

Original Research Article

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Effect of Endophytic Bacteria on Tomato Plant Growth Promotion and Tomato Early Blight Management

B. Sai Sushma*, B. Vidya Sagar, S. Triveni and G. Uma Devi

Department of Plant Pathology, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad, India

*Corresponding author

ABSTRACT

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Early blight caused by *Alternaria solani* is a predominant constraint causing major yield losses in tomato. Twenty four isolates of endophytic bacteria were obtained from different plant tissues including root, stem and fresh leaves of tomato plants. The isolates were tested for their antagonistic activity against the causal organism of early blight in tomato, *A. solani* under in vitro conditions. The isolate EBT22 displayed maximum antifungal activity with an inhibition of 62.66 per cent. The five potential endophytic isolates were further tested for their effect on plant growth parameters of tomato cv. Arka Vikas and the isolate EBT18 recorded highest germination (88.88 per cent), root length (18.67 cm), shoot length (15.91 cm) and vigour index (3302). The isolate EBT18 recorded minimum PDI of 20.30 per cent when applied as seed treatment + soil drench with a per cent disease reduction of 57.94 compared to control under glass house conditions.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops in India. It is a good source of vitamins, minerals, organic acids, essential amino acids and dietary fibers. The productivity of tomato is low due to several abiotic and biotic stresses. Among the biotic factors associated with low yield, diseases occupy a predominant position. Tomato is attacked by many diseases as early blight (*Alternaria solani*), damping off (*Pythium ultimum*, *Phytophthora*), leaf spots (*Septoria* and *Stemphylium*), bacterial fruit canker (*Clavibacter michiganense* subsp.

michiganense), spotted wilt (virus), mosaic (virus) and nematodes in its growth stages. Among the fungal diseases, early blight caused by *Alternaria solani* (Ellis and Martin) Sorauer is the most destructive disease which causes severe damage during all the stages of plant development.

The disease appears in three stages causing collar rot, leaf blight and stem canker and becomes a serious problem during fruiting period in *kharif* (Datar *et al.*, 1981). The symptoms first appear on older leaves and then progress to younger leaves and on the fruits, the symptoms appear similar to those of leaf symptoms with dark concentric rings.

The pathogen causes seedling blight in tomato that result in development of dark lesions on rootlets. The calyx and blossom may also get affected by *Alternaria solani* in susceptible genotypes (Pandey *et al.*, 2003). Fungicides like mancozeb and captan are used to manage *Alternaria* blight. The most widely used approach for management of the disease is application of fungicides, leads to accumulation of fungicide residues, development of resistance in the pathogen, environmental pollution, and reduction of beneficial microbe population. Hence, the study was carried out to develop an ecofriendly management strategy for early blight disease in tomato by using endophytic bacteria.

Endophytic bacteria can be defined as those bacteria that can be extracted from surface sterilized plant tissue and do not harm the plant. After they gain entry into the plant, they may be either localised at the point of entry or spread through the plant (Hallman *et al.*, 1997). Endophytes enter into plant tissue primarily through the root zone. Aerial portions of the plant may also aid in entry of endophytes (Kobayashi and Palumbo, 2000). Endophytes synthesize bioactive compounds like alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols and phenols that stimulate plant growth and increase resistance to plant pathogens (Rosenblueth and Romero, 2006). Use of endophytic bacteria can be considered as a new source of biocontrol agents in the plant disease management (Backman *et al.*, 2008), as they share the same ecological niche as that of plant pathogens, which makes them suitable for biocontrol (Ryan *et al.*, 2008).

The present study evaluates the potential endophytes under in vitro and glass house conditions against *Alternaria* blight of tomato.

Materials and Methods

Geographical location

All the experiments were carried out in the glasshouse and laboratory at Department of Plant Pathology, and Department of Microbiology and Bioenergy, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana state.

Collection of diseased samples

Tomato leaves and fruits that showed early blight symptoms were collected from tomato fields in Agricultural Research Institute, Professor Jayashankar Telangana State Agricultural University, Hyderabad. The samples were brought in polythene bags to the laboratory for isolation and identification of the pathogen.

Isolation of pathogen

The isolation of the pathogen from early blight infected tomato leaves was carried out under aseptic conditions by the tissue segment method. Small bits of tomato leaves comprising of both diseased and healthy tissues were cut with a sharp sterilized blade. These bits were surface sterilized by immersing in 1% Sodium hypochlorite for one minute and then washed with three changes of sterile water and then blot dried on sterile paper towels. Later, they were transferred to Potato Dextrose Agar plates and incubated at 25±1° C for seven days.

Antagonistic activity of endophytic bacteria against *Alternaria solani* in vitro

The antifungal activity of all the isolated endophytic bacteria against *A solani* on PDA was evaluated by following dual-culture technique. Each individual treatment was

replicated three times and the colony diameter of the pathogen was measured 6 days after incubation at $25 \pm 2^\circ\text{C}$. The inhibition rate (Vincent, 1947) of the pathogen was calculated using the formula

$$R = (CD - TD / CD) \times 100$$

Where,

R = per cent inhibition of mycelial growth,
CD = Radial growth of test pathogen in control (mm), TD = Radial growth of test pathogen in treatment (mm)

Plant growth promotion of tomato by endophytic bacteria

The plant growth-promoting activity of the potential endophytic bacteria was assessed based on seedling vigour index by the standard roll towel method (ISTA, 1993).

The germination percentage, root length and shoot length of the individual seedlings was calculated. The vigour index of the seedlings was calculated using the formula given below (Abdul-Baki *et al.*, 1973).

Vigour index = (mean root length + mean shoot length) x % germination.

Evaluation of endophytic bacteria against *Alternaria solani* under glass house conditions

The tomato variety Arka Vikas was used in the field trial for the evaluation of potential endophytic bacteria against early blight disease. Seed treatment of the endophytic bacterial isolates was done prior to sowing. The seeds were inoculated with a bacterial inoculum of 10^8 CFU/ml. Soil drench with the bacterial suspension was carried out ten days after transplanting the seedlings. For the soil drench, the surrounding soil was drenched

with 100 ml of bacterial inoculum and for foliar spray, the inoculum was sprayed on to the surface of all the leaves after transplanting. The experiment was conducted in factorial design with nine treatments and four replications.

Results and Discussion

Screening of endophytic bacteria against *Alternaria solani* under *in vitro* conditions

Most of the bacteria were found to inhibit the pathogen by 31 to 63 per cent (Table 1). Among all the isolates, five isolates (EBT3, EBT8, EBT14, EBT18 and EBT22) were highly effective in inhibiting the mycelial growth of the pathogen. Maximum per cent inhibition was recorded by the isolate EBT22 (62.66) per cent while minimum (32.04) inhibition recorded by the isolate EBT24.

The isolate EBT8 and EBT18 showed 60.99 per cent and 53.84 per cent reduction over control. The isolates EBT3 and EBT 14 were on par with each other with per cent inhibition of 51.07 and 52.17 per cent respectively. The bacterial isolate EBT15 inhibited *A. solani* by 31.33 percent.

Among thirty four bacterial isolates tested for antagonism against phytopathogens, the gram positive isolates showed strong inhibition (Ghai *et al.*, 2007) and similarly in our current study, *Bacillus* isolates have inhibited the pathogen to a greater extent compared to other isolates.

The isolate EBT18 has also recorded siderophore production which might be the reason for the antagonistic activity of EBT18 against *A. solani* under *in vitro* (53.84 per cent inhibition) and glass house conditions. Similarly, the endophytic bacteria *Bacillus stratosphericus* strain LW-O3 isolated from *Lilium wardii* was found to be antagonistic

towards four fungal pathogens namely, *Botryosphaeria dothidea*, *Botrytis cinerea* and *Fusarium fujikuroi* and *Fusarium oxysporum* with an inhibition percentage of $74.56 \pm 2.35\%$, $71.91 \pm 2.87\%$, $69.54 \pm 2.73\%$ and $65.13 \pm 1.91\%$ respectively (Khan *et al.*, 2020).

Effect of endophytic bacteria on tomato plant growth

Germination percent

The results showed that on treatment with endophytic bacteria alone, isolate EBT18 has shown highest germination per cent of 88 per cent (Table 2). The isolates EBT22 and EBT8 were on par with each other with a germination per cent of 74.67 and 74 per cent respectively. The isolate EBT14 recorded 72 per cent germination followed by isolate EBT3 which has shown 70 per cent of germination.

When endophytic bacteria were tested for growth promotion, the *Bacillus subtilis* strain EPC016 and EPC5 have recorded a germination per cent of 93.5 and 88.5 per cent respectively (Subramaniam *et al.*, 2012).

The effect of eight endophytic bacteria isolated from weed plants were tested for promotion of growth parameters of tomato and all the bacterial strains increased germination up to 83 per cent but the *Bacillus* spp. strain PF3 was found to be most promising (Haddad *et al.*, 2013).

When the isolates of endophytic bacteria were combined with the pathogen and applied to the seed, the isolate EBT18 has shown highest germination per cent (80%) followed by the isolate EBT14 (71.67 %). The least germination per cent was recorded by isolates EBT3 and isolate EBT8 with germination of 62 and 64 per cent respectively.

Root length and shoot length

Of the five isolates tested alone, the isolate EBT18 has shown maximum root length of 18.67 cm and shoot length of 15.91 cm respectively. The isolate EBT8 and isolate EBT14 were on par with each other with root lengths of 14.63 cm and 14.50 cm, shoot lengths of 12.37 cm and 12.56 cm respectively. The isolate EBT22 recorded a root length of 12.26 cm and shoot length of 10.80 cm. However, the isolate EBT3 has recorded minimum root and shoot length of 10.88 cm and 8.17 cm.

Similar results were obtained when endophytic bacteria were applied, with the highest shoot length of 18.4 cm by the *Bacillus* isolate EPC016 and the highest root length of 16.1 cm by the *Bacillus* isolate EPC5 (Subramaniam *et al.*, 2012). Inoculation of endophytic bacteria on chilli seedlings resulted in a maximum shoot length of 21.4 cm by the isolate BETS 14 and a minimum shoot length of 13.5 by the isolate BECS1 (Amareesan *et al.*, 2012).

The isolates of endophytic bacteria when applied in combination with *A. solani*, the highest root length and shoot length were recorded by the isolate EBT22 with a root length of 15.37 cm and shoot length of 13.05 cm followed by EBT14 which showed a root length of 13.70 cm and shoot length of 12.02 cm. The isolate EBT22 when applied along with *A. solani* has shown root and shoot length of 11.01cm and 8.88 cm. The isolates EBT8 and EBT3 have shown a root length of 9.20 cm and 8.23 cm, a shoot length of 8.79 cm and 6.20 cm respectively.

Vigour index

The results revealed that when endophytic bacteria were applied alone to the seed, the isolate EBT18 recorded highest seedling

vigour index of 3302 followed by the isolate EBT14 that showed vigour index of 2492 (Table 2). A vigour index of 2014.77 was recorded by the isolate EBT8. The isolates EBT3 and EBT22 showed a vigour index of 1352 and 1714.73 respectively.

In the treatment of endophytic bacteria plus *A. solani*, the isolate EBT18 showed highest seedling vigour index of 2402.13 followed by the isolate EBT14 which has shown a vigour index of 1510.60. The least vigour index was recorded by the isolate EBT3 when combined with *A. solani* (728.33) which is less than the uninoculated control (1073.55).

The bacterial strain *Bacillus cereus* EHR1 has shown highest vigour index of 3603 in tomato followed by a vigour index with the respective values of 3247 and 3213 by *Pseudomonas* spp. PS1 and *Bacillus amyloliquefaciens* OS4 (Haddad *et al.*, 2013). The endophytic *Bacillus* isolates that have shown vigour index values of 3225.75 and 2814.30 respectively (Subramaniam *et al.*, 2012)].

It can be inferred (Table 2) that all the isolates when applied alone were found effective in promoting the growth parameters of tomato plant and all the endophytic bacterial isolates except EBT3 and EBT22. The isolate EBT8, isolate EBT14 and isolate EBT18 were effective in inhibiting the growth of pathogen and thereby resulting in enhancement of plant growth.

The superiority of the isolate EBT18 in plant growth might be due to the highest (+++) production of IAA by the isolate that resulted in plant growth promotion in standard roll towel method under *in vitro* conditions. Similarly, CEFR-3 (*Pseudomonas aeruginosa*), an endophytic bacteria isolated from chilli fruit for plant growth promotion produced higher amount of IAA and

increased the growth of the plant (Allu *et al.*, 2014).

Evaluation of endophytic bacteria against *Alternaria solani* under glass house conditions

All the treatments were effective in reducing the disease index of early blight caused by *A. solani*. However, the magnitude of disease reduction varied from treatment to treatment.

Among the treatments, the per cent disease index ranged from 20.30 per cent to 38.82 per cent in comparison with 48.33 per cent in control. The isolate EBT18 recorded minimum PDI of 20.30 per cent when applied as seed treatment + soil drench with a per cent disease reduction of 57.94 when compared to control whereas the highest PDI (38.82) was recorded by the isolate EBT3 when applied as foliar spray with a disease reduction of 19.60 per cent.

Among the five potential isolates of endophytic bacteria, the isolate EBT18 was found to be superior in reducing the per cent disease index of tomato early blight caused by *A. solani* irrespective of the treatments imposed followed by the isolate EBT14, except in treatments where the endophytic bacteria was applied as seed treatment + foliar spray. When the isolates were applied alone, the seed treatment recorded a PDI ranging from 28.05 to 36.27 per cent with the minimum (28.05) disease index by the isolate EBT18 and maximum (36.27) by the isolate EBT3. The foliar application resulted in a disease index in the range of 26.46 to 38.82 per cent with the minimum disease index by the isolate EBT18 and maximum disease index by the isolate EBT3. Soil drench recorded a per cent disease index ranging from 24.85 to 36.88 with the minimum disease index by the isolate EBT18 and maximum disease index by the isolate EBT3.

Table.1 Effect of endophytic bacteria on radial growth of *Alternaria solani*

S.No	Isolate	Radial growth of <i>A. solani</i>	Percent reduction over control
1	EBT1	69.90	0.00 (0.00)*
2	EBT2	69.90	0.00 (0.00)
3	EBT3	34.20	51.07 (45.59)
4	EBT4	47.40	32.18 (34.55)
5	EBT5	46.60	33.33 (35.25)
6	EBT6	69.90	0.00 (0.00)
7	EBT7	69.90	0.00 (0.00)
8	EBT8	27.26	60.99 (51.33)
9	EBT9	69.90	0.00 (0.00)
10	EBT10	69.90	0.00 (0.00)
11	EBT11	44.50	36.33 (37.05)
12	EBT13	47.13	32.57 (34.78)
13	EBT14	33.43	52.17 (46.23)
14	EBT15	48.00	31.33 (34.02)
15			0.00

	EBT16	69.90	(0.00)
16	EBT17	46.73	33.14 (35.13)
17	EBT18	32.26	53.84 (47.18)
18	EBT19	69.90	0.00 (0.00)
19	EBT20	44.80	35.90 (36.80)
20	EBT21	69.90	0.00 (0.00)
21	EBT22	26.10	62.66 (52.31)
22	EBT23	47.66	31.80 (34.31)
23	EBT24	48.20	31.04 (33.83)
24	EBT25	69.90	0.00 (0.00)
25	Control	69.90	0.00 (0.00)
CD @ 5%			2.50
SEm±			0.87
CV			6.30
*Figures in the arcsine transformed values			

Table.2 Effect of endophytic bacteria on growth parameters of tomato cv. Arka Vikas

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling vigour index
T1- EBT3+As	62.00 (51.92) *	8.23	6.20	728.33
T2- EBT3	70.00 (56.77)	10.88	8.17	1352.25
T3- EBT8+As	64.00 (53.11)	9.20	8.79	1108.26
T4- EBT8	74.00 (59.33)	14.63	12.37	2014.77
T5- EBT14+As	71.67 (57.83)	13.70	12.02	1510.60
T6- EBT14	72.00 (58.03)	14.50	12.56	2492.53
T7- EBT18+As	80.00 (63.42)	15.37	13.05	2402.13
T8- EBT18	88.00 (69.74)	18.67	15.91	3302.00
T9- EBT22+As	67.33 (55.13)	11.01	8.88	1067.39
T10- EBT22	74.67 (59.83)	12.26	10.80	1714.73
T11- As	42.67 (40.76)	7.31	5.69	405.81
T12- Control	68.00 (55.53)	8.94	7.50	1073.55
CD	2.88	1.15	1.14	73.8
SEm±	0.98	0.39	0.39	25.1
CV (%)	3.76	5.64	6.66	2.72

As-=*Alternaria solani*'
 *Figures in parenthesis are arcsine transformed values

Table.3 Effect of endophytic bacteria on per cent disease index (PDI) of tomato early blight caused by *Alternaria solani*

Treatment	Isolates of potential endophytic bacteria				
	EBT3	EBT8	EBT14	EBT18	EBT22
T ₁ - Seed treatment	36.27 (37.01)*	32.63 (34.82)	32.49 (34.73)	28.05 (31.96)	34.19 (35.76)
T ₂ - Foliar spray	38.82 (38.52)	34.21 (35.78)	30.88 (33.74)	26.46 (30.94)	31.94 (34.39)
T ₃ - Soil drench	36.88 (37.37)	33.66 (35.44)	28.96 (32.54)	24.85 (29.89)	34.44 (35.91)
T ₄ - Seed treatment + foliar spray	38.24 (38.18)	31.35 (34.03)	36.74 (37.29)	26.30 (30.84)	34.38 (35.88)
T ₅ - Seed treatment +soil drench	33.13 (35.12)	36.68 (37.26)	32.05 (34.46)	20.30 (26.76)	36.80 (37.32)
T ₆ - Foliar spray + soil drench	34.04 (35.67)	34.02 (35.66)	28.63 (32.33)	26.19 (30.76)	30.96 (33.79)
T ₇ - Seed treatment + foliar spray + soil drench	35.49 (36.55)	31.94 (34.39)	28.49 (32.24)	24.58 (29.70)	30.12 (33.27)
T ₈ - Mancozeb	16.66 (24.06)	16.66 (24.06)	16.66 (24.06)	16.66 (24.06)	16.66 (24.06)
T ₉ - Control	48.33 (44.02)	48.33 (44.02)	48.33 (44.02)	48.33 (44.02)	48.33 (44.02)

*Figures in the parenthesis are arcsine transformed values

Fig.1 Antagonistic activity of different isolates of endophytic bacteria against *Alternaria solani*



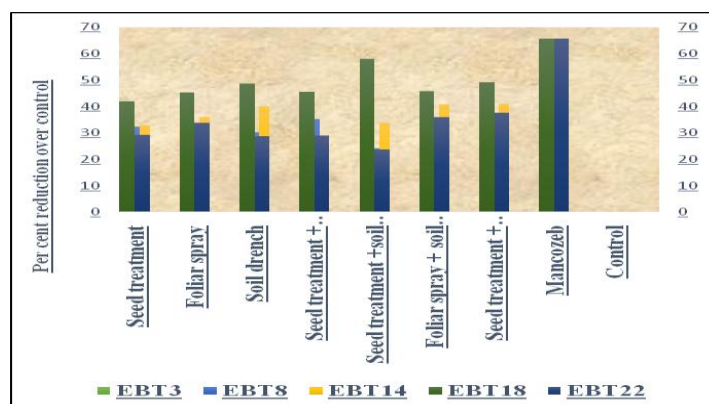
Table.4 Effect of endophytic bacteria on per cent disease control (PDC) of tomato early blight caused by *Alternaria solani*

Treatment	Isolates of potential endophytic bacteria				
	EBT3	EBT8	EBT14	EBT18	EBT22
T ₁ - Seed treatment	24.88 (29.88)*	32.42 (34.68)	32.72 (34.85)	41.94 (40.34)	29.19 (32.66)
T ₂ - Foliar spray	19.60 (26.21)	29.14 (32.63)	36.00 (36.83)	45.23 (42.25)	33.88 (35.58)
T ₃ - Soil drench	23.62 (29.03)	30.25 (33.31)	40.07 (39.25)	48.55 (44.15)	28.75 (32.41)
T ₄ - Seed treatment + foliar spray	20.81 (27.09)	35.11 (36.32)	23.92 (29.25)	45.55 (42.43)	28.85 (32.47)
T ₅ - Seed treatment +soil drench	31.39 (34.05)	24.07 (29.36)	33.68 (35.45)	57.94 (49.56)	23.76 (29.07)
T ₆ - Foliar spray + soil drench	29.55 (32.91)	29.60 (32.94)	40.73 (39.64)	45.79 (42.56)	35.87 (36.76)
T ₇ - Seed treatment + foliar spray + soil drench	26.48 (30.92)	33.95 (35.61)	41.03 (39.81)	49.13 (44.48)	37.62 (37.81)
T ₈ - Mancozeb	65.56 (54.05)	65.56 (54.05)	65.56 (54.05)	65.56 (54.05)	65.56 (54.05)
T ₉ - Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	26.88 (29.35)	31.12 (32.10)	34.861 (34.35)	44.41 (39.98)	31.50 (32.31)

Factors	CD	SE (d)	SE (m)
Treatments (T)	1.527	0.771	0.545
Isolates (I)	1.138	0.575	0.406
T X I	3.414	1.724	1.219

* Figures in the parenthesis are arcsine transformed values

Fig.2 Effect of potential isolates of endophytic bacteria on per cent disease reduction of early blight caused by *Alternaria solani*



When two application methods were combined, Seed treatment + foliar spray recorded a per cent disease index in the range of 26.30 to 38.24 and the minimum disease index was recorded by the isolate EBT22 and maximum disease index was shown by the isolate EBT3. Seed treatment + soil drench recorded a per cent disease index in the range of 20.30 to 33.13 with the minimum index shown by the isolate EBT22 and maximum by the isolate EBT3. Foliar spray + soil drench recorded a per cent disease index in the range of 26.19 to 34.04 with the minimum disease index by the isolate EBT18 and maximum index by the isolate EBT3.

When seed treatment, foliar spray and soil drench were combined together, it resulted in a disease index in the range of 24.58 to 35.49 with the minimum disease index by the isolate EBT18 and maximum disease index was recorded by the isolate EBT3.

It is evident (Table 3 and 4) that there is significant difference between the isolates EBT3, EBT14 and EBT18 and the isolates EBT8 and EBT22 are on par with each other. The isolate EBT18 was found to be superior among all the isolates followed by the isolate EBT14 evaluated for the management of early blight under the glass house conditions.

The effectiveness of three delivery methods for introducing endophytic bacteria into tomato plants and also the efficacy of delivery methods in reducing bacterial wilt of tomato were studied and the results indicated that the highest suppression resulted from the isolate B2 which gave 71.88 per cent reduction when applied as seed treatment + soil drench (Algam *et al.*, 2005).

The isolate EBT18 has also recorded siderophore production which might be the reason for antagonistic activity of EBT18 against *A. solani* under *in vitro* (53.84 per

cent inhibition) and glass house conditions. When the endophytic bacteria isolated from *Solanum elaeagnifolium* stem were screened for growth promotion of tomato plants and suppression of *Fusarium* wilt, the inhibitory effect of *B. tequilensis* strain SV104 may be due to the siderophore production ability of the strain (Abdallah *et al.*, 2016). *Streptomyces* strain AzR-051 isolated from *Azadirachta indica* A. Juss was found to produce higher amounts of IAA and siderophore which might be possible reason for plant growth promotion of tomato seedlings and significant antagonistic activity against *A. alternata* (Verma *et al.*, 2011).

In conclusion this study illustrates the importance of endophytic bacteria in plant growth promotion as well as antifungal activity. All the isolated endophytic bacteria were evaluated for their antagonistic effect against *Alternaria solani* under *in vitro* using dual culture technique. Among all the isolates, five isolates (EBT3, EBT8, EBT14, EBT18 and EBT22) were highly effective in inhibiting the mycelial growth of *Alternaria solani*. The isolate EBT22 recorded maximum inhibition of 62.66 per cent and the isolate EBT8 was better in inhibiting *A. solani* by 60.99 per cent. Minimum inhibition of 32.04 per cent was recorded by the isolate EBT24. The five potential isolates that were found to be potential antagonists against *A. solani* in dual culture technique were tested for plant growth promotion and reduction of per cent disease index of early blight disease under glass house conditions.

This research concluded that the endophytic bacteria from *Lycopersicon esculentum* Mill produced one or more characteristics that are different involved in plant growth promotion and showed antagonistic activity against *Alternaria solani*. Hence, it is proposed that the potential strain observed in the present study can be deployed as bio-inoculant to

increase tomato growth and to control early blight disease.

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