carboxylase(ACC) deaminase(Vurukonda, Vardharajula, Shrivastava, & SkZ, 2016). PGPR from different samples soil like phyllosphere, Rhizosphere and rhizoplane are shown to be phosphate solubilization, Nitrogenase activity, and indole acetic acid (IAA) production, and acting as a biofertilizer in rice

cultivations(Mwajita, Murage, Tani, & Kahangi, 2013). plants rely on microflora for the absorption of dissolved nonreactive minerals(recalcitrant). (Jacoby, Peukert, Succurro, Koprivova, & Kopriva, 2017). The microbes associated with Curcuma longa L shows antimicrobial activity against human pathogens E.coli, P. aeroginosa, and S.aureus.(Mandale, Dagar, & Dagar, 2017) and also PGPR improves salt tolerance enhancing stem height, stem and Rhizome biomass, alleviating the curcumin content in curcuma longa L (A. Kumar, Vandana, et al., 2016). Mohite isolated and characterized the rhizospheric soil microorganisms for indole acetic acid production and its effect on wheat plants and conclude that IAA producing bacteria will be functioned as efficient biofertilizer(Mohite, 2013). Microbes are also present within plant tissue known as endophytes isolated from the rhizome of zingiber officinale and found to produce an IAA, ACC deaminase and siderophores as a plant growth promoting harmone(Jasim, Joseph, John, Mathew, & Radhakrishnan, 2014). Endophytes are also isolated from the rhizome of curcuma longa L and after characterization found to be B.Cereus, B. Thuringenesis, Bacillus sp. Pseudomonas putida, clavibacterium Michiganesis produces Indole Acetic Acid and solubilize phosphate (A. Kumar, Singh, et al., 2016). Endophytes are also associated with leaf, stem and roots and produce plant growth hormone.(Deshmukh, Patil, Kale, & Dudhare, 2018). Total 576 endophytic isolates were isolated from the leaves, stem, roots of rice and found that the associates synthesize Auxin, siderophore and solubilizes phosphate which helps in developing plants growth and also induces a systemic resistance for antifungal activity against F. Oxysporum and R. solani.(Ji, Gururani, & Chun, 2014)

Indole acetic acid is the product of l-tryptophan metabolism and showed that increase in the concentration of l-tryptophan there is increase in the IAA production also shows that significant increase in plant root biomass in inoculation with IAA compared to uninoculated(Matter et al., 1985),it is responsible for the root proliferation Cell growth and metal homeostasis, ubiquin mediated proteolysis (Woodward & Bartel, 2005).Indole acetic acid(IAA) exert the effect like primary root and lateral root initiation, apical dominance phenomenon, morphogenesis in leaves, and development in vascular bundles in plants. It is also found that IAA affects were species specific i.e. positive or negative effects in roots depend upon species of plants.(Fu et al., 2015). A microbial community like paenibacillus sp, klebsiella sp, Bacillus cereus, B. Thuringenesis, Bacillus sp, B. pumilis, pseudomonas putida, and Clavibacterium michiganensis, are found as endophytic in turmeric plants.(A. Kumar et al., 2017).

In the present work emphasis on isolate and screen IAA producing native community including Bacteria, Actinomycetes, and Fungi associated with curcuma longa L both Rhizospheric and endophytic interactions, screen the isolate for the maximum amount of IAA production.

MATERIALS AND METHODS:

1) Media preparation for isolation:

For isolation of rhizospheric bacteria Nutrient agar media used with antifungal agents(flucanozole-75µg/ml, Ketoconazole-75µg/ml) to control the fungal contamination(Devi & Ramanjaneyulu, 2016). Fungi were isolated by using a potato dextrose agar with 50 mg/l chloramphenicol (diversity et al., 2014). And actinomycetes were isolated by using actinomycetes isolation agar ((Gms/Litre) Sodium caseinate-2.000, L-Asparagine-0.100, Sodium propionate-4.000, Dipotassium phosphate-0.500 Magnesium sulphate-0.100 Ferrous sulphate 0.001 Agar-15.000 Final pH(at 25°C)-8.1±0.2.)("Actinomycete Isolation Agar," n.d.) with cycloheximide (50µg/ml) and nystatin (50µg/ml). (Damam, Moinuddin, & Kausar, 2016).

2) Isolation of microbial community:

The rhizospheric communities were isolated by collecting soil samples associated with rhizosphere of Curcuma longa L from the crop of vishnupuri, taluka and district Nanded (431606). Serial dilution technique was used as follows for isolation of rhizospheric microorganisms: 10 g of rhizospheric soil was added in the 90 ml of sterile distilled water making 10-1 dilution and incubated on a rotary shaker for 10 at 120 rpm. (Mohite, 2013). The 10-1 dilution were further serially diluted up to 10-7. 1 ml from each dilution was spread on a nutrient agar plate, actinomycetes isolation agar, potato dextrose agar (PDA) for the

isolation of rhizospheric bacteria, actinomycetes, Fungi respectively. The plates were incubated for 300C for the 7 to 10 days for actinomycetes(Muleta & Assefa, 2018), at 260C to 300C for 3-5 days(N. V. Kumar, Rajam, & Rani, 2017) and at 300C for 24 to 48 hrs for bacteria(Singh & Prasad, 2014). The aseptic condition should be maintained during all procedure was carried out in.

The isolation of endophytes was carried out by collecting the sample of Rhizome, stem, and leaves of turmeric. The parts were gently washed with tap water especially Rhizome and then surface sterilized by using 70% ethanol for 3 min, rinse with sterile distilled water then 0.5 % NaOCl and 70% C2H5OH for 30 sec. Later sterilizing treatment procedure the samples were gently washed with two to three times with sterile distilled water(Deshmukh et al., 2018). The isolation of endophytic community takes place by aseptically drying, and sliced the Rhizome, stem, and leaf uniformly and transfers to nutrient agar plates for bacterial isolation(Deshmukh et al., 2018), on potato dextrose agar plates for fungal isolation(Diversity et al., 2014),and actinomycetes isolation agar for isolation of actinomycetes(Gangwar, Rani, & Sharma, 2012). All plates are incubated for the same time as described in the isolation of rhizospheric fungi, bacteria, and actinomycetes. The colonies from rhizospheric soil and endophytes are examined as follows: The bacterial colonies developed after incubation was characterized according to colony morphology and biochemical characteristics. These were examined as reported by Bergey's manual of determinative bacteriology. (A. Kumar, Singh, et al., 2016). The fungal colonies were identified by colony characteristics and microscopic examining their cultural characteristic (Tolulope, Adeyemi, Erute, & Abiodun, 2018). The actinomycetes colony were separated by observing small, dry, and the powdery colony with highly slow growth rate were selected and transferred to a fresh medium to obtain a pure culture. (Damam et al., 2016). The colonies were elected and streaked on newly prepared media slants i.e. bacterial colonies on Nutrient agar, fungal colonies on potato dextrose agar (PDA). And actinomycetes on actinomycetes isolation agar. (Sheik, Maqbul, S, & Ms, 2017).

Total 54 isolates were isolated in pure culture from rhizospheric soil and endophytic region of stem, rhizome, leaves. These were further estimated for Indole acetic acid production.

Production of Indole acetic acid :

Cultures were estimated for indole acetic acid production by supplementation of L-tryptophan 300 µg/ml in nutrient broth for bacteria , actinomycetes isolation broth for actinomycetes, and potato dextrose broth for fungi(Kesaulya, Baharuddin, Zakaria, &Syaiful, 2015) (A. Kumar, Singh, et al., 2016), and adjusted the pH 6.4 for fungi and 7.4 for bacteria and 7.6 for actinomycetes. All these were incubated on a rotary shaker at 30 0C for bacteria and actinomycetes, and 28 0C for fungi. (Taylor, Krishnapura, & Belur, n.d.).

Culture's were estimated for the indole acetic acid production after 2-3 days for bacteria(Pant & Agrawal, 2014), 7 days for actinomycetes(Harikrishnan, Shanmugaiah, & Balasubramanian, 2014), and 3 days for fungi(N. V. Kumar et al., 2017). After incubation, the cultures of all broth were centrifuged at 00 rpm for 10 min(A. Kumar, Singh, et al., 2016). 1 ml of supernatant was blended with Salkowaski reagent (50 ml, 35 % of perchloric acid, 1 ml 0.5 M FeCl3 solution) 4 ml(Pant & Agrawal, 2014). After mixing incubate for 25 min at room temperature as incubation will be finished there is color changes to pink indicates production of IAA(N. V. Kumar et al., 2017). The pink color is the indication of Indole acetic acid(IAA) production hence for quantitative estimation was done by spectrophotometrically at 530 nm wavelength and the concentration was measured by using standard graph adapted by using standard IAA.(N. V. Kumar et al., 2017).

RESULTS AND DISCUSSION.

Isolation of Bacteria, fungi, and actinomycetes:

In the present study total, 54 isolates were isolated on the basis of colony morphology from the rhizospheric and endophytic region including bacteria, fungi, and actinomycetes. Isolation of all isolates was carried out according to the standard procedures of isolation described in the materials and methods. From all 11 were found to be actinomycetes, 29 are fungi and 14 are bacteria from endophytic and rhizospheric soil associated with Curcuma longa L.

The isolates number and type of organisms are presented in table below.

Table.1: Isolates number of isolates and type of microorganisms

Sr. no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Actinomycetes	2	15	19	30	35	38	43	44	46	47	54																		
Fungi	1	3	5	7	9	10	16	20	21	23	24	26	27	31	33	34	36	37	39	40	41	42	45	48	49	50	51	52	53
Bacteria	6	8	11	12	13	14	17	18	22	25	28	29	32	4															

Screening for IAA production:

Production of IAA was estimated by using a Salkowski reagent. The pink color development in the test sample was selected for the quantitative estimation of IAA. (Singh & Prasad, 2014). Auxin is a phytoharmone responsible for plant growth and development. (Kesaulya et al., 2015)

Quantitative estimation of IAA:

Out of 54 isolates, 4 isolates of actinomycetes shows a positive result and higher IAA production, also 8 from fungi and 2 from bacteria. Isolates number 19,30,35,and 38, are shown higher production from actinomycetes, isolate number 1,7,9,21,23,24,37,39, from fungi, and 6,12 were from bacteria shows higher production.

Out of 54 isolates, 14 isolates showed the maximum amount of IAA only selected for the quantitative estimation out of that isolate number 9,23,24,37,39 are the highest IAA producers. Measurably they are fungi which produce the maximum amount of IAA than the actinomycetes and bacteria. Maximum IAA production was recorded in isolate HB8 (5.816 mg l-1) as compared to other isolates. (Kesaulya et al., 2015) maximum IAA produced(362.53 ± 3.29 µgml−1) after 18 d of incubation at 30◦ C under rotary shaking condition, (Taylor, Bose, Shah, & Keharia, 2013). The maximum amount of IAA produced by the fungi are isolate number 23, actinomycete isolate number 38 and bacteria isolate number 6. Production of IAA by major isolates was presented below.

Table.2: Production of IAA

Sr. no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Isolate number	19	30	35	38	1	7	9	21	23	24	37	39	6	12
IAA production (µg/ml)	7	4	3	18	20	12	65	24	74	70	71	45	30	27

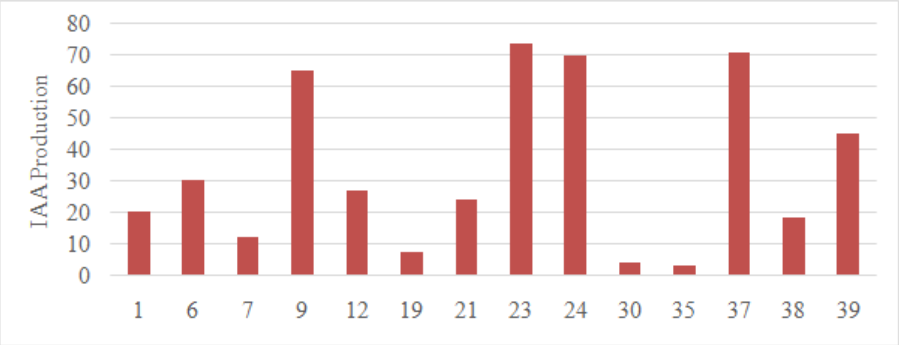

FIGURE 2: DIAGRAM: EFFICIENCY OF IAA PRODUCTION BY FUNGALISOLATE 23, BACTERIAL ISOLATE 6 AND ACTINOMYCETE ISOLATE 38.

CONCLUSION:

Indole acetic acid is a phytoharmone responsible for plant growth and development (Woodward & Bartel, 2005). Microbial isolates are all contributing the plant growth and development by the production of indole acetic acid including bacteria (Mohite, 2013), actinomycetes (Anwar, Ali, & Sajid, 2016)and fungi(N. V. Kumar et al., 2017). The cumulative result of microbial consortium responsible for plant growth and development. (Choure & Dubey, 2012). Among all fungi are the most efficient indole acetic acid producer than actinomycete and bacteria but all are producers of plant hormone indoleacetic acid hence their cumulative effect helps the growth and development of Curcuma longa L. Indole acetic acid producers are associated as an Endophyte as well as in rhizospheric soil of Curcuma longa L. Hence the consortium of all these are adequately acting as a biofertilizer (Vanegas, 2010).

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