Evaluation of bacterial endophytes of tomato plant for bio-control and growth promoting potential

ABSTRACT

The study was conducted at Indira Gandhi Krishi Vishwavidyalaya, Raipur, during 2020-2023 with the objective to evaluate endophytic bacteria of tomato plants. A total of 24 endophytic bacterial isolates (ESR1, EBR2, EAR4 ERR5, EAS12 EJS11 ERS13, ESL17, EBL18, ERL21, and EML24) obtained from roots(R), fresh leaf(L) and stems(S)of tomato plants collected from several locations i.e., Surajpur(S), Balrampur(B), Jashpur(J), Ambikapur(A), Raipur college campus(R), Pirda(P), Chhokranala(C) and Mungeli(M). Significant quantity of IAA produced by these isolates *in-vitro* after 24 hours of incubation with added precursor Tryptophan ranging from 8.80 µg ml^{-1} to 18.00µg ml^{-1} being significantly maximum in EAR4 followed by ERR5, EAS12. Siderophore test was positive in EBR2, ERR5 and EJS11. Using the *in vitro* dual culture technique, seven isolates were found to have antagonistic activity against *Fusarium oxysporum* out of which EJS11, and EAS12 isolates showed highest inhibition percentage of 67.5 and 65.11 respectively. Plant infection studies of endophytic bacteria in tomato revealed that a significant increased shoot dry weight (60%) was obtained with ESL17 followed by ESR1(58.08%) and EJS11(56.22%). Shoot N content was found maximum (3.12%) with ESR1 followed by EML24 and EAS12.EAS12has shown highest germination% (97.5) and seedling vigour index (2774) was maximum in EJS11 followed by EAS12. Considering these, promising isolates were ESR1, EBR2, EAR4, ERR5, EJS11, EAS12 and EML24. The endophytic bacteria from tomato have better potential; may be applied for growth promoter, also may be used as commercial fungicides looking towards economic and environmental impacts.

Key words: Tomato, Endophytic bacteria, IAA, Antagonistic activities

Introduction

Tomato (*Solanum lycopersicum* L.) is widely grown worldwide and of major importance for agricultural industry.(Olaniyi JO, *et al*., 2010).Tomatoes are commonly consumed in daily diets. It is a major source of antioxidants, like lycopene, and great sources of vitamin C, potassium, folate and vitamin K. (Soytong, M. *et al*., 2021). The tomato (*Solanum lycopersicum*L.) a food

that is considered protective, plays a significant role in human economies because to its high nutritional value added products and ability to be produced in a variety of agro-climatic situations. It is regarded as one of the most significant vegetables in the world, coming in second behind potatoes. Both raw and processed forms of its fruits are consumed. (ANON, 2002).Area, production and productivity of tomato crop in India (2020-21)was recorded as 8.4 Lakh Ha, 211.81 Lakh MT and 25.1 MT/Ha. (Horticultural Statistics at a Glance, 2021).Area, production and productivity of Tomato crop in Chhattisgarh state (2020-21) was 61.17 Ha, 1138.82 MT and 18.62 MT/Ha.(Horticultural Statistics at a Glance, 2021).Tomato is one of the important horticultural crops cultivated by farmers and is one of the high economic value commodities with significant export potential. This plant carotene, flavonoids, lycopene, vitamins, andβ-contains becomes one of the most widely consumed vegetables in the world.(Ahmed B, *et al*., 2018).

Plant growthpromoting bacteria (PGPB) are the most promising alternative source for chemical fertilizers which has a significant impact on crops improvement through biochemical, physiological and molecular mechanisms. (Palacios *et al*.,2014).Endophyte bacteria are those which live and associate with plant tissues without causing any symptoms in plants.(Hardoim PR, *et al*., 2015).Endophytes can also enhance the plant growth-promoting (PGP) traits of crops either by a direct or indirect mechanism. (Ahmed *et al*., 2022). Endophytic bacteria can produce nutritive metabolites and antibiotics or promote induced systemic resistance (ISR) in plants as a critical defence pathway. (Rat *et al*., 2021).The existence of bacteria endophyte in the tissue of plants involved in producing substances hypergrowth, anchoring nitrogen, mobilizing phosphate, and inducing plant resistance to pathogens disorders.(Baccari C, *et al*., 2018). In addition, the endophyte bacteria play a role in helping plants to grow and adapt to environmental conditions gripped by drought to produce several compounds that can promote the plants growth helping them to adjust to environmental conditions.(Mishra A, *et al*., 2018). It was further reported that the endophyte bacteria have the potential to improve the viability and vigor index. (Suryanto D, *et al*., 2018).Many endophytic bacteria are directly or indirectly involved in plant growth and development. Endophytic bacteria live in plant tissues without causing substantive harm to the host or gaining any benefit other than a non-competitive environment inside the host. (Patel H A, *et al*., 2012).Use of endophytic bacteria can be considered as a new source of bio-control agents in the plant disease management. (Backman *et al*., 2008).

Materials and Methods

Collection of plant material

A Survey was carried out for collection of tomato (*Solanum lycopersicum* L.)seedlings and isolation of endophytic bacteria was done from root, stem and leaves of tomato plant. Four weeks old healthy tomato plants were collected from eight different agricultural fields in Surajpur, Balrampur, Jashpur, Ambikapur, Raipur, Pirda, Chhokranala and Mungeli districts of Chhattisgarh, India. Random sampling was done by carefully uprooting the plants from field. Healthy tomato plant samples were collected. Samples **(**root, stem and leaves of tomato plants) were taken to the laboratory in sealed sterile plastic bag and stored at 4°C and processed within 4 hrs of collection.

Isolation of endophytic bacteria from different plant parts (root, stem and leaves) of tomato plants

Collected tomato roots, stems and leave samples were rinsed properly in running tap water to remove soil and dust particles followed by washing with double distilled water before processing. After which surface sterilization was done under laminar air flow.For eliminating surface microbes, surface sterilization carried out. Root, stem, leaf portions were cut to 0.5-1cm pieces, then sample tissues individually disinfected by soaking in 70% (v/v) ethanol for 1 min, subsequently immersed in 1% sodium hypochlorite w/v for 10 min; followed by washing with sterile water and then washed with 70% ethanol for 30s. Then they were rinsed three times with sterile distilled water (SDW) to remove surface sterilization agents and air-dried aseptically on sterile filter papers.Then small pieces (0.5-1.0 cm in length) of each sampled organ (root, stem and leaf samples) plated on nutrient agar (NA) media, incubated at 31°C for 48 h for maximum recovery of bacterial colonies.(Abdallah R.A.B.*et al*., 2018). The efficiency of surface sterilization process was checked according to Hallmann*et al*., (1997).

Plant growth promoting activity

IAA production

The bacterial cultures were inoculated in nutrient broth with tryptophan (5 µg/ml), and incubated at 28±2°C for 5 days. Cultures were centrifuged at 3,000 rpm for 30 min. Two ml

of the supernatant was mixed with two drops of orthophosphoric acid and 4 ml Salkowski's reagent (1 ml of 0.5 M FeCl□ on 50 ml of 35% perchloric acid). Incubated at 28ºC for 30 min. Development of red color indicates Indole 3-acetic acid (IAA) production; the optical density (OD) was read at 530 nm using a spectrophotometer. The level of IAA produced was estimated from IAA standard curve and expressed as micrograms per milliliter (Patten and Glick 1996).

Siderophore production

Chrome azurol S (CAS) assay was used to detect siderophores produced by endophytic bacteria. Siderophore production was tested on petridishes contained CAS agar. Pure isolates of endophytic bacteria were spotted on CAS agar plates and incubated at 28±2°C for 5 days in the dark. The colonies with orange zones were considered as positive for siderophore production. The control plates of CAS agar were incubated under the same conditions as described above and no color change in the CAS - blue agar was observed, after incubation period of 3-5days. (B. Sai Sushma *et al*., 2020).

In vitro antagonistic activity test of tomato-endophytic bacteria against *Fusarium oxysporum*

Antagonistic activity of isolated bacterial endophytes against *Fusarium* was evaluated by using dual culture technique on petridish containing Martin Agar medium. The agar plug of pathogen (A 6 mm mycelial disc from a 7 day old PDA culture of fungal pathogen) was placed at the center of culture medium and two days old fresh culture of isolated endophytic bacterial strains were uniformly streaked on Martin agar medium on the left and right sides of the pathogen at 2 cm. length from the edge of the Petri dish then incubated at 28°C for 4 days. Control plates were streaked with sterile distilled water. Evaluation of inhibition of fungal mycelial growth was assessed when pathogen grown full in control plate. The diameter of the fungal growth was measured 5 days after incubation and expressed as percent growth inhibition over control.

The percent inhibition was calculated by using the formula:

$I = (C - T)/C \times 100$

Where,

- I is the percent inhibition of mycelial growth over the control
- C is diameter of the mycelial growth of fungal pathogen colony in the control plate and
- T is diameter of the mycelial growth of fungal pathogen colony in endophytic bacteria inoculated plate.

The experiment was carried out in three independent replicates. (Amaresan N, *et al*.,2012).

Screening of beneficial endophytic bacterial isolates by plant infection test

All isolates obtained from healthy tomato plant was cultured in nutrient broth (NB) and incubated on rotary shaker for 48 hr. Tomato seeds were treated separately with culture before sowing. Also 10 ml of bacterial suspensions were added separately to different poly-bags containing tomato seedlings at 3 day of age grown in sterilized mixture of soil and sand in (3:1 ratio). The experimental design was completely randomized design (CRD) with 03 replications. After 7 days of inoculation with endophytic bacteria, the seedlings survived and the growth data was collected.The growth of plants were kept for 30 days. The growth data including stem height, shoot weight, root weight, total weight and number of leaves at 30 DAS. Data were calculated for growth index of seedling vigor index (svi) as following formula (PanisaPrasom*et al.*, 2017).

- Seed germination study: Percentage of seed germination in each treatment at 30 days after sowing.
- Germination (%) = Total number of seed germinated/Total no. of seeds sown x 100
- Seedling vigor index (svi) = (mean root length + mean shoot length) x % germination

Statistical analysis

The data were statistically analysed using ANOVA for completely randomized design (CRD).The significant difference was tested through F-test at 5% level of significance. The standard error of means (SEm±) and CD were calculated where F-test was significant for comparing treatment means (Panse and Shukhatme, 1978).

Results and Discussion

A total of 24 isolates of endophyte bacteria have been isolated from tissues of roots, leaf and stems of tomato plants obtained from several locations of Chhattisgarh.

All the 24 bacterial endophytes were characterized based on the different morphological characteristics. Out of 24 isolates tested, 18 isolates were gram positive and 6 were gram negative. This indicated that majority of the bacteria observed in this study belong to gram positive *bacilli*.Also studied by Amaresan*et al*.,(2012) that abundance of *Bacillus* in tomato plants was reported as out of eight endophytic bacteria from tomato plants. Similar type of isolation of endophytic bacteria was carried out by Inuwa*et al*., (2017) who isolated sixteen endophytic bacteria from roots and leaves of lemon grass.B. Sai Sushma *et al*., (2020),twenty four isolates of endophytic bacteria were obtained from different plant tissues including root, stem and fresh leaves regions of tomato plants.

Phytohormone (IAA) production capacity

In the present study, a total of 24 bacterial isolates were tested for quantitative assay of IAA. The quantity of IAA produced was determined by measuring the OD values at 530 nm. Among all the twenty-four isolates tested, IAA production varied with tryptophan supplementation and results are presented in the table 1. The data (Table 1) indicated that in the presence of tryptophan the highest IAA production was shown by the isolate EAR4 (18.0µg/ml) followed by the isolate ERR5 (17.4 µg/ml), while minimum amount of IAA production was recorded by the isolate ERL21 (8.80 µg/ml) followed by ERS13 (9.00 µg/ml). IAA enhances the development of root system and thus resulting in high water and nutrient uptake (Patten *et al.,* 1996).There were significant differences ($P<0.05$) among the bacterial isolates in their potential for production of IAA. Similar studies have also been carried out by Patel H. A. *et al*., (2012). IAA is one of the most important and physiological active auxin and provides greater access to soil nutrients and water uptake. IAA also acts as a signaling molecule in bacteria, promoting favourable effects on plant health, including phytostimulation and plant immunity (Barbieri P. *et al*., 1993).

Table: 1 Quantitative estimation of Indole acetic acid (IAA) produced by endophytic bacterial isolates of tomato plants

Sl. No.	Name of isolates	IAA Production (µg ml^{-1})

1	ESR1	16.30
2	EBR2	15.50
3	EJR3	12.00
4	EAR4	18.00
5	ERR5	17.40
6	EPR6	12.00
7	ECR7	10.20
8	EMR8	13.20
9	ESS9	11.00
10	EBS10	10.80
11	EJS11	11.30
12	EAS12	16.60
13	ERS13	9.00
14	EPS14	15.90
15	ECS15	9.10
16	EMS16	11.30
17	ESL17	11.40
18	EBL18	10.60
19	EJL19	11.60
20	EAL20	9.20
21	ERL21	8.80
22	EPL22	13.30
23	ECL23	11.90
24	EML24	16.00
	SE(m) ±	1.077
	C.D. (0.05%)	3.071

Siderophore production

Three bacterial isolates have shown significantly prominent positive response as per siderophore testing which were EBR2, ERR5 and EJS11. However 08 endophytic bacterial isolates were also seen as producing clear zones though not prominent as above three isolates. While except these

11 isolates, others were found negative for production of siderophore. Siderophores are small organic molecules produced by microbes including endophytic bacteria, under iron limiting conditions. The siderophore production was found to become of the mechanisms to outcompete the pathogens. By synthesizing siderophores bacterial endophytes capture iron from the iron limiting environment and supply it to plants for development (Schwyn*et al*., 1987). Siderophore production by endophytic microorganisms facilitates in colonization of bacteria to the host tissue from rhizospheric zone (Loaces*et al.*, 2011).

Among twenty-four endophytes isolates of tomato ESR1, EBR2, EAR4 ERR5, EAS12 EJS11 ERS13, ESL17, EBL18, ERL21, and EML24 isolates were found promising over other isolates due to outstanding production of IAA and siderophores.

Antagonistic activities test against *Fusariumoxysporum*

A total of 24 isolated bacterial endophytes were tested for antagonistic activity against *Fusariumoxysporum*under *in vitro* using dual culture method.Results revealed that among of these, only seven endophytic bacterial isolates showed antagonistic activity to inhibit/suppress the growth of *Fusarium oxysporum*. These isolates were EJS11, ERS13, ERR5, and EBR2 inhibited the growth of fungus, including more than 30 percent. The best isolates were EJS11, and ERS13, which had the highest inhibition percentage of 67.5 and 65.11, respectively.The widest inhibition zone was produced by EBR2 and ERR5 isolate.The details of isolate wise antagonistic activities are presented in Table 2. Different mechanisms used by these microbes to antagonize the pathogen directly by production of antifungal metabolites or indirectly by competing for space and nutrients and activating the immune system of the plant. (Chaturvedi *et al.*,2016). Various studies have reported that bacterial endophytes have successfully reduced the impact of pathogens on the host-plant *in-vivo* (Neerja. 2010; Amaresan*etal*., 2012 and Nandhini*et al*., 2012). Devi, N. O*et al*., (2022).

Table: 2 Antagonistic activities of endophytic bacterial isolates of healthy tomato plants to suppress the growth of *Fusarium oxysporum*

Isolates	Inhibitory effect against *Fusarium oxysporum*	
	Diameter of colony (cm)	**% Growth inhibition**
ESR1	7.48	16.88
EBR2	6.33	29.66

EJR3	7.85	12.77
EAR4	7.63	15.22
ERR5	6.07	32.55
EPR6	8.00	11.11
ECR7	8.36	7.11
EMR8	8.38	6.88
ESS9	7.64	15.11
EBS10	7.95	11.6
EJS11	2.92	67.5
EAS12	3.14	65.11
ERS13	8.72	3.11
EPS14	7.87	12.55
ECS15	8.52	5.33
EMS16	8.87	3.33
ESL17	7.83	13.00
EBL18	8.63	4.11
EJL19	8.89	1.22
EAL20	7.74	14.00
ERL21	8.95	0.55
EPL22	7.91	12.11
ECL23	8.28	8.00
EML24	7.65	15.00
Control	9	0.00
SE(m) ±	0.348	0.730
C.D. (0.05%)	0.991	2.081

Plant infection test with endophytic bacterial isolates based on growth and seedling vigor of tomato crop

Twenty four isolates of endophytic bacteria isolated from each part of healthy tomato plants (root stem and leaves) was found to have a variety of morphological colony characteristics and biochemical characteristics. In addition, 24 isolates of endophytic bacteria were tested on performance of tomato seedlings. Comparative plant growth-promoting ability of endophytic bacterial isolates were studied by giving as inoculation treatments on tomato seedlings.

In the present study, the effect of the endophytic bacterial isolates on the growth parameters of tomato seedlings i.e.; plant height, biomass accumulation, no. of leaves, seedling vigour at 30 days post treatment were observed.All plant growth parameters (plant height, aerial part and root fresh weight, and maximum root length), at 30 days post-treatment, revealed a significant ($P \leq 0.05$) variation in depending on tested bacterial treatments.The results in this experiment showed that most endophytic bacteria did not harm tomato seedlings, also promote the seedlings growth and seedlings vigour. Tomato plants inoculated with endophytic bacteria showed significantly increased growth attributes; germination%, shoot height, number of leaves, seedlings fresh weight and dry weights seedling vigour index. Those isolates including EAS, EAR, EML, EJS, ERR, EBR and ESR. The details of data were shown in Table 3 (a) and Table 3(b).

A significant increase in plant height, by 30.23% compared to control, was noted on tomato plants treated with the isolates EAR4. The highest shoot dry weight increment (60%) was obtained using ESL17 inoculation treatment; followed by those performed with ESR1 (58.08%) and EJS11 (56.22%) isolates. Significantly the maximum root length in ESR1 increase from 8.0 to13.2cm over control. As per number of leaves/plant concerned the maximum was found (9.66) with ESR1. Nitrogen content of shoot was found maximum in (3.12%) with ESR1 isolate followed by EML24 and EAS12 Endophytic bacterial isolate, EAS12 has shown highest germination % (97.5) and seedling vigour index (2774) was maximum in EJS11, in tomato followed by EAS12, endophytic bacterial isolate with a vigour index of 2652. Based on growth parameters, germination percentage and seedling vigour index values of tomato plants, ESR1, EAR4, EJS11, EAS12, ESL17 and EML24 were the efficient plant growth-promoting endophytic bacterial isolates were observed. Also considering antagonistic activity, production of IAA and siderophores ESR1, EBR2, EAR4, ERR5, EJS11, EAS12 and EML24 endophytic bacterial isolates were found promising in tomato as compared to other isolates. Similar result was obtained by P. Prasom and P. Sikhao*et al*., (2017). Similar result was obtained by B. Sushma *et al*.,(2020).

Table: 3(a) Effect of seed treatment with endophyte bacteria on germination percentage (GP), vigor index and growth performances of tomato at 30 days after treatment

Endophytic Bacterial Isolates	Shoot height (cm)	Maximum Root length (cm)	No. of leaves/plant	Germination %	Seedling vigor index	Nitrogen content of Shoot (%)

Control	12.0	8.0	6.66	78.75	1575.0	2.07
ESR1	16.7	13.2	9.66	92.00	2566.8	3.12
EBR2	16.2	11.2	9.6	93.75	2568.8	2.72
EJR3	12.8	9.5	8.66	81.25	1811.9	2.20
EAR4	17.2	11.4	8.66	96.25	2752.8	2.76
ERR5	15.9	10.5	9.33	93.00	2455.2	2.87
EPR6	12.4	8.40	7.33	88.62	1843.3	2.56
ECR7	16.2	8.10	8.6	77.5	1833.3	2.56
EMR8	12.5	9.1	9.6	88.75	1917.0	2.94
ESS9	12.5	13.0	8.66	90.37	2304.4	2.59
EBS10	15.4	9.6	8.33	84.75	2118.8	2.67
EJS11	17.0	12.2	9.3	95.00	2774.0	2.57
EAS12	16.0	11.2	8.66	97.5	2652.0	2.85
ERS13	14.6	9.5	7.66	95.00	2289.5	3.05
EPS14	13.4	8.5	7.66	87.00	1905.3	2.83
ECS15	13.4	9.0	8.33	86.25	1932.0	2.01
EMS16	15.0	11.2	8.00	91.25	2390.8	2.85
ESL17	17.0	12.0	8.00	86.5	2508.5	2.65
EBL18	15.5	9.5	8.00	89.25	2231.3	2.64
EJL19	16.2	11.0	7.66	85.00	2312.0	2.56
EAL20	11.5	8.2	7.60	87.5	1723.8	1.96
ERL21	14.3	9.2	8.66	89.00	2091.5	2.67
EPL22	13.2	9.2	8.33	90.00	2016.0	2.87
ECL23	14.0	8.9	7.30	80.00	1832.0	2.66
EML24	16.2	11.0	9.30	91.75	2495.6	2.90

SE(m)±	0.87	0.79	0.77	1.44	26.41	0.008
C.D. (0.05%)	2.48	2.24	N/A	4.10	75.25	0.006

Table: 3 (b) Effect of seed treatment with endophyte bacteria on biomass accumulation in tomato at 30 days after treatment

Isolates	Shoot fresh wt(g)	Root fresh wt(g)	Total fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)	Total dry wt (g)
Control	1.93	0.66	2.59	0.197	0.054	0.251
ESR1	4.69	1.5	6.19	0.470	0.420	0.890
EBR2	3.52	1.52	5.04	0.429	0.455	0.884
EJR3	3.09	1.5	4.59	0.281	0.479	0.760
EAR4	3.32	1.62	4.94	0.349	0.222	0.571
ERR5	4.49	2.2	6.69	0.433	0.498	0.931
EPR6	3.10	1.25	4.35	0.284	0.170	0.454
ECR7	2.41	0.51	2.92	0.202	0.067	0.269
EMR8	1.80	0.47	2.27	0.197	0.053	0.250
ESS9	3.79	1.13	4.92	0.384	0.196	0.580
EBS10	3.16	0.89	4.05l	0.287	0.136	0.423
EJS11	4.2	1.07	5.28	0.450	0.189	0.639
EAS12	3.78	1.66	5.44	0.366	0.345	0.711
ERS13	1.46	0.44	1.90	0.157	0.062	0.219
EPS14	3.60	1.68	5.28	0.334	0.257	0.591
ECS15	1.53	0.87	2.40	0.196	0.157	0.353
EMS16	3.38	0.99	4.37	0.304	0.171	0.475
ESL17	4.81	1.26	6.07	0.503	0.200	0.703
EBL18	2.14	0.675	2.82	0.226	0.166	0.392

EJL19	3.23	0.83	4.06	0.337	0.188	0.525
EAL20	1.91	1.05	2.96	0.173	0.171	0.344
ERL21	2.56	0.42	2.98	0.250	0.067	0.317
EPL22	3.03	1.22	4.25	0.286	0.197	0.483
ECL23	1.75	0.91	2.66	0.248	0.186	0.434
EML24	3.55	0.95	4.50	0.350	0.193	0.543
SE(m)±	0.034	0.028	0.019	0.001	0.001	0.001
C.D. (0.05%)	0.097	0.080	0.053	0.004	0.003	0.003

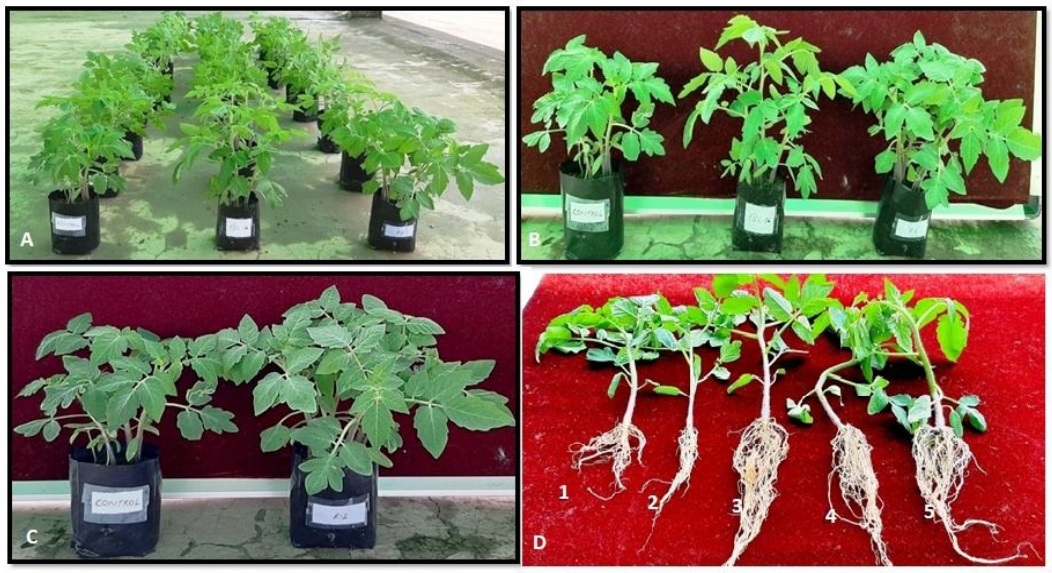

Plate: 1 Effect of endophytic bacterial isolates recovered from tomato plants on aerial part and root growth of tomato plant at 30 DAS compared to control.

(A) Overview of plant infection test in Tomato seedlings with endophytic bacteria
(B) Comparison between tomato seedlings inoculated with endophytic bacteria (b) ESL17, (c) EBR2 and (a) control
(C) Comparison between tomato seedlings inoculated with endophytic bacteria (ERR5) and control
(D) Endophytes treated plants and control plants. Plant roots inoculated with bacterial endophytes (3)ESL17, (4) EBR2, (5) ERR5 and control (1 and 2)

Conclusion

The evaluation of bacterial endophytes from tomato plants for bio-control and growth promoting potential reveals their significant role in sustainable agriculture. These beneficial microorganisms enhance plant (seedling) growth by producing phyto-hormones, facilitating nutrient uptake and improving stress tolerance. Furthermore, their bio-control properties, such as the production of antimicrobial compoundsand competition with pathogens, make them effective agents in reducing plant diseases.The findings underscore the potential of bacterial endophytes as eco-friendly alternatives to chemical fertilizers and pesticides. Integrating these microbial allies into agricultural practices can enhance crop productivity while minimizing environmental impact.

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