Journal of Biological Control, 32(4): 257-263,2018, DOI: 10.18311/jbc/2018/21771

Research Article

Evaluation of phyllosphere antagonistic bacteria on the management of Fusarium ear rot of maize caused by *Fusarium verticillioides*

KONDA SAMEER, G. SOBANBABU, K. G. SABARINATHAN, V. K. PARTHIBAN and V. RAMAMOORTHY
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Thoothukudi District, Tamil Nadu, India
Corresponding author E-mail: rvrmoorthy@yahoo.com

ABSTRACT: *Fusarium verticillioides* is an insidious fungal pathogen of maize associated with diseases such as ear rot and kernel rot. It attacks the phyllosphere region of the maize plant especially on inflorescence and cobs. Thus, this study was conducted to isolate potential phyllosphere colonizing antagonistic microbes for the management of Fusarium ear rot. Four phyllosphere colonizing antagonistic bacteria were found to be effective in suppression of the growth of *Fusarium verticillioides.* Based on 16S rDNA analysis, these bacterial isolates were identified as *Pseudomonas aeruginosa* isolate 1*, P. aeruginosa* isolate 2*, Bacillus subtilis* isolate and *B. amyloliquefaciens*. Among these four phyllosphere bacteria tested against *F. verticillioides,* the maximum inhibition of mycelial growth of *F. verticillioides* and production of volatile compounds was exhibited by *P. aeruginosa* isolate 1. Application of *P. aeruginosa* isolate -1*,* as seed treatment @ 10 g/kg of seeds, soil application @ 5 g/pot and foliar spray @ 0.2 per cent, recorded the minimum PDI with the maximum disease reduction over control. Thus, the present study showed that *P. aeruginosa* isolated from agricultural ecosystem could be a potential phyllosphere antagonistic bacterium for the management of maize ear rot disease.

Keywords: *Fusarium verticillioides, Fusarium ear* rot*, Phyllosphere* antagonistic bacteria*, Pseudomonas aeruginosa,* 16S rDNA Analysis

(Article chronicle: Received: 09-06-2018; Revised: 10-11-2018; Accepted: 25-12-2018)

INTRODUCTION

Maize crop is subjected to attack by several fungal, bacterial and viral pathogens. Among many fungi, *Fusarium* and *Aspergillus* are frequently reported as mycotoxigenic fungal genera. Among the several toxigenic fungi, *Fusarium verticillioides* (Sacc.) Nirenberg (Teleomorph: *Gibberella moniliforme* Wineland) causes the ear and kernel rot disease in maize. *F. verticillioides* is more common in regions with hot and dry weather and infection especially occurs mainly during pollination (Reid *et al*., 1999). The disease symptoms appear on kernels as moldy growth which may be initially white, pink or salmon colored. Later the infected kernels may turn to tan or brown in color (Payne *et al*., 1999). Presently, attempts on management of plant diseases are focused on the biological control method as an alternative method to chemical management due to the residual effect, environmental pollution and health hazards. Several biocontrol agents were isolated from rhizosphere and evaluated for the management of soil borne fungal and bacterial pathogens. But, their use on the management of foliar diseases was not successful due to their inability to survive on the phyllosphere region because of erratic atmospheric temperature variation, exposure to direct sunlight, UV irradiation and low availability of moisture and nutrient in the phyllosphere region (Vorholt, 2012). These attributes led to focus on finding the phyllosphere colonizing anti-microbial agent for the control of foliar/phyllosphere pathogens (Innerebner *et al.,* 2011). *Fusarium verticillioides* causes infection mainly on aerial parts of the plant (tassels and cobs). Thus, it would be wise to find phyllosphere colonizing antagonistic microbes that could better survive in the dynamic atmospheric conditions of phyllosphere region compared to the rhizosphere region for the management of this disease.

MATERIALS AND METHODS

Isolation and identification of pathogen

The freshly infected maize cobs showing typical symptoms of *Fusarium* ear rot disease were collected from Thoothukudi district of Tamil Nadu, India. The surface sterilized kernel bits were placedon Petridish containing potato dextrose agar medium (PDA) and incubated at 25 ± 2º C for 5 days. The growing fungal colony of each kernel bit was sub cultured and purified by single hyphal tip method (Tutte, 1969). Different isolates of *Fusarium* spp. were characterized and virulent

isolate/species was selected based on their growth, asexual reproduction and pathogenicity. They were identified by analyzing their conidial characters and molecular techniques by amplifying specific ITS region using specific primer followed by sequencing. The primers used for amplification of ITS region are ITS1- 5′TCCGTAGGTGAACCTGCGG3′(F) and ITS4 -5′ TCCTCCGCTTATTGATATGC3′(R).

Isolation of phyllosphere colonizing bacteria

The healthy leaves from different plants were collected and washed with 100 ml of sterile water containing 0.01% Tween 20. The potential antagonistic phylloplane microbes were screened directly by co-cultivating 100 µl of conidial suspension of *F. verticillioides* (1x 10^5 conidia/ml) and 1 ml of phyllosphere microbial suspension mixture in the PDA medium (Andrew *et al.,* 2002). The medium was mixed with the conidial + phyllosphere microbial suspension thoroughly and incubated at 25°C for 2-3 days. The microbial colony that showed clear inhibition zone around its colony was picked and used for further study.

Biochemical and molecular characterization of phyllosphere antagonists

The array of biochemical test has been performed to characterize the bacterial antagonists *viz.,*gram staining (Gerhardt *et al.,* 1981), KOH test (Suslow *et al.,* 1982), pigment production (Schaad, 1992), growth at 4°C and 45°C; starch hydrolysis (Seely and Vandemark, 1981), hydrogen cyanide production (Lorck, 1948) and siderophore production assay (Schwyn and Neilands, 1987). The genomic DNA from potential antagonistic bacterial isolates were isolated using the standard protocol of Cetyltrimethyl Ammonium Bromide (CTAB) method. The molecular confirmation of the bacterial strains was done through amplification of 16S rDNA intervening sequence by PCR using specific 16S rRNA region primers *viz*., 27f - 5′ AGAGTTTGATCTGGCTCAG 3′; 1115r - 5′ AGGGTTGCGCTCGTTG 3′ and 1525r - 5′ AAGGAGGTGWTCCARCC 3′.

Phylogenic analysis was carried out using MEGA 6 software by comparing the 16S-rDNA sequence. Based on the 16S-rDNA analysis in BLAST search program, other closely related *Pseudomonas* spp, *viz*., *P. aeruginosa, P. syringae, P. fluorescens, P. putida, P. stutzeri,* were selected for phylogenetic study with *P. aeruginosa* isolate 1.16S - rDNA-sequence data were obtained from the GenBank and their GenBank ID numbers were indicated in Fig. 2C. The 16S – rDNA sequences of each species were aligned by Clustal W program. Phylogeny analysis was done by neighbourhood joining method.

In vitro* evaluation of phyllosphere bacteria against *Fusarium verticillioides

The antagonistic effect against the pathogen was tested by dual culture technique by placing 9 mm of Fusarium fungal disc at one end and streaking loopful of the actively growing culture of the test bacterium at the other end (Dennis and Webster, 1971). The efficacies of phyllosphere bacterial antagonist were compared with the *P. fluorescens* (Pf 1) and *B. subtilis* (Bs2) obtained from Department of Plant Pathology, TNAU, Coimbatore. The growth inhibition per cent was also calculated using the following formula.

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc=Average diameter of fungal growth (cm) in control; Dt=Average diameter of fungal growth (cm) in treatment; PI=Per cent inhibition over control

Effect of volatile compounds of the antagonistic bacteria against *Fusarium verticillioides*

Nutrient Agar (NA) medium and PDA medium were prepared in separate conical flask about 100 ml. The media were sterilized and allowed to cool to bearable level and 20 ml of the medium was poured into sterilized Petri plates (90 mm) separately and allowed to solidify. On NA medium plate, the actively growing antagonistic bacteria were streaked. On PDA medium plate, *F. verticillioides* was inoculated at the centre of the plate by placing a nine mm actively growing culture disc cut from five day old culture of *F. verticillioides* by means of a sterilized cork borer. The two bottom plates*viz.,* NA medium plate and PDA medium plate were closed together wrapped with parafilm completely. Control plate was kept with non-streaked NA medium plate. The plates were incubated at 28°C. Three replications were maintained for each treatment. The radial growth of the mycelium was measured when the mycelial growth covered the Petri plates in control.

Effect of bacterial antagonists on maize ear rot disease under pot culture conditions

Pot culture experiment was conducted on the management of ear rot disease using effective *Pseudomonas* spp. and *Bacillus* spp. with maize variety COH (M) 6. The seeds were treated with talc formulation of bacterial antagonists at the rate of 10 g/kg of seed at the time of sowing. Soil application of bacterial antagonists was done at the rate of 5g/pot by thoroughly mixing with well decomposed FYM and vermicompost was applied 30 days after transplanting (Vidhyasekaran and Muthamilan, 1995). Foliar application of bacterial antagonists was given at 0.2 per cent on 45 and 60 days after transplanting. The plants were inoculated at flowering stage (at the time of silk formation) with the conidial suspension of *F. verticillioides.* The observation on germination per cent was made 20 days after sowing and PDI

was calculated at harvest.

Effect of bacterial antagonists on maize ear rot disease under field conditions

For the field study, the seeds were sown in the field in the recommended spacing (60 x 25 cm) and fertilizer dosage (250:75:75 kg of NPK/ha). Seed treatment at 10g/kg of seed at the time of sowing, soil application at 2.5 kg/ha at 30 days after sowing and foliar application at 0.2% talc formulation during boot leaf, anthesis and milky stage were applied. The observation was made on germination per cent at 20 days after sowing and PDI was calculated at harvest.

RESULTS AND DISCUSSION

Isolation and identification of *Fusarium verticillioides*

Three different isolates of *Fusarium* spp. viz. *Fusarium* sp. KS2, *Fusarium* sp. KS3 and *Fusarium* sp. KS4 were isolated from the infected corn cobs and they were identified at genus level as *Fusarium* based on production ofmicroconidia and macroconidia. Microscopic observation of conidial suspension produced on carboxymethylcellulose medium revealed that the pathogen typically produced three celled macroconidia and one celled micro conidia. This showed that these three isolates of pathogen belong to *Fusarium* genus. Among these three isolates, *Fusarium* sp. isolate KS4 grew vigorously and caused the maximum PDI in pathogenicity study (data not shown). To identify the fungus at species level, DNA from *Fusarium* sp KS4 were isolated using CTAB method and ITS sequence was amplified at the size of 700 bp PCR fragments and PCR product was sequenced at Eurofins genomics Pvt. Ltd (Fig. 1A and B). When the ITS sequence of the *Fusarium* sp isolate KS4 was BLAST searched in the database, the output data matched at 99 per cent DNA sequence homology with *Gibberella moniliforme* which is the perfect state of *F. verticillioides.* Similar to the present study, Visentin *et al*., (2009) reported that *Fusarium* ear rot of maize is incited by several species of *Fusarium* spp. and among them, *F. verticillioides* was the most frequently isolated species from the infected maize ears. Sreenivasa (2006) reported that ITS sequence analysis was used as molecular tool for the identification and species confirmation of *Fusarium* spp. infecting maize.

Characterization of phyllosphere antagonistic bacteria

Phyllosphere antagonistic bacteria were isolated from leaves of calotropis, acalypha and opuntia plants (Table 1) and selected based on inhibitory zone around their colony on the co-cultivation plates and they were again confirmed by dual culture techniques. Since these plants are perennial, several microbes with antagonistic properties were found. Attempts were also made to isolate phyllosphere antagonistic

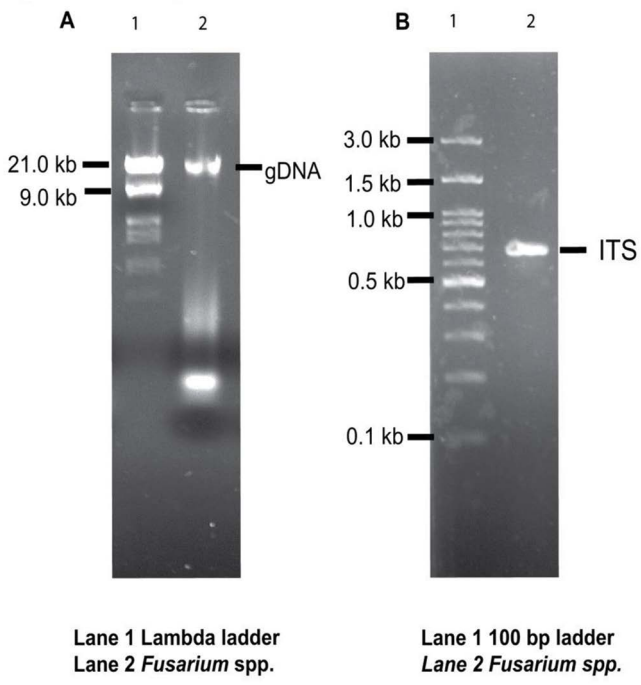

Fig. 1. A. Genomic DNA isolated from *Fusarium* sp. KS4. B. ITS PCR product amplified from *Fusarium verticillioides.*

Table 1. List of phyllosphere antagonistic bacteria isolated from different crops

S. No.	Isolates	Phyllosphere bacteria	Identified method
1	Calotropis	*P. aeruginosa* isolate -1	16s rRNA sequence analysis
2	Calotropis	*P. aeruginosa* isolate-2	16s rRNA sequence analysis
3	Opuntia	*B. amyloliquefaciens*	16s rRNA sequence sequence
4	Acalypha	*B. subtilis*	16s rRNA sequence sequence

microbe from annual plants such as rice, maize and sorghum. But no antagonistic microbe was found from the leaves of the annual plants. The best isolates showing strong inhibitory effect against *F. verticillioides* were selected further. Based on the biochemical tests, two isolates were identified as *Pseudomonas* spp. and other two were identified as *Bacillus* spp.

16S rDNA sequence analysis is one of the commonly used molecular methods for the identification of bacteria at species level. 16S rDNA from two putative *Pseudomonas* spp. and two putative *Bacillus* spp. were isolated using CTAB method. Single band of intact genomic DNA was visualized on the agarose gel (Fig. 2A). 16S rDNA region of these bacterial isolates was amplified with primer pairs 27f and 1525r and 27f and 1115 and PCR fragments with the size of 1500 bp and 1100bp were obtained respectively (Fig. 2B). Based on the 16s ribosomal

sequence analysis, phyllosphere antagonistic bacteria were identified as *B. subtilis* isolate 1 (accession number - MH715473), *B. amyloliquefaciens* (accession number - MH715478), *P. aeruginosa* isolate -1 (accession number -MH715475) and *P. aeruginosa* isolate -2 (accession number - MH715410).

In the present study, *P. aeruginosa* isolate 1 was found to be the most effective phyllosphere colonizing antagonistic bacteria as described below. But *P. aeruginosa* is being considered as opportunistic human pathogen. Thus, phylogeny analysis was carried out to analyse the genetic relatedness of *P. aeruginosa* isolate 1 with other *Pseudomonas* spp. associated with human and plant system. Based on 16S-rDNA sequence similarity, *P. aeruginosa* isolate 1, other *P. aeruginosa* isolates and *P. stutzeri* isolates falls into the same clade. Other species

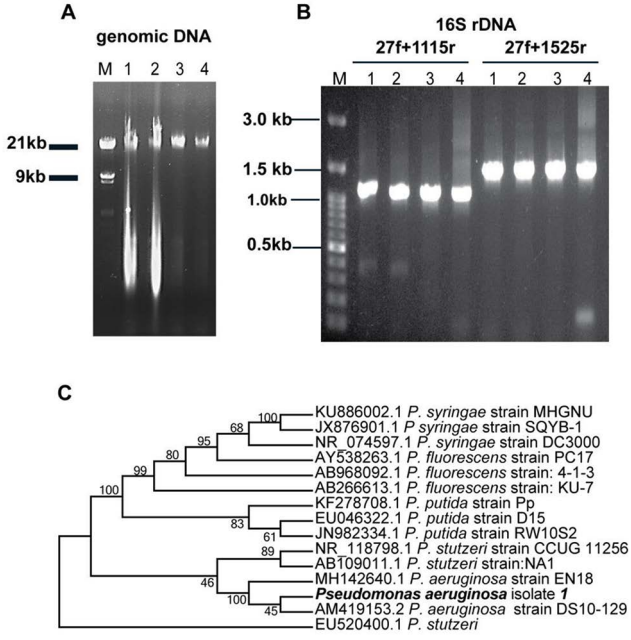

Fig. 2. A. Isolation of genomic DNA and amplification of 16S rDNA region from phyllosphere colonizing antagonistic bacteria. Lane M-LamdaDNA ladder; Lane 1-*Pseudomonas aeruginosa* isolate 1; Lane 2-*P. aeruginosa* isolate 2; Lane 3-*Baclillsu subtilis* isolate 1; Lane 4-*B. amyloliquefaciens.*
B. Amplification of 16S rDNA using primer pair 27f +1115r and 27f+1525r. Using primer pair 27f+1115r, the 16S rDNA – PCR product size of 1100 bp was obtained. Using primer pair 27f+1525r, the 16S rDNA – PCR product size of 1500 bp was obtained.Lane M- 100 bp DNA ladder; Lane 1-*Pseudomonas aeruginosa* isolate 1; Lane 2-*P. aeruginosa* isolate 2; Lane 3-*Baclillsu subtilis* isolate 1; Lane 4-*B. amyloliquefaciens.*
C. Phylogeny analysis. Phylogenetic experiments were carried out using 16S-rDNA sequences of *P. aeruginosa* isolate 1with 16S-rDNA sequences of other *Pseudomonas* spp. ID in parenthesis indicates the GenBank number referring the 16S-rDNA sequence of the corresponding organism.

of *Pseudomonas* such as *P. fluorescens, P. putida* and *P. syringae* were found to be distantly related to *P. aeruginosa* isolate 1 (Fig. 2 C).

Evaluation of efficacy of phyllosphere antagonistic bacteria against *Fusarium verticillioides* under *in vitro*

Among the phyllosphere bacteria tested against the mycelial growth of *F. verticillioides,* the maximum per cent inhibition over control was shown by *P. aeruginosa* isolate -1(72.22 %) followed by *P. aeruginosa* isolate -2 showing 62.22 per cent inhibition over control. *B. subtilis* Bs2 showed the least mycelial growth inhibition (Table.2; Fig. 3). Similar to the present study, Cavaglieri *et al.* (2005) reported that among the various bacterial isolates, *B. subtilis*, isolated from maize was demonstrated to be the most effective in reducing mycelial growth of *F. verticillioides in vitro* and also under field conditions. Nourozian *et al.* (2006) reported that *B. subtilis*, *P. fluorescens* and *Streptomyces* sp. were found to inhibit the mycelial growth of *Fusarium.* Recently, it has been reported that phenazine producing strains of *P. aeruginosa* inhibited the mycelial growth of fungi such as *F. graminearum, F. oxysporum, Rhizoctonia solani, Magnaporthe grisea, , Rhizopus microsporus, Aspergillus niger,* and *Alternaria alternate* (Zhou *et al*., 2015 and Uzair *et al*., 2018)

Effect of volatile compound production by phyllosphere antagonists against *Fusarium verticillioides*

The isolated phyllosphere bacterial antagonists were tested for the production of volatile compounds active against the growth of *F. verticillioides.* The maximum inhibition

Table 2. *In Vitro* evaluation of phyllosphere antagonistic bacteria against *Fusarium verticillioides*

S. No	Phyllosphere bacteria	Mycelial growth (mm)*	Per cent reduction over control
1	*P. aeruginosa* isolate -1	25.00^{e}	72.22
2	*P. aeruginosa* isolate-2	34.00^{d}	62.22
3	*B. amyloliquefaciens*	45.67^{c}	49.26
4	*B. subtilis*	48.67^{c}	45.93
5	*B. subtilis*Bs2	54.00^{b}	40.00
6	*P. fluorescens*Pf1 (Standard check)	47.33^{c}	47.41
7	Control	90.00^{a}	
	CD 5% (p=0.05)	3.42	

The treatment means are compared using Duncan's Multiple Range Test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (p=0.05)

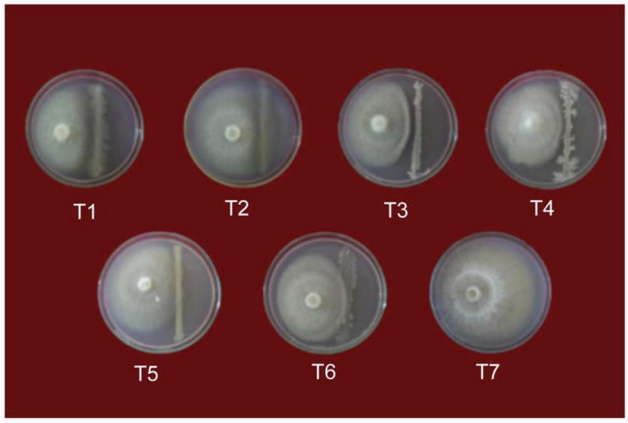

Fig. 3. Efficacy of phyllosphere colonizing antagonistic bacteria against the mycelial growth of *Fusarium verticillioides* under in vitro conditions. T1 – *Pseudomonas aeruginosa* isolate1, T2 – *P. aeruginosa* isolate2, T3 – *Bacillus amyloliquefaciens*. T4 – *B. subtilis* 1, T5 – *P. fluorescens* (Pf 1) T6 – *B. subtilis* Bs2 and T7 – Control

was recorded by *P. aeruginosa* isolate -1(74.44 %) followed by *P. aeruginosa* isolate -2(73.33) which were on par with each other. *B. amyloliquefaciens* recorded 41.85 per cent inhibition over control. The minimum inhibition was shown by *B. subtilis* Bs2 with 21.48 per cent inhibition over control. The test culture *P. fluorescens* Pf1 showed 68.89 per cent inhibition over control (Table 3).

Efficacy under potted conditions

The result obtained from the experiment revealed that *P. aeruginosa* isolate-1 applied as seed treatment @ 10 g/kg of seeds, soil application @ 5 g/pot and foliar spray @ 0.2 per cent recorded the minimum PDI of 19.83 which accounted for 71.03 per cent reduction over control followed by the *P. aeruginosa* isolate-2 which recorded PDI of 21.02 with 69.30 per cent reduction over control (Table 4). The germination percentage under glass house conditions was significantly different between the various treatments tried in this study. The germination was maximum in the seeds treated with *P. aeruginosa* isolate 1 (81.14 %) followed by *P. aeruginosa* isolate 2 (72.29%).

Efficacy in field

In field experiment, among the treatments, *P. aeruginosa* isolate-1 (Table 4) recorded the minimum PDI of 10.13 which accounted for 76.06% per cent disease reduction over control followed by *P. aeruginosa* isolate-2 (PDI of 11.97) which accounted for 71.70 per cent reduction over control (Table 5). Thus the present study showed that both *P. aeruginosa* isolate-1 and *P. aeruginosa* isolate-2, when applied as seed treatment @ 10 g/kg of seeds, soil application @ 5 g/pot and foliar spray @ 0.2 per cent, were found to suppress the Fusarium ear rot disease and showed the minimum PDI. In accordance with the present results, Nayaka *et al.* (2008) reported that seed treatment and foliar

Table 3. Effect of volatile compounds of phyllosphere antagonistic bacteria on growth of *Fusarium verticillioides*

S. No	Treatments	Mycelial growth (mm)*	Per cent inhibition over control*
1	*P. aeruginosa* isolate -1	23.00^{d}	74.44
2	*P. aeruginosa* isolate-2	24.00^{d}	73.33)
3	*B. amyloliquefaciens*	52.33^{c}	41.85
4	*B. subtilis*	58.67^{c}	34.81
5	*B. subtilis*Bs2	70.67^{b}	21.48
6	*P. fluorescens*Pf1 (Standard check)	28.00^{d}	68.89
7	Control	90.00^{a}	
	CD (p=0.05)	7.31	

***Mean of three replications**

The treatment means are compared using Duncans Multiple Range Test (DMRT).

In a column, means followed by a common letter (s) are not significantly different (p=0.05)

Table 4. Effect of phyllosphere antagonistic bacteria on maize ear rot under pot culture conditions

S. No	Treatments	PDI	% reduction over control	Germination (per cent)
1	*P. aeruginosa* isolate -1	19.83^{g}	71.03	81.14^{a}
2	*P. aeruginosa* isolate-2	21.02^{f}	69.30	72.29^{ab}
3	*B. amyloliquefaciens*	22.58^{e}	67.01	59.21^{cd}
4	*B. subtilis*	27.48^{c}	59.86	46.92^{cd}
5	*B. subtilis* Bs2	34.24^{b}	49.99	39.23^{e}
6	*B. amyloliquefaciens* + *P. aeruginosa* isolate -1	24.70^{d}	63.92	50.77^{bcd}
7	*P. fluorescens* Pf1	23.56^{de}	65.58	59.21^{abcd}
8	Carbendazim	22.40^{e}	67.29	63.85^{abc}
9	Control	68.47^{a}	0.014	68.48^{ab}
	CD 5% (p=0.05)	1.16		19.01

The treatment means are compared using Duncans Multiple Range Test (DMRT).

In a column, means followed by a common letter (s) are not significantly different (p=0.05)

Table 5. Effect of phyllosphere antagonistic bacteria on maize ear rot under field conditions

S. No	Treatments	PDI	% reduction over control	Germination (percent)*
1	*P. aeruginosa* isolate -1	10.13^{e}	76.06	83.86^{a}
2	*P. aeruginosa* isolate-2	11.97^{de}	71.70	77.71^{ab}
3	*B. amylolique-faciens*	13.79^{de}	67.42	71.39^{a}
4	*B. subtilis*	25.64^{c}	39.39	57.29^{bc}
5	*B. subtilis*Bs2	29.95^{b}	29.22	48.87^{d}
6	*B. amylolique-faciens* + *P. aeruginosa* isolate -1	24.27^{c}	42.64	61.33^{b}
7	*P. fluorescens* Pf1	21.67^{c}	48.79	75.15^{a}
8	Carbendazim	15.24^{d}	64.98	65.69^{bc}
9	Control	43.52^{a}	-	62.00^{b}
	CD 5% (p=0.05)	4.11	-	17.23

The treatment means are compared using Duncans Multiple Range Test (DMRT).
In a column, means followed by a common letter (s) are not significantly different (p=0.05)

spray in maize with *P. fluorescens* reduced the incidence of *F. verticillioides* under field conditions. Application of *P. aeruginosa* induced the various defence related compounds in tomato plants and protected the plants against various pathogens (Durairaj *et al*., 2017). Similarly, Rhamnolipids of *P. aeruginosa* triggered the defence reaction in *Brassica napus* and protected the plants against *Botrytis cinerea* infection without causing physiological disorders (Monnier *et al*., 2018).

This study clearly showed that phyllosphere colonizing antagonists viz. *P. aeruginosa* and *Bacillus* spp. were effective for the management of ear rot of maize. Based on the results of the present study and also recent publications on use of *P. aeruginosa*as a biocontrol agents (Durairaj *et al*., 2017; Monnier *et al*., 2018; Uzair *et al*., 2018 and Zhou *et al*., 2015), it is concluded that though *P. aeruginosa*is a human opportunistic pathogen, it can be used as a biocontrol agent in commercial scale after studying its biosafety measures, because it is frequently isolated from agricultural soil-ecosystem and associated with crop plants.

REFERENCE

Andrews JH, Spear RN, Nordheim EV. 2002. Population biology of *Aureobasidium pullulans* on apple leaf surfaces. *Can J Microbiol*. **48**: 500-513. https://doi.org/10.1139/w02-044 PMid:12166677

Cavaglieri L, Orlando J, Rodríguez MI, Chulze S, Etcheverry M. 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides* in vitro and at the maize root level. *Res Microbiol*. **156**: 748-754. https://doi.org/10.1016/j.resmic.2005.03.001 PMid:15950130

Dennis C, Webster J. 1971. Antagonistic properties of species groups of *Trichoderma*. III. Hyphal interactions. *Trans Brit Mycol Soc*. **57**: 363-369. https://doi.org/10.1016/S0007-1536(71)80050-5

Durairaj K, Velmurugan P, Park JH, Chang WS, Park YJ, Senthilkumar P, Choi KM, Lee JH, Oh BT. 2017. Potential for plant biocontrol activity of isolated *Pseudomonas aeruginosa* and *Bacillus stratosphericus* strains against bacterial pathogens acting through both induced plant resistance and direct antagonism. *FEMS Microbiol Lett*. **364** (23) fnx225. https://doi.org/10.1093/femsle/fnx225 PMid:29069329

Gerhardt P, Murray RGE, Costelow RN, Nexter EW, Wood WA, Dreig NP, Phillips GB. 1981. *Manual of methods of general bacteriology.* American Society of Microbiol. p. 400-450.

Innerebner G, Knief C, Vorholt JA. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Appl Environ Microbiol*. **77**: 3202-s3210. https://doi.org/10.1128/AEM.00133-11 PMid:21421777 PMCid:PMC3126462

Lorck H. 1948. Production of hydrocyanic acid by bacteria. *Physiol Plant*. **1**: 142-146. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x

Monnier N, Furlan A, Botcazon C, Dahi A, Mongelard G, Cordelier S, Clément C, Dorey S, Sarazin C, Rippa S. 2018. Biotechnological potential of a rhizosphere *Pseudomonas aeruginosa* strain producing phenazine-1-carboxylic acid and phenazine-1-carboxamide. *Front Plant Sci*. **9**:1170. https://doi.org/10.3389/fpls.2018.01170 PMid:30135699 PMCid:PMC6092566

Nayaka SC, Sahankar ACU, Reddy MS, Niranjana SR, Prakash HS, Shetty HS, Mortensen CN. 2008. Control of *Fusarium verticillioides*, cause of ear rot of maize, by *Pseudomonas fluorescens*. *Pest Manag Sci*. **65**: 769-775. https://doi.org/10.1002/ps.1751 PMid:19347968

Nourozian J, Etebarian HR and Khodakaramian G. 2006. Biological control of *Fusarium graminearum* on wheat

by antagonistic bacteria Songklanakarin. *J Sci Technol*. **28**: 29-38.

Payne GA. 1999. Ear and kernel rots. In: White, DG (Ed.). *Compendium of Corn Diseases*. Third Edition. American Phytopathological Society, St. Paul, MN, USA. 44–49. PMid:10216195

Reid LM, Hamilton RI, Mather DE. 1996. Screening Maize for Resistance to *Gibberella* Ear Rot. Agriculture and Agri-Food. Canadian Technological Bulletin Publication, 196-5E.

Schaad NW. 1992. *Laboratory guide for identification of plant pathogenic bacteria*. APS Press, St. Paul, Minnesota. P. 164.

Schwyn B, Neilands JB. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal Biochem*. **160**: 47-56.https://doi.org/10.1016/0003-2697(87)90612-9

Seely W, Vandemark J. 1981. *Microbes in action. A laboratory manual of microbiology*. The W.H. Freeman and Company Inc. (3rd edition) Sanfrancisco.

Sreenivasa MY. 2006. Molecular detection of fumonisin producing *Fusarium* species of freshly harvested maize kernels using Polymerase Chain Reaction (PCR). *Taiwania-Taipei* **51**(4): 251.

Suslow TV, Schroth MN, Isaka M. 1982. Application of rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathol*. **72**: 917-918. https://doi.org/10.1094/Phyto-72-917

Tutte J. 1969. *Plant Pathological methods*. Fungi and bacteria. Burgess publishing company, U.S.A. 229.

Vidhyasekaran P, Muthamilan M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis*. **79**: 782-786. https://doi.org/10.1094/PD-79-0782

Visentin I, Tamietti G, Valentino D, Portis E, Karlovsky P, Moretti A, Cardinale F. 2009. The ITS region as a taxonomic discriminator between *Fusarium verticillioides* and *Fusarium proliferatum*. *Mycol Res*. **113**: 1137-1145. https://doi.org/10.1016/j.mycres.2009.07.011 PMid:19631741

Uzair B, Kausar R, Bano SA, Fatima S, Badshah M, Habiba U, Fasim F. 2018. Isolation and molecular characterization of a model antagonistic *Pseudomonas aeruginosa* divulging In Vitro plant growth promoting characteristics. *Biomed Res Int.* 6147380 https://doi.org/10.1155/2018/6147380

Vorholt JA. 2012. Microbial life in the phyllosphere. *Nat Rev Microbiol*. **10**: 828-840. https://doi.org/10.1038/nrmicro2910 PMid:23154261

Zhou L, Jiang HX, Sun S, Yang DD, Jin KM, Zhang W, He YW. 2016. Biotechnological potential of a rhizosphere *Pseudomonas aeruginosa* strain producing phenazine-1-carboxylic acid andphenazine-1-carboxamide. *World J Microbiol Biotechnol*. **32**(3): 50. https://doi.org/10.1007/s11274-015-1987-y PMid:26873561