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Molecular Identification of *Fusarium* spp. from Wilt-infected Tomato and Brinjal Plants in Selected Regions of Sri Lanka and Endophytic Bacteria as a Potential Option for Disease Management

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***ABSTRACT**: Fusarium wilt caused by Fusarium spp. is an economically important fungal disease of tomato and brinjal production areas in Sri Lanka. The study was carried out to identify Fusarium isolates infecting tomato and brinjal, and endophytic antagonists bacteria against Fusarium wilt pathogen. The infected tomato and brinjal samples were collected from farmers' fields in Matale, Kandy, Nuwara Eliya and Badulla districts and PCR was conducted using primers specific for species, races and formae speciales. Eight, nine and five isolates were identified as Fusarium solani, Fusarium oxysporum f. sp. lycopersici race 1 and Fusarium oxysporum f. sp. radicis-lycopersici, respectively from the wilt-infected tomato and brinjal collected from the four districts. Thirty endophytic bacterial isolates were isolated from healthy tomato and brinjal stems were antagonistic against Fusarium oxysporum f. sp. lycopersici and Fusarium solani. Molecular identification revealed that Pseudomonas geniculata strain ICPH-14, Pseudomonas sp. strain SB 904 (E7), Delftia tsuruhatensis strain MTQ 1, Stenotropomonas maltophilia strain ATCC 13637, Stenotropomonas pavanii strain ICB 89, and Bacillus velezensis strain C19 were among the potential endophytic antagonists.*

***Keywords*:** *Antagonism, endophytes, formae speciales, Fusarium, 16S rRNA*

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* and *Fusarium solani* is an economically important fungal disease of Solanaceous crops throughout the world. As *F. oxysporum* species are phylogenetically diverse and well known as a mycotoxin producer, its precise identification is of prime concern (Irzykowska *et al*., 2012). *Fusarium oxysporum* has two important pathogenic formae speciales, namely, *F. oxysporum* f. sp. *lycopersici* (FOL) which is grouped into three races (1, 2 and 3) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) causing *Fusarium* wilt and *Fusarium* crown and root rot, respectively and have been encountered more frequently in tomato-producing countries (Can *et al*., 2004). Identification of *Fusarium* species is traditionally based on fungal morphology. It is time-consuming and requires taxonomical expertise (Khosrow, 2016). Molecular markers are technically easy to use, informative and presents conclusive results in the identification of species and subspecies of fungi. The control of *Fusarium* wilt of tomato (*S. lycopersicum* L.) and brinjal (*S. melongena* L.) is difficult due to expression of field symptoms at a later stage of crop

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growth and it shows limited effectiveness to synthetic fungicides. Moreover, the survival of its resting structures, i.e., chlamydospores in the soil for many years without a host, and limits the suppressive effect of crop rotation (Vethavalli and Sudha, 2012). Biological control using endophytic bacteria is one of the alternative control methods used in integrated plant diseases management, supporting agricultural sustainability (Purnawati *et al*., 2014). Endophytic bacteria live in various tissues and organs of healthy plants. They establish a mutualistic relationship with the plants through many mechanisms to adapt to their living environments and enhance resistance to disease by producing several compounds that promote growth of plants (Gao *et al*., 2010). So far in Sri Lanka, very few studies related to biological control of root and stem rots of annual crops have been reported, and they have been focused on root rot of bean and root and stem rot of cucumber (Abeysinghe, 2007). However, no attempts have been made in Sri Lanka to explore the potential of endophytic bacteria to use as a biological control agent against vascular pathogens in general or in *Fusarium* wilt of Solanaceous crops in particular.

Molecular identification of species and subspecies of causal fungi associated with *Fusarium* wilt of Solanaceous crops in Sri Lanka has become a prime concern as it aids in effective disease management. The objectives of the present study were; (i) isolatation and molecular identification of the causal pathogens of *Fusarium* wilt of selected Solanaceous crops cultivated in Sri Lanka, (ii) isolation and identification of indigenous endophytic bacteria at molecular level, and (iii) to assess the efficacy of endophytic bacterial isolates against *Fusarium* wilt causing pathogen/s under *in vitro* conditions.

MATERIALS AND METHODS

Collection of plant samples

A total of 90 plant samples (42 brinjal and 48 tomato) showing leaf yellowing, wilting and root rot-like symptoms were collected from farmers' fields at various locations of major Solanaceous growing areas namely Kandy, Matale, Nuwaraeliya and Badulla districts in Sri Lanka during January to June 2017 (Table 1). Disease incidence and field symptoms of affected plants from different locations were recorded.

Table 1. Details of the plant samples showing wilt symptoms collected from various locations

District	Locations of sample collection	No. samples collected		
		Tomato	Brinjal	Total No.
Kandy	Gannoruwa, Marassana, Galaha, Manikhinna, , Madamahanuwara	24	13	37
Matale	Madawala ulpotha, Naula Haththotamuna, Pallepola	5	8	13
Badulla	Bandarawela, Divithotawela, Keppetipola	12	11	23
Nuwara Eliya	Hanguranketha, Rahangala, Mandaramnuwara	7	10	17
Total		48	42	90

Isolation of *Fusarium* spp.

Fusarium spp. were isolated from vascular tissues of stems of wilted tomato and brinjal plants. Affected stems of the collected plant samples were washed under running tap water to remove dirt and soil particles. The stems were split lengthwise and cut into thin small pieces (≤ 1 cm). The cut pieces were surface sterilized with 70% ethanol for two minutes then rinsed with sterile distilled water. Cut pieces were placed on PDA medium amended with Amoxicillin (500 g/L) to suppress bacterial growth and were incubated at room temperature (28 °C ± 2) for 1-2 days. After 2 days, sub culturing was done to obtain pure cultures of *Fusarium*. All the isolates were maintained as axenic cultures by single spore isolation and those purified isolates were kept at 4 °C by culturing them on PDA slants for further use.

Confirmation of Pathogenicity

Pathogenicity of 79 *Fusarium* isolates which were obtained from either tomato or brinjal was tested using tomato (var. Thilina) and brinjal (var. Thinnaweli purple). The seedlings were transplanted in plastic pots (8 cm diameter) containing a sterilized soil mixture (1:1 top soil : compost) under greenhouse conditions at Horticultural Crops Research and Developmental Institute, Gannoruwa. A set of 20 days old ten seedlings of tomato and brinjal, which were, were inoculated separately with each *Fusarium* isolate at a concentration of 1 $x10^6$ (spores/mL) adopting a root inoculation technique (Maitlo *et al*., 2016). Another set of 20 days old seedlings (n = 10) were inoculated with sterile distilled water to serve as the control. Disease incidence (DI) was recorded as the percentage of infected plants. Disease severity (DS) was assessed as number of plants affected using a visual disease scale of 0 - 2, where 0 = no symptoms, 1 = leaf yellowing, without wilt, 2 = yellowing, wilt, vascular discoloration and dead (Pavlou and Vakalounakis, 2005). The plants were observed for the development of wilt symptoms at weekly intervals after inoculation. Wilted plants which were randomly selected were used for re-isolation of the pathogen and pathogenicity was confirmed according to Koch's postulates.

DNA extraction

Genomic DNA was extracted from 22 pathogenic *Fusarium* isolates (as determined by the Koch's postulates) using CTAB procedure (Grover *et al*., 2012). These 22 pathogenic isolates of *Fusarium* represented the two wilt-infected crops and the four districts. Extracted genomic DNA of the 22 isolates were subjected to PCR using species and race specific primers (Table 2) as described by Hirano and Arie (2006).

PCR conditions

PCR amplification was performed with specific primer sets (Table 2) and the reaction mixture contained a total volume of 10 µl of PCR master mixture (25 units Taq DNA polymerase, 200 µM of each dNTP and 1× PCR buffer and 1.5 mM $MgCl_2$) (Promega, USA), 0.8 µl of each primer (10 mM), 0.5 µl of diluted (1:10) DNA template and 2.9 µl of sterilized distilled water. PCR program included an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C with a final extraction at 72 °C for 10 min. PCR products were resolved in 2% agrose gel for 45 min at 80 V and visualized with a gel documentation system (ENDURO™GDS, USA). G 571A 1kb ladder (Promega, USA) was used as a DNA size marker.

Table 2. Primers used in the study to identify species, forma speciales and races of *Fusarium*

Primer code	Primer sequence (5'→3')	Expected product size (bp)	Specificity*
uni-f	ATCATCTTGTGCCAACTTCAG	670 - 672	*F. oxysporum*
uni-r	GTTTGTGATCTTTGAGTTGCCA		
sp13-f	GTCAGTCCATTGGCTCTCTC	445	FOL (races 1 and 3)
sp13-r	TCCTTGACACCATCACAGAG		
sp 23-f	CCTCTTGTCTTTGTCTCACGA	518	FOL (races 2 and 3)
sp 23-r	GCAACAGGTCGTGGGGAAAA		
sprl-f	GATGGTGGAACGGTATGACC	947	FORL
sprl-f	GATGGTGGAACGGTATGACC		
TEF-Fu3f	GGTATCGACAAGCGAACCAT	~ 400	*F. solani*
TEF-Fu3r	TAGTAGCGGGGAGTCTCGAA		

* FOL - *F. oxysporum* f.sp. *lycopersici* FORL- *F. oxysporum* f. sp. *radicis-lycopersici*

DNA sequencing and homology search

The resulted in PCR products were sequenced at Asiri Centre for Genomic and Regenerative Medicine, Asiri Surgical Hospital, Colombo 5, Sri Lanka and were subjected to DNA homology search with available nucleotide databases (BLAST; http: www.ncbi.nlm.gov/ BLAST/).

Isolation of endophytic bacteria

Solanaceous plants having no visible symptoms were also collected from the locations from where wilt-infected plants were collected to isolate bacterial endophytes. Isolation was done using stems of 40 healthy brinjal and tomato plants collected from farmers' fields. The stem of each plant was initially washed under tap water for 10 min, to remove adhering soil particles and subsequently was rinsed three times with sterilized distilled water. The base of the stems of each healthy plant was cut into small pieces and surface sterilized by immersing in 70% ethanol for 1-2 min, followed by two washes in sterile distilled water. After blot drying each part was placed on nutrient agar (NA) medium and was incubated at room temperature (28 °C ± 2 °C) for 24 - 48 hr. Single bacterial colonies were isolated following streak plate technique. Pure isolates of bacteria were preserved in 15% glycerol and stored at -80 °C. Isolates of the endophytic bacteria were characterized following conventional methods including colony appearances and biochemical properties. To differentiate Gram positive and negative bacteria, a loop full of each isolated bacterium was placed on a glass slide with a drop of 3% (v/v) KOH solution, stirred for 10 seconds and was observed for the formation of slime threads (Purnawati *et al*., 2014).

***In vitro* screening of antagonistic endophytic bacteria against pathogenic *Fusarium* isolates**

Thirty different isolates of endophytic bacteria were preliminarily screened for the *in vitro* antagonistic activity against two pathogenic *Fusarium* isolates (representing a highly pathogenic isolate from each crop, based on pathogenicity test) by dual-culture technique on

Potato Dextrose Agar (PDA) with some modifications. Briefly, the mycelial plug of 5 mm, pathogenic fungus collected from the edge of actively growing colonies were placed on the center of the plates containing fresh PDA medium. A loopful of each endophytic bacterial isolates (from 1-day old culture) was streaked on three sides opposite to the fungal disc at a distance of 10 mm from the edge of the plate. The plates inoculated with the pathogen alone were maintained as control. Each treatment (i.e. endophytic bacterial isolates) was replicated six times according to a complete randomized design. The plates were incubated at room temperature (28 ± 2 °C) for seven days. The percent inhibition of growth of each *Fusarium* isolate was calculated in comparison to the fungal growth in control plates. Colony growth of each *Fusarium* isolate was recorded and percent inhibition of fungus (growth reduction over control) was calculated by using following formula (Vethavalli and Sudha, 2012).

$$I = [(C - T)/C] \times 100 \qquad \text{(1)}$$

where, I is the percentage inhibition of mycelial growth, C is the radial growth of fungus in the control and T is radial growth of the pathogen in treatment. Data were analyzed using analysis of variance (ANOVA) for the completely randomized design, and mean separation was performed using Fisher's Protected Least Significant Difference (LSD) method.

Extraction of genomic DNA from endophytic bacteria

Bacterial DNA was extracted from six effective endophytic bacterial isolates as determined by the *in vitro* antagonistic test according to the modified protocol by Sun *et al*. (2008). The quality of the DNA obtained from six endophytic bacterial isolates was verified on a 1% agarose gel.

Molecular identification of endophytic bacteria

The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 492R (5'-TACGGYTACCTTGTTACGACTT-3') which will amplify an approximately 1500 bp PCR product (Sun *et al*., 2008). PCR amplification was done with an initial denaturation at 94 °C for 4 min followed by 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 58 °C, 1 min extension at 72 °C and final extension step was 10 min at 72 °C. The PCR products obtained by amplification were analyzed on a 2% (w/v) agarose gel and the PCR products were sequenced at Asiri Centre for Genomic and Regenerative Medicine, Asiri Surgical Hospital, Colombo 5, Sri Lanka. A homology search with the 16S rDNA sequences of the isolates were performed using BLAST program from GenBank database (http: www.ncbi.nlm.gov/ BLAST/).

RESULTS AND DISCUSSION

Isolation of causal pathogens of *Fusarium* wilt

Most of the tomato and brinjal fields showed infection at a later stage of the crop growth and the disease incidence ranged from 10-70%. Few tomato fields at Manikhinna, Galaha and Mandaramnuwara reported infected plants at an early stage of the crop growth (2-3 weeks after transplanting) with > 50% disease incidence showing leaf yellowing, wilting and root rot symptoms and was suspected as *Fusarium* crown and root rot disease. A total of 79 *Fusarium* isolates were successfully recovered from disease stems of tomato and brinjal plants collected from 90 field samples. Out of 79 *Fusarium* isolates, 34 isolates were from

Kandy, 22 were from Nuwara Eliya, 12 were from Matale and 11 were from Badulla districts.

Confirmation of pathogenicity

The results of the pathogenicity tests carried out with the two crops using 79 *Fusarium* isolates are presented in Table 3. The *Fusarium* isolates were categorized into four groups viz., Highly pathogenic (19 isolates), Moderately pathogenic (23), low pathogenic (31) and Non-pathogenic (six isolates) based on disease severity scale of Pavlou and Vakalounakis (2005). All isolates were successfully re-isolated from the plants showing wilt symptoms and confirmed the pathogenicity of each *Fusarium* isolate.

Table 3. Pathogenic variation of 79 *Fusarium* isolates based on pathogenicity test

District	Total isolates tested	No. of isolates			
		Non-pathogenic	Low pathogenic	Moderately pathogenic	Highly pathogenic
Matale	12	0	4	2	6
Kandy	34	4	14	11	5
Badulla	14	0	3	4	4
Nuwaraeliya	3	2	12	6	4
Total	79	6	31	23	19

Molecular identification of selected *Fusarium* spp

Out of the 22 *Fusarium* isolates obtained from wilt-infected tomato and brinjal plants representing the four districts, 14 gave PCR products of approximately 675 bp with universal primers (uni-f and uni-r) confirming *F. oxysporium* (Figure 1). The ITS region was successfully amplified by the fungal-specific universal primer pairs *TEF-Fu3f* and *TEF-Fu3r* and expected PCR product of ~ 400 bp (Figure 2) was obtained by the other eight *Fusarium* isolates, confirming that they are *F. solani*.

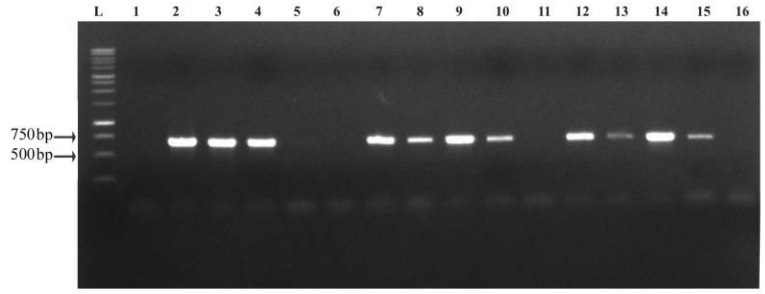

Figure 1. PCR amplicons of *uni-f* and *uni-r* primers for *F. oxysporum*
Lanes L: DNA size marker, 1: Fu1, 2: Fu33, 3: Fu 34, 4: Fu 37, 5: Fu 40, 6: Fu 10, 7: Fu 22, 8: Fu 23, 9: Fu 29, 10: Fu 60, 11: Fu 11, 12: Fu 12, 13: Fu19, 14: Fu 20, 15: Fu 76, 16: negative control (Water)

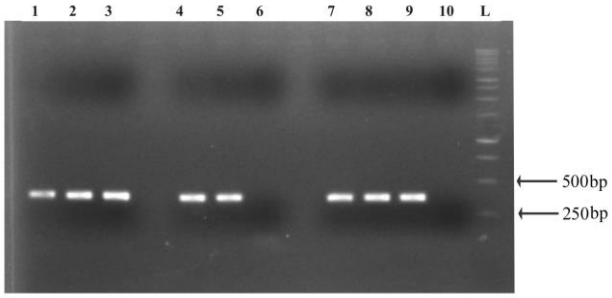

Figure 2. PCR amplicons of *TEF-Fu3f* and *TEF-Fu3r* primers for *F. solani*
Lanes L: DNA size marker, 1: Fu 1, 2: Fu 38, 3: Fu 40, 4: Fu 2, 5: Fu 10, 6: Fu 22, 7: Fu 11, 8: Fu 17, 9: Fu 71, 10: negative control (water)

Among the 14 *F. oxysporium* isolates, five isolates, namely two (i.e. Fu22 and Fu25) from Manikkhinna area of Kandy district, Fu32 from Haththotaamuna of Matale district, Fu 19 from Helahalpae of Badulla district and Fu76 from Labuhena of Nuwaraeliya district resulted in PCR amplicons of an approximate size of 947 bp with *sprl* primers and they were identified as *F. oxysporum* f. sp. *radicis-lycopersici* (Figure 3). Remaining nine *F. oxysporium* isolates (four from Matale, two from Kandy and two from Badulla districts) gave PCR amplicons of 445 bp with *sp13* primers specific to *F. oxysporium* f. sp. *lycopersici* (FOL) races 1 and 3 (Figure 4). However, none of the above nine *F. oxysporium* isolates gave fragments with *sp23* primers (FOL, races 2 and 3). It was confirmed that all nine isolates belong to *F. oxysporium* f. sp. *lycopersici* (FOL) race 1. This is the first study to report the identification of the *F. oxysporium* f. sp. *lycopersici* (FOL) race 1 from Solanaceous crops grown in Sri Lanka.

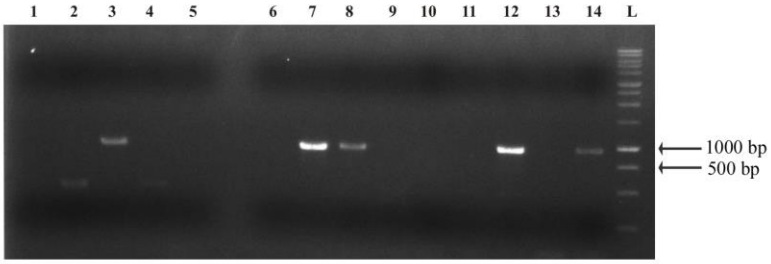

Figure 3. PCR amplicons of specific primers *sprl f & r* for *F. oxysporum* f. sp. *radicis-lycopersici* isolates
Lanes L: DNA size marker, 1: Fu7, 2: Fu 33, 3: Fu 32, 4: Fu 34, 5: Fu 37, 6: Fu 22, 7: Fu 23, 8: Fu 25, 9: Fu 29, 10: Fu 60, 11: Fu 12, 12: Fu 19, 13: Fu 20, 14: Fu 76

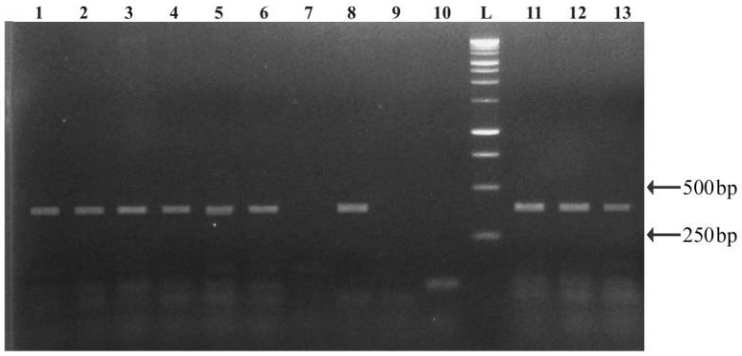

Figure 4. PCR products resulted in by specific primers (sp 13 f & r) to detect races of *F. oxysporium* f. sp. *lycopersici* isolates
Lanes L: DNA size marker 1: Fu 32, 2: Fu 33, 3: Fu 34, 4: Fu 37, 5: Fu 7, 6: Fu 12, 7: Fu 19, 8: Fu 20, 9: Fu 76, 10: Fu 23, 11: Fu 22, 12: Fu 29, 13: Fu 60

Table 4. Results of homology search of selected *Fusarium* isolates

Isolate No.	Best homologue given by BLAST	Query coverage (%)	E value	Identity (%)	Sequence ID of the best homologue
Fu 19	*Fusarium oxysporum* f. sp. *radicis lycopersici* pgx 4	95	0.0	96	AB256796.1
Fu 29	*Fusarium oxysporum* f. sp. *trachephilum* pq1	100	0.0	98	MAFF235726
Fu 33	*Fusarium oxysporum* f. sp. *eustomae fuslis* 31/09	96	0.0	97	KJ361536.1
Fu 60	*Fusarium oxysporum* f. sp. *lycopersici* FOL 14	95	0.0	97	KP404108.1
Fu 40	*Fusarium solani* TRR-1	99	e-137	97	KY659068.1

Based on the molecular identification, it is clear that different *Fusarium* species (i.e. *F. oxysporum and F. solani*) and several formae speciales of *F. oxysporum* (i.e. f. sp. *radicis lycopersici*, f.sp. *trachephilum*, f.sp. *eustomae fuslis* and f.sp. *lycopersici*) are responsible for the development of wilt in tomato and brinjal in Sri Lanka (Table 3).

Isolation of endophytic bacteria

Thirty endophytic bacterial isolates were isolated from healthy tomato and brinjal stems, namely 5 isolates from Kandy, 2 from Nuwara Eliya, 11 from Matale and 12 from Badulla districts. Results of the 3% KOH test revealed that 26 out of the 30 bacterial endophytes were Gram negative.

In vitro screening of antagonistic endophytic bacteria against pathogenic *Fusarium* isolates

Results revealed that 10 out of the 30 bacterial endophyte isolates which were tested for their antagonistic ability against Fu 60 isolate (*F. oxysporum* f. sp. *lycopersici*) showed more than 30% inhibition of the fungal colony growth. The bacterial endophytes which gave more than 30% colony growth inhibition of Fu 60 were further tested against *F. oxysporum* f. sp. *trachephilum* (Fu 29) and *F. solani* (Fu 11). Results presented in Table 5 indicate that all tested bacterial endophytes showed varying levels of inhibitory effect against the growth of the two *F.oxysporum* formae speciales (Fu 29 and Fu 60) and *F. solani* (Fu 11). Among the ten isolates, six coded as E 2, E 7, E 8, E 10, E 24 and E 25 were found to be highly antagonistic (50 - 67%) against three *Fusarium* isolates (Fu 29, Fu 60 and Fu 11).

Table 5. Percentage mycelia growth inhibition of different Fusarium species by different endophytic bacterial isolates under in vitro conditions

Endophyte isolate	Location	*F. oxysporum* f. sp. *trachephilum*	*F. oxysporum* f. sp. *lycopersici*	*F. solani*
E 2	Gannoruwa	54.95^{bc}	48.17^{de}	51.85^{a}
E 7	Divithotawela	49.15^{c}	61.80^{b}	51.48^{a}
E 8	Divithotawela	64.51^{ab}	57.02^{c}	50.19^{a}
E 10	Helahalpae	64.51^{ab}	47.33^{e}	55.55^{a}
E 14	Helahalpae	29.73^{e}	29.71^{f}	29.89^{c}
E 17	Naula	34.44^{d}	34.17^{g}	33.39^{c}
E 19	Hathotaamuna	33.36^{d}	46.12^{e}	30.19^{c}
E 24	Pallepola	67.74^{a}	50.37^{c}	51.59^{a}
E 25	Pallepola	61.29^{b}	64.70^{a}	50.00^{a}
E 26	Udadelthota	32.99^{f}	42.51^{d}	39.43^{b}
Control		0.00^{i}	0.00^{f}	0.00^{d}
CV		3.13	2.15	6.29
LSD		2.14	1.46	4.07

***** *Means in a column followed by same letter do not significantly differ according to LSD Test (P<0.05)*

Molecular identification of endophytic bacteria

Results of homology search revealed the best matching organisms for endophytic isolates E 2, E 7, E 8, E 10, E 24 and E 25 are *Pseudomonas geniculata* strain (E 2), *Bacillus velezensis* strain C19 (E 25), *Pseudomonas sp.* strain SB 904 (E 7), *Delftia tsuruhatensis* (E 8), *Stenotropomonas maltophilia* strain ATCC 13637 (E 10) and *Stenotropomonas pavanii* strain ICB 89 (E24), respectively.

Based on previous research by Rania *et al*. (2016), *Stenotrophomonas maltophilia* str. S37 isolated from *Datura stramonium* exhibited 44% inhibition of *Fusarium oxysporum* f. sp. *lycopersici*. Further, Bacillus species have reported strong inhibition of *Fusarium oxysporum*, over 70% mycelia growth reduction compared to control treatment under *in vitro* conditions (Narayan *et al*., 2013). Wang *et al*. (2016) have reported that *Bacillus amyloliquefaciens* can show growth promotion and antagonistic activity through the production of antifungal lipopeptides and volatile compounds against *F. oxysporum*.

Table 6: Results of homology search of endophytic bacterial isolates

Isolate No.	Blast research results	Query coverage (%)	E value	Identity (%)	Sequence ID
E2	*Pseudomonas geniculata* strain ICPH - 14	99	0.0	96	KX611373.1
E7	*Pseudomonas sp. strain* SB 904	96	0.0	95	MG491639.1
E8	*Delftia tsuruhatensis* strain MTQ	97	4e-10	86	HQ143604.1
E10	*Stenotropomonas maltophilia* strain ATCC 13637	81	3e-116	93	NR112030.1
E24	*Stenotropomonas pavanii* strain ICB 89	97	0.0	96	NR116793.1
E25	*Bacillus velezensis* strain C19	99	0-0	98	KU681039.1

Pseudomonas geniculata, a Gram negative bacterium isolated from chickpea has reported its ability to promote plant growth (Gopalakrishnan and Sirinivas, 2015). Several previous studies have shown that *Stenotrophomonas maltophilia* isolated from *Datura stramonium*, plays important roles in agricultural production as a plant growth-promoting bacterium, which could suppress disease development by secretion of antibiotics such as maltophilin and xanthobaccin which have antifungal activity, however is inactive against bacteria (Rania *et al*., 2016). *Stenotrophomonas pavanii* (E24) isolated from Brazilian sugarcane is an endophytic bacterium that is capable of nitrogen fixation (Patricia *et al*., 2011). *Bacillus velezensis* (E25) (*Bacillus amyloliquefaciens* subsp. *plantarum*) is a plant-associated bacterium, which stimulates plant growth and produces secondary metabolites that suppress soil-borne plant pathogens. Therefore, the endophytic bacterial isolates of the present study agrees with the identity of previous reports related to potential endophytic bacteria of *Fusarium* spp. However, *S. maltophilia* has also been reported as nosocomial opportunistic pathogens (Mukhopadhhyay *et al*., 2003), hence, caution is needed when selecting potential endophytic bacterial antagonists for *in vivo* studies, though they are effective under *in vitro* conditions.

CONCLUSIONS

The present study identified the causal pathogens of *Fusarium* wilt of tomato and brinjal as *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici*. Molecular identification revealed the presence of four formae speciales, namely *lycopersici, radices-lycopersici, trachephilum* and *eustomae* in infected plants. All the isolates of *Fusarium oxysporum* f. sp. *lycopersici* identified in the present study belong to Race 1 and it is the first report from Solanaceous crops grown in Sri Lanka. The Endophytic bacterial isolates which were identified as *Pseudomonas geniculata* strain (E2), *Bacillus velezensis* strain C19 (E 25), *Pseudomonas* sp. strain SB 904 (E7), *Delftia tsuruhatensis* (E8), *Stenotropomonas maltophilia* strain ATCC 13637 (E10) and *Stenotropomonas pavanii* strain ICB 89 (E24) were potential antagonists of *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium solani*.

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