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RESEARCH ARTICLE

ANTAGONISTIC ACTIVITY OF ENDOPHYTIC MICROORGANISMS AGAINST BACTERIAL WILT DISEASE OF TOMATO

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A B S T R A C T

Bacterial wilt caused by *Ralstonia solanacearum* is one of the major constraints for the cultivation of tomato in Kerala and cent per cent crop loss has been estimated in susceptible varieties. Biocontrol using endophytic microorganisms is one of alternative control methods to support agriculture sustainability. The objective of these experiments are to isolate endophytes from root and stem of healthy tomato plants from 16 locations of north, central and south Kerala and to estimate their biocontrol potential against the bacterial wilt pathogen. Among 154 endophytic isolates obtained, 12 out of 79 bacteria, 16 out of 68 fungi, and four out of seven actinomycetes were antagonistic to*R. solanacearum*in *in vitro*. Among them, five bacteria, eight fungi, and two actinomycetes were promising *in planta*.

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INTRODUCTION

Bacterial wilt disease incited by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* is one of the most destructive diseases of solanaceous crops in tropics, subtropics, and warm temperate regions of the world. In Kerala, the yield loss due to the bacterial wilt incidence ranged from 20 to cent per cent depends upon the varieties (Sadhankumar 1995). Biocontrol of plant pathogen is becoming an important component of integrated disease management. In view of the hazardous impact of pesticides and other agrochemicals on the ecosystem, biocontrol of plant diseases as an alternate strategy has received increasing attention in recent years. Therefore, the focus on the management of plant diseases has been shifted from chemical pesticides to more ecofriendly biopesticides to reduce environmental hazards and minimize the risk of development of pesticide resistant strains of plant pathogens.

A novel method of biological control using endophytes has entered the arena of disease management with attempts to make the plant, defend itself from the pathogens. The beneficial effects that the endophytes can confer on plants have made their role highly significant in biological control of diseases in various crops (Bargabus *et al*. 2004; Kloepper *et al*. 2004).Therefore, an attempt was made to study the efficacy of the endophytic microorganisms isolated from tomato against bacterial wilt disease.

MATERIALS AND METHODS

Collection of samples

Healthy tomato plants were collected from 16 different locations representing north, central and south Kerala namely Padanakkad (Kasaragod District), Panniyur (Kannur), Malappuram and Tavanur (Malappuram), Ozhalapathy and Eruthempathy (Palakkad), Vellanikkara, Mannuthy and Cherumkuzhy (Thrissur), Kalamassery (Ernakulam), Kumarakom (Kottayam), Alappuzha and Kayamkulam (Alappuzha), and Kallambalam, Vellayani and Amburi (Thiruvananthapuram).

Isolation of endophytes from the samples of different locations

The endophytic microorganisms *viz.* bacteria, fungi and actinomycetes were isolated from the samples collected from different locations using the standardized dilution factors for each organism and as per the protocols. The predominant microbial colonies were selected, purified and the pure cultures were maintained on potato dextrose agar (PDA) slants for further studies. The isolates were numbered representing the place of collection, plant part from which it was isolated, the type of organism and the Arabic numerals in serial order.

In vitro evaluation of isolated endophytes for antagonistic activity against R. solanacearum

The isolated endophytes were tested for their antagonistic reaction against *R. solanacearum* adopting simultaneous antagonism method. Two day old bacterial, five day old fungal and seven day old actinomycetes cultures were used for *in vitro* evaluation. The culture media which favour the growth of both antagonists and the pathogen *viz.*PDA for fungi and actinomycetes and NA for bacteria were used.

In vitro evaluation of endophytic bacteria against R. solanacearum

A total of 79 bacterial endophytes obtained from different

locations were screened against *R. solanacearum* by point inoculation technique. For the preliminary evaluation, the bacterial suspension of the pathogen was prepared by adjusting the concentration to 10^8cfu/ml and 0.1 ml of the suspension was spread on NA medium using a glass spreader. A loopful of four different antagonistic organisms were spotted on the bacterial lawn at four equidistant points of 2 cm from the plate periphery. Plates were incubated at 28 ± 2^0C for 48-72 h and observed for the inhibition of the pathogen. The organisms that showed antagonistic reaction were selected for further studies.

Bacterial isolates, which showed antagonistic reactions in the initial screening, were tested individually by agar well method. Bacterial lawn was prepared on nutrient agar mediated plates. Eight mm sized wells were made at the centre of the plates using a sterile cork borerand these wells were filled with 50 µl of 48 h old endophytic bacterial suspension. Plates with pathogen alone served as control. Petridishes were incubated for 48 h with three replications for each antagonist. The diameter of inhibition zone was measured and the per cent inhibition was calculated using the formula suggested by Vincent (1927).

$$\text{Per cent Inhibition} = \frac{C - T}{C} \times 100$$

Where C = Radial growth of pathogen in control (cm)
T = Radial growth of pathogen in treatment (cm)

In vitro evaluation of fungal endophytes against R. solanacearum

The isolated 68 fungal endophytes were screened against *R. solanacearum*. In the initial screening, the pathogen was spread on PDA mediated Petri plates and four different candidate organisms were placed simultaneously at four corners of the culture plates at equidistant points. These plates were incubated at 28 ± 2^0Cfor five days. Those organisms showing antagonistic reactions were selected for further studies.

The antagonistic fungi selected from preliminary screening were then tested individually for its antagonistic property. Eight mm mycelial discs of selected fungi were placed individually at the centre of the PDA medium seeded with the pathogen and the plates were incubated at room temperature for five days. Plates with endophytic fungi alone served as control. Observations were taken till full growth in control plates, and percent inhibition of the pathogen was calculated.

In vitro evaluation of endophytic actinomycetes against R. solanacearum

Seven endophytic actinomycetes were tested against *R. solanacearum.* For preliminary screening, four mm discs of the antagonists were placed at four corners on the bacterial lawn at equidistance and incubated for seven days at room temperature.

The promising antagonists were selected and tested individually using agar well method in triplicates. The diameter of the inhibition zone was measured and the percent inhibition of the pathogen was calculated.

Screening of selected endophytes for antagonistic potential under in planta condition

The promising endophytes selected from *in vitro* studies were evaluated for their antagonistic activity using a highly susceptible variety, PKM-1under *in planta* condition. Nursery was raised in earthern pots containing sterilized potting mixture consisting soil, sand, and cow dung @ 2:1:1. The potting mixture was sterilized using 4 per cent formaldehyde solution and covered with a polythene sheet for 10 days. It was then kept open for two days with intermittent raking to remove the traces of formaldehyde fumes. This potting mixture was used for raising the nursery and for growing tomato seedlings in polybags.

For the preparation of antagonist suspensions, the selected endophytic organisms were grown in different liquid media like potato dextrose broth (PDB) for fungi, nutrient broth (NB) for bacteria, and Kenknight broth for actinomycetes and incubated for 7, 2, and 14 days respectively.

Twenty five day old seedlings were planted in black polybags of size 30 cm x 20 cmcontaining sterilized potting mixture. The antagonists were applied to the soil at the time of planting @ 30ml/plant having concentrations of 10^6 spores/ml for fungi, 10^8cfu/ml for bacteria and 10^5cfu/ml for actinomycetes. Challenge inoculation of the pathogen was done with fresh bacterial ooze suspension having concentration of OD_{600} = 0.3 @ 10 ml/plant by soil drenching with wounding at 30days after planting. This procedure was adopted in all other *in planta*/ pot culture experiments. Three replications with 12 plants in each were maintained for the experiments. Wilt incidence was recorded 10 days after inoculation. Per cent wilt incidence was calculated using the following formula.

$$\text{Per cent wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants observed}}$$

Statistical analysis

Statistical analysis was performed using SPSS 20 software program. The data were analysed using Duncan's Multiple Range Test (DMRT).

RESULTS

Isolation and enumeration of endophytic microbial population from collected samples

Endophytic microorganisms were isolated from both root and stem of healthy tomato plant samples collected from 16 locations. The isolated microbial population varied with the plant samples and the population was higher in root as compared to stem samples. Microbial population varied significantly with the samples collected from different locations.

From the enumerated microbial population, the predominant colonies of 154 microorganisms consisting of 79 bacteria, 68 fungi, and seven actinomycetes were selected. Among these, 44 bacteria, 42 fungi and four actinomycetes were from root and 35, 26 and three respectively from stem samples.

In vitro evaluation of endophytes against R. solanacearum

A preliminary *in vitro* screening was carried out with the isolated 154 endophytes including 79 bacteria, 68 fungi and seven actinomycetes.

Table 1 *In vitro* evaluation of bacterial endophytes against *Ralstoniasolanacearum*

Sl. No	Isolates	* Per cent inhibition
1	PRB-1	40.56^{defg}
2	PyRB-2	13.63^{no}
3	PyRB-4	16.11^{lmno}
4	MRB-1	20.00^{kl}
5	MSB-1	48.33^{b}
6	TRB-1	41.11^{def}
7	TSB-1	14.78^{mno}
8	TSB-2	20.00^{kl}
9	TSB-3	18.33^{klmn}
10	ORB-1	35.00^{h}
11	ORB-2	36.11^{gh}
12	OSB-3	41.96^{de}
13	VSB-1	41.67^{de}
14	VSB-2	41.11^{def}
15	VRB-3	40.00^{efg}
16	VRB-4	18.89^{klm}
17	VRB-5	25.56^{ij}
18	MyRB-3	14.18^{mno}
19	CSB-1	43.07^{cde}
20	EkRB-1	53.89^{a}
21	EkSB-1	40.85^{def}
22	EkSB-2	33.07^{h}
23	KuSB-1	27.78^{i}
24	KuSB-2	36.67^{fgh}
25	KuRB-1	34.44^{h}
26	KuRB-3	49.45^{b}
27	KuRB-4	46.67^{bc}
28	KRB-2	45.00^{bcd}
29	KaSB-1	22.22^{jk}
30	AmRB-1	12.78^{o}
31	AmRB-3	14.74^{mno}

* Mean of three replications Treatment means with same alphabet in superscript, do not differ significantly

In vitro evaluation of bacterial endophytes against R. solanacearum

Among 79 bacteria screened, 31 isolates showed antagonistic reaction against *R. solanacearum*. It is observed from Table 1 that, per cent inhibition of the pathogen varied from 12.78 to 53.89 with maximum by EkRB-1 (53.89 %) followed by KuRB-3 (49.45 %) and MSB-1 (48.33 %) and the lowest inhibition was exhibited by AmRB-1 (12.78 %). Five isolates *viz.* OSB-3, KuRB-3, KuRB-4, KRB-2 and EkSB-1 also showed high pigment production. Based on per cent inhibition (> 40 %), 12 bacterial endophytes were selected for *in planta* studies.

In vitro evaluation of endophytic fungi against R. solanacearum

Out of 68 fungi tested, 27 showed antagonistic property against the pathogen and the results are presented in Table 2. It is found that, the per cent inhibition of the pathogen ranged from 11.67 to 66.67 with maximum by ARF-2 (66.67 %) followed by ERF-1 (60 %). The isolates PSF-1, MyRF-1, VyRF-1, ASF-3 and VSF-3 also showed antagonistic activity with more than 50 per cent inhibition.

Most of the isolates showed both lysis and overgrowth type of antagonism. Isolates PSF-1, MRF-2, TRF-2, ESF-2, VRF-1 and CSF-1 were found to produce metabolites. Based on per cent inhibition and metabolite production, 16 fungal endophytes were selected. Of the seven actinomycetes screened, only four isolates showed antagonistic activity against *R. solanacearum* of which the isolate, ORA-1 recorded maximum inhibition (27.22 %) and was on par with VRA-1 (26.67 %) (Table 3).

Table 2*In vitro* evaluation of fungal endophytes against *R. solanacearum*

Sl. No	Isolates	* Per cent inhibition	Mechanism	Other observations		
				Growth	Sporulation	Metabolite production
1	PRF-1	41.67^{fgh}	L	++	+	-
2	PRF-2	44.44^{fg}	L & O	+++	++	-
3	PSF-1	59.44^{abc}	O	++	++	++
4	PyRF-1	21.11^{jkl}	L & O	++	+	-
5	PyRF-2	36.11^{ghi}	L	++	+	-
6	MRF-2	16.67^{l}	L & O	+	++	++
7	TRF-2	44.44^{fg}	L & O	+++	++	+++
8	TRF-3	26.67^{ijk}	O	+++	++	-
9	TSF-1	21.11^{jkl}	L & O	+++	++	-
10	ESF-2	11.67^{l}	L	++	++	++
11	ERF-1	60.00^{ab}	L & O	++	++	-
12	VSF-I	27.22^{ijk}	L	+++	++	-
13	VSF-3	50.00^{cdef}	L & O	++++	+++	-
14	VRF-1	12.22^{l}	L	+++	+++	++++
15	MyRF-1	55.00^{bcd}	L	++	+	-
16	CRF-1	31.67^{i}	L & O	++	+++	-
17	CSF-1	42.78^{fg}	L & O	+++	+++	++++
18	EkSF-2	32.78^{hi}	L	+++	+++	-
19	ARF-1	30.00^{ij}	L & O	++	+	-
20	ARF-2	66.67^{a}	L & O	+++	+++	-
21	ASF-1	28.89^{ijk}	L	++	+++	-
22	ASF-2	45.56^{defg}	L & O	+++	+++	-
23	ASF-3	51.11^{bcdef}	L & O	++	++	-
24	VyRF-1	54.44^{bcde}	L	+++	+++	-
25	AmSF-1	19.44^{kl}	L	++	++	-
26	KaRF-3	45.00^{efg}	L & O	++	++	-
27	KaSF-2	43.33^{fg}	L	++	++	-

* Mean of three replications; Treatment means with same alphabets in superscript, do not differ significantly; Growth:++++ - Very fast, +++ - Fast, ++ - Average, + - Slow Sporulation: +++ - Good, ++ - Average, + - Poor ; Metabolite: ++++ - Very high, +++ - High, ++ - Medium, - - Nil; L- lysis O-Overgrowth

In vitro evaluation of endophytic actinomycetes against R. solanacearum

All the four isolates, which showed inhibitory effect on the pathogen, were selected for *in planta* experiment.

Table 3 *In vitro* evaluation of endophyticactinomycetes against *R.solanacearum*

Sl. No	Isolates	Per cent inhibition
1	PyRA-1	21.11 [b]
2	ORA-1	27.22 [a]
3	VRA-1	26.67 [a]
4	AmRA-1	16.67 [b]

* Mean of three replications
Treatment means with same alphabet in superscript, do not differ significantly

Screening of selected endophytes against the bacterial wilt disease under in planta condition

Sixteen fungi, 12 bacteria and four actinomycetes selected from *in vitro* experiments were screened against the bacterial wilt pathogen under *in planta* condition using highly susceptible variety, PKM -1 and the wilt incidence recorded at 10 days after inoculation are furnished in Table 4, 5 and 6.

Table 4 *In planta* screening of bacterial endophytes against *R.solanacearum*

Tr. No.	Bacterial endophytes	*Per cent wilt incidence 10 DAI	Per cent efficiency over control
1	PRB-1	44.44^{cde} (0.73)	52.94
2	MSB-1	44.44^{cde} (0.73)	52.94
3	TRB-1	38.89^{cde} (0.67)	58.82
4	OSB-3	77.78^{ab} (1.09)	17.64
5	VSB-1	22.22^{e} (0.48)	76.47
6	VSB-2	55.56^{bcd} (0.84)	41.17
7	CSB-1	38.89^{cde} (0.67)	58.82
8	EkRB-1	27.78^{de} (0.47)	70.59
9	EkSB-1	27.78^{de} (0.54)	70.59
10	KuRB-3	61.11^{bc} (0.90)	35.29
11	KuRB-4	66.67^{abc} (0.96)	29.41
12	KRB-2	50.00^{bcde} (0.79)	47.06
13	Control	94.44^{a} (1.29)	--

* Mean of three replications DAI – Days after inoculation
Treatment means with same alphabets in superscript, do not differ significantly
Figures in parenthesis are arc-sine transformed values

In planta screening of bacterial endophytes against bacterial wilt pathogen

Data presented in Table 4 showed that, all bacterial endophytes were superior to control in reducing wilt incidence. The incidence varied from 22.22 to 77.78 against 94.44 per cent in control at 10 DAI. The isolate, VSB-1 showed lowest incidence of 22.22 per cent followed by EkRB-1 and EkSB-1(27.78 %) with 76.47 and 70.49 per cent efficiency over control respectively. Five bacterial isolates which showed less than 40 per cent incidence were selected for mutual compatibility test.

Table 5*In planta* evaluation of fungal endophytes against *R. solanacearum*

Tr. No	Fungal endophytes	*Per cent wilt incidence 10 DAI	Per cent efficiency over control
1	PRF-2	55.56^{cde} (0.84)	44.44
2	PSF-1	38.89^{de} (0.67)	61.11
3	MRF-2	66.67^{bc} (0.96)	33.33
4	TRF-2	88.89^{ab} (1.22)	11.11
5	ESF-2	61.11^{cd} (0.91)	38.89
6	ERF-1	33.33^{e} (0.61)	66.67
7	VSF-3	33.33^{e} (0.61)	66.67
8	VRF-1	38.89^{de} (0.67)	61.11
9	MyRF-1	22.22^{f} (0.48)	77.78
10	CSF-1	27.78^{f} (0.55)	72.22
11	ARF-2	72.22^{bc} (1.02)	27.78
12	ASF-2	61.11^{cd} (0.90)	38.89
13	ASF-3	38.89^{de} (0.67)	61.11
14	VyRF-1	77.78^{bc} (1.09)	22.22
15	KaRF-3	38.89^{de} (0.67)	61.11
16	KaSF-2	72.22^{bc} (1.02)	27.78
17	Control	100.00^{a} (1.37)	--

* Mean of three replications
DAI – Days after inoculation
Treatment means with same alphabets in superscript, do not differ significantly
Figures in parenthesis are arc-sine transformed values

In planta evaluation of fungal endophytes against the pathogen

Data furnished in Table 5 revealed that, all fungal endophytes were superior to control in reducing disease incidence, which varied from 22.22 to 88.89 per cent against cent per cent in control at 10 DAI. Among the 16 fungal endophytes tested, all isolates except four showed high wilt incidence. MyRF-1 isolate showed minimum incidence of 22.22 per cent with 77.78 per cent efficiency over control and was on par with CSF-1, which showed 27.78 per cent incidence and 72.22 per

Table 6 *In planta* evaluation of endophyticactinomycetes against *R.solanacearum*

Tr. No	Actinomycetesendophytes	*Per cent wilt incidence 10 DAI	Per cent efficiency over control
1	AmRA-1	83.33^{a} (9.13)	16.67
2	ORA-1	33.33^{b} (5.69)	66.67
3	VRA-1	38.89^{b} (6.25)	61.11
4	PyRA-1	88.89^{a} (9.45)	11.11
5	Control	100.00^{a} (10.02)	--

* Mean of three replications DAI – Days after inoculation
Treatment means with same alphabets in superscript, do not differ significantly
Figures in parenthesis are square root transformed values

cent disease reduction. The other two isolates, VSF-3 and ERF-1 showed 33.33 per cent incidence. Four isolates, which showed less than 40 per cent incidence, were also selected for further studies.

In planta evaluation of endophytic actinomycetes against the pathogen

The four actinomycete isolates showed wilt incidence ranging from 33.33 to 88.89 per cent at 10 DAI with minimum by ORA-1 (33.33%) recording 66.67 per cent efficiency over control and was on par with VRA-1 (38.89%) showing 61.11 per cent disease reduction (Table 6). Isolates, AmRA-1 and PyRA-1 were least effective which recorded more than 80 per cent incidence.

DISCUSSION

Plants are in continuous association with microbes which interact with them in positive, negative or neutral ways. Endophytes are microbial entities that colonize living plant tissues and most of them act as symbionts in the host plant. These endophytes could become better biocontrol agents compared to rhizospheric microbes because they do not compete for nutrition and/or niche in apoplast. *In vitro* studies are useful for identifying likely candidates for biocontrol and for understanding the mechanisms by which they inhibit pathogens (Mejia *et al.* 2008). Hence in this study, endophytic isolates obtained were screened for *in vitro* inhibitory effect on the bacterial wilt pathogen, *R. solanacearum*. Of the 79 endophytic bacteria, 31 were able to exert antagonism with 12.78 to 53.89 per cent inhibition with maximum exhibited by EkRB-1, a bacterial endophyte from Ernakulam. Sturz *et al*.(1998) isolated endophytic bacteria from potato and clover of which 74 percent showed varying levels of *in vitro* antibiosis to the clover and potato pathogens. Mathew (2006) also observed the inhibitory effect of endophytic bacteria isolated from black pepper on *R. solanacearum*of chilli. Nawangsih *et al.* (2011) reported the antagonistic effect of 17 endophytic bacteria isolated from tomato stem on the pathogen,*R. solanacearum*. FENG *et al.* (2013) also reported that, out of 41 endophytic bacterial isolates obtained from tomato, only six were found to have *in vitro* antagonistic property against *R. solanacearum*. Similar findings have been recorded by Purnawati *et al.* (2014) who also noticed the antagonistic activity of the bacterial endophytes of tomato against *R. solanacearum*.

In the screening of 68 fungal endophytes, 27 showed antagonism ranged from 11.67 to 66.67 per cent inhibition of which ARF-2, a fungal endophyte from Alappuzha showed maximum antagonistic activity on the bacterial wilt pathogen. Many workers have reported the antagonistic ability of endophytic fungi against different pathogens. Haiyan *et al.* (2005) observed that, of the 130 endophytic fungi isolated from chinese medicinal plants evaluated, only 30 per cent exhibited antagonistic activity. Similarly, Mathew (2006) also observed *in vitro* antagonistic activity of endophytic *Trichoderma* spp. against *R. solanacearum* of chilli. Kim *et al.* (2007) also reported that, endophytic fungi isolated from vegetable plants showed *in vitro* antagonism against *Pythiumultimum*, *P.infestans* and *P. capsici*.

In vitro evaluation of seven endophytic actinomycetes showed varying level of antagonism ranging from 16.67 to 27.22 per cent inhibition with maximum by Ozhalapathy isolate, ORA-1. The antagonistic effect of endophytic actinomycetes against *R. solanacearum* has already been reported by earlier workers. Moura *et al.* (1998) observed that, endophytic actinomycetes isolated from the root tissues of tomato showed cent per cent inhibition of the pathogen. Moura &DE-Romero (1999) also noted high inhibitory effect of endophytic actinomycetes isolated from various hosts against *R. solanacearum.* This study also supports the findings of Sreeja (2011) who recorded 8.14 - 29.25 per cent inhibition of *R. solanacearum* with the endophytic actinomycetes of tomato from various locations of Kerala.

The ability of microorganisms to inhibit the pathogen under *in vitro* condition does not necessarily imply the same ability *in vivo*. Hence, it has become pertinent to evaluate the efficiency of selected endophytes under *in planta* condition. Among the endophytic isolates, 16 fungi, 12 bacteria and four actinomycetes which exhibited better antagonism in the *in vitro* experiment, were evaluated for their performance in plants. *In planta* evaluation showed marked variation in the level of disease reduction brought about by these isolates and five bacteria, eight fungi and two actinomycetes which showed higher efficiency, recording more than 50 per cent reduction over control were selected. There are number of ways by which antagonists suppress the growth of pathogen of which production of volatile metabolites, lytic enzymes, induction of host resistance and antagonistic proteins are important. Similarly, Mathew (2006) and Sreeja (2011) also studied the efficacy of endophytic fungi, bacteria, and actinomycetes against *R. solanacearum* of chilli and tomato under *in planta* condition. Summing up the findings of *in vitro* and *in planta* experiments, it is observed that, the isolates which showed high antagonistic activity under *in vitro* condition were not that effective in *in planta* condition except the bacterial isolate EkRB-1, fungal isolate MyRF-1, and actinomycete ORA-1, which performed well under both conditions. The lack of correlation between *in vitro* and *in vivo* effectiveness of biological control had already been observed by Ran *et al.* (2005) who also reported that, fluorescent pseudomonads sometimes succeeded as a biocontrol agent *in vitro* or under controlled conditions but failed under pot and field conditions. However, the present studies reveal the possibility of exploitation of endophytic microorganisms for the better management of bacterial wilt disease.

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References

1. Bargabus R.L., Zidack N.K., Sherwood J.E., Jacobsen B.J. (2004): Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. Biological Control, 30:342-350.

2. Feng H., Li Y., Liu Q.(2013): Endophytic bacterial communities in tomato plants with differential resistance to *Ralstonia solanacearum*. *African Journal of Microbiological Research*,7: 1311-1318.
3. Haiyan Li., Qing C., Zhang Y.,Zhao Z. (2005): Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. World *Journal of Microbiology and Biotechnology*,21: 1515-1519.
4. Kim H. Y., Choi G. J., Lee H. B., Lee S. W., Lim H. K., Jang K. S., Son S.W., Lee S. O., Cho K. Y., Sung N.D.,Kim J.C. (2007): Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. Letters in Applied Microbiology, 44:332-337.
5. Kloepper J.W., Ryu C.M., Zhang S. (2004): Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology, 94: 1259-1266.
6. Mathew S.K. (2006): Biocontrol consortium for the management of bacterial wilt of chilli and *Phytophthora* rot of black pepper and vanilla.(KSCSTE project) Annual Report, Kerala Agricultural University, Thrissur, 28p.
7. Mejia L. C., Rojas E. I., Maynard Z., Van Bael S., Arnold A. E., Hebbar P., Samuels G. J., Robbins N., Herre E. A. (2008):Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biological Control, 46: 4-14.
8. Moura A. B., da-Romeiro R. S., Naves M. C. P. (1998): Bioassay for mass assessment of antagonistic actinomycetes against *Ralstonia solanacearum* on tomato crop. Pesquisa- Agro pecuaria-Brasileira, 3 (2): 2065-2072.
9. Moura A.B., da-Romeiro R. S. (1999):*In vitro* evaluation of actinomycetes as antagonists to *Ralstonia solanacearum*. Ciencia Agrotechnology, 23(2): 281-28.
10. Nawangsih A. A., Damayanti I., Wiyono S., Kartika, J. G. (2011): Selection and characterization of endophytic bacteria as biocontrol agents of tomato bacterial wilt disease. HAYATI *Journal of Biosciences*, 18(2): 66.
11. Purnawati A., Sastrahidayat I. R., Abadi A. L., Hadiastono, T. (2014):Endophytic Bacteria as Biocontrol Agents of Tomato Bacterial Wilt Disease. *Journal of Tropical Life Science*,4 (1): 33-36.
12. Ran L. X., Liu C. Y., Wu G. J., Van Loon L. C., Bakker P. A. H. M. (2005): Suppression of bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. in China. Biological Control, 32(1): 111-120.
13. Sadhankumar P.G. (1995): Incorporation of resistance to fruit cracking in a bacterial wilt resistant genetic background in tomato. Ph.D thesis, Kerala Agricultural University, Thrissur, 151p.
14. Sreeja S.J. (2011): Bioefficacy of endophytic actinomycetes on plant growth promotion and management of bacterial wilt in tomato. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 73p.
15. Sturz A.V., Christie B.R., Matheson B.G. (1998): Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. Canadian Journal of Microbiology, 44: 162-167.
16. Vincent J.M. (1927): Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159: 850.

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