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Molecular characterization of effective PGPRs from rhizosphere of banana against tip over disease caused by *Erwinia carotovora* subsp. carotovora

Soumya D Rotti and MR Ravikumar

Abstract
In recent years, there has been a reverse interest in the search of plant growth promoting rhizobacterias (PGPRs) for sustainable crop production. Banana is an economically important tropical fruit crop, which is subjected to infection by fungai, bacteria, virus and nematodes. A total of 134 PGPRs were isolated from rhizosphere of banana. Twelve out of 64 isolates of *Bacillus* spp. and seven out of 70 isolates of *Pseudomonas* spp. were found to effective against the *Erwinia carotovora* subsp. *carotovora in vitro*. Among them most effective isolate of PGPRs were further subjected for molecular characterization. The molecular studies confirmed them to be as *Bacillus pumilis, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Pseudomonas putida*.

Keywords: Plant growth promoting rhizobacterias, Banana, *Erwinia carotovora* subsp. *carotovora*, *in vitro*, *Bacillus pumilis*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Pseudomonas putida*.

Introduction
Biological control of plant pathogens by antagonistic microorganisms is a potential non-chemical means (Harman, 1991) [7] and is known to be a cheap, effective and eco-friendly method for the management of crop diseases (Cook and Baker, 1983) [5]. The use of biological control agents as an alternative to fungicides and bactericides is increasing rapidly in the present day agriculture due to the deleterious effects of chemical pesticides. Efforts to control plant diseases with antagonistic bacterial agents have been made successfully (Chen *et al*., 1995) [3]. *Bacillus* spp. have special characteristics that make them good candidates as biological control agents. Members of the genus Pseudomonas have long been known for their potential to reduce the plant disease and they have gained considerable importance as potential antagonistic microorganisms (Pant and Mukhopadhyay, 2001) [9]. Among these, the bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *Pseudomonas* ssp. And *Bacillus* spp. have been successfully used for biological control of several plant pathogens (Ramamoorthy *et al*., 2001) [10] and biological control using PGPR strains especially from the genus *Pseudomonas* is an effective substitute for chemical pesticides to suppress plant diseases (Compant *et al*., 2005) [4]. The soil bacteria that aggressively colonize the root zone and promote plant growth are generally termed as Plant Growth Promoting Rhizobacterias (PGPRs). Gechemba *et al.* (2016) [6] reported that the tropical banana rhizosphere harbor's a wide diversity of antagonastic bacteria that may not only aid in beneficial symbiotic relationships but also stimulate the plant growth by suppressing pathogenic organisms.
Tip over is one of the important disease of banana caused by *Erwinia carotovora* subsp. *carotovora* causing yield losses upto 65.28 percent (Totagi, 2012) [12] and the disease is transferred through tissue cultured materials, infected seedlings, soil and water.

Material and Methods
Isolation of PGPRs from the rhizosphere of banana plant
Rhizospheric soil samples were collected from the neighbouring healthy plants of banana in the field. The collected soil were transferred to sample collection bags, antagonistic bacterium was isolated by following serial dilution and Pour plate method by using Hicrome Bacillus agar, Nutrient agar and King's B media.

Isolation of *Pseudomonas* spp.
Fluorescent Pseudomonads were isolated from soil using a specific media *viz.*, King's B (KB)

medium following serial dilution and pour plate technique was done. The plates were incubated at 28 ± 1 ºC for 24 h. Colonies were observed under UV light. The fluorescent colonies observed under UV light were picked up, was purified by repeated streaking on same medium and checked for their fluorescence. Further well isolated single colonies were transferred to 20 percent glycerol stock for preservation.

Isolation of *Bacillus* spp.

Different species of *Bacillus* were isolated from soil using a specific media *viz.*, Hicrome bacillus agar medium following serial dilution and plating technique was done. Then the plates were incubated at 28 ± 1 ºC for 48 h. Colonies formed were picked up and purified by repeated streaking on the Nutrient agar medium. Well isolated colonies were transferred to 20 percent glycerol stock for preservation.

In vitro* evaluation for efficacy of isolated PGPRs against *Erwinia carotovora* subsp. *carotovora

Isolated PGPRs were evaluated for their efficacy against the growth of *Erwinia carotovora* subsp. *carotovora* by well diffusion method. A heavy suspension of *Erwinia carotovora* subsp. *carotovora* was multiplied in nutrient broth (20 ml) was mixed with lukewarm nutrient agar medium in flask. The inoculated flasks were incubated at 28 ± 1 ºC for 48 h. The bacterial suspension was then seeded to the lukewarm nutrient agar medium. The seeded medium was poured into the sterilized Petri plates and was allowed to solidify. Then, a well with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and a volume (20-100 µL) of the isolated PGPRs cultured in the nutrient broth was introduced into the well. The inoculated plates were incubated at 28 ± 1 ºC for 48 h. The observations for the production of inhibition zone around the PGPRs was measured by taking mean diameter of the zone formed and then were analyzed statistically.

Molecular characterization of effective PGPRs

The total genomic DNA from pure culture of the different isolates of bacteria was extracted by the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray and Thompson, 1980) [8] with some modifications. PCR amplification of rDNA sequences were conducted by using the universal primers (16S rDNA for bacteria). Finally the amplified products of the representative samples were sent for sequencing. The obtained sequence results were analyzed using Basic Local Alignment Search Tool (BLAST) algorithm available at http://www.ncbi.nlm.nih.gov.

Results and Discussion

Isolation of PGPRs from rhizosphere of banana

Number of isolates collected from rhizosphere of banana varied from one place to other place. A total of 134 isolates were collected from surveyed area. Among the total isolates, 64 isolates were identified as *Bacillus* spp. and remaining 70 isolates were identified as *Pseudomonas* spp. Similarly, Apastambh *et al.* (2016) [1] isolated 8 strains of fluorescent pseudomonas and 4 strains of Bacillus from Banana rhizosphere.

In vitro* evaluation of isolated PGPRs against *Erwinia carotovora* subsp. *carotovora

Among 64 isolates of isolated *Bacillus* spp. 12 isolates were found to be effective compared to other isolates (Table 1 & Fig 1). Among the 70 isolates of isolated *Pseudomonas* spp. 7 isolates were found to be effective compared other isolates (Table 2 & Fig 2). Among 12 effective isolates of *Bacillus* spp. maximum inhibition (16.67 mm) was observed by Belagavi isolate 13 and minimum inhibition (12.00 mm) was observed by Haveri isolate 5. Among 7 effective isolates of *Pseudomonas* spp. maximum inhibition (18.00 mm) was observed by Belagavi isolate 8 and minimum inhibition (12.00 mm) was shown by Dharwad isolate 2. *Pseudomonas* spp. was found to suppress *Erwinia carotovora* subsp. *carotovora* with maximum inhibition (18.00 mm) whereas, *Bacillus* spp. showed the maximum inhibition (16.67 mm). Hence, it indicated that *Pseudomonas* spp. are more efficient than *Bacillus* spp. Similarly Snehalatharani and Khan (2009) [11] reported that the efficacy of three antagonistic microorganisms. *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Among antagonistic microorganisms, *Pseudomonas aeruginosa* was found to be most effective *in vitro* conditions followed by *Pseudomonas fluorescens*.

Molecular characterization of the effective PGPRs

Out of 12 effective isolates of *Bacillus* spp. 4 most effective isolates and of 7 effective isolates of *Pseudomonas* spp. 6 most effective isolates were characterized molecularly. The isolated DNA was amplified at 1500 bp (plate 1). Molecular characterization of effective *Bacillus* spp. were identified as *Bacillus cereus* (Belagavi isolate 13 and Vijayapur isolate 2), *Bacillus subtilis* (Vijayapur isolate 7) and *Bacillus pumilis* (Vijayapur isolate 8). These results were in similar with the results of biochemical characterization. Molecular characterization of effective *Pseudomonas* spp. were identified as *Pseudomonas aeruginosa* (Belagavi isolate 8, Belagavi isolate 9, Haveri isolate 3, Belagavi isolate 4 and Vijayapur isolate 3) and *Pseudomonas putida* (Bagalkote isolate 5). Balayogan and Marimuthu (2014) isolated and molecularly characterized the potential plant growth promoting *Bacillus cereus* GGBSTD1 and *Pseudomonas* spp. GGBSTD3 from Vermisources.

1. Belagavi isolate 13

The Microbe was found to be most *Bacillus cereus* strain LB8, Sequence ID (Accession no.): MH187637. The next closest homologue was found to be *Bacillus cereus* strain SML_M123, Sequence ID: MG937670.

>16SRDNAF

CATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTT
ATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGG
GTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA
CCGGGGCTAATACCGGATAACATTTTGAACCGCATG
GTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTAT
GGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGT
AACGGCTCACCAAGGCAACGATGCGTAGCCGACCTG
AGAGGGTGATCGGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT
CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC
GTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGG
CACCTTGACGGTACCTAACCAGAAAGCCACGGCTAA
CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGC
AGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTG
AGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCG
GTGAAATGCGTAGAGATATGGAGGAACACCAGTGG

CGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGG
CGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTA

2. Vijayapur isolate 7

The Microbe was found to be most *Bacillus subtilis* strain GF14, Sequence ID (Accession no.): MG976623. The next closest homologue was found to be *Bacillus subtilis* strain GF3, Sequence ID: MG976621.

>16SRDNAF

TGCAGTCGAGCGGACAGGCGGGAGATTTGCGTCTCT
CTATGTTAGCGACGGACGGACGAGTGACACGTGTAT
CACCTGCCTGCCAGACTGGGAGGATTCCTACTCGCC
GGAGCTAATACCGGATAGTTCCTTGTACCGGGGGAA
GGGGGGAAGAAAGACAGCTCCCACTGTTGCTTACGG
ATGGACCCGATTAGCATTATCTAGGTGGAGAGGCTC
CGGCTCACCAAGGCGACGATGCGGATCCGACCTGAA
AGGGTGATCCTGCACACTGGGACTGACCACACTCCT
AACTCCTACGGGAGGGGGGAATTGGAATCTTGGCAA
TGGACTGATCCTGACGGACAACGCCGCATGAGTGAT
GAAGGTTTTCGGATCACTTTTTCTGTTGTTAGGAATA
ACCCCGCTGAGAACTGCTTGCACCTTGACTGCACCT
AACCAGAAAGCCACCTATAACTACGTGCCAGCAGCC
GCGGTAATACGGAAGTGGCAAGCGTTAATCGGAATT
ATTGGGCGTAAAGGGCTCGCATGCGGATTCTTAATG
CTGATGTGAAAGCCCCCGGCTCAACACTGGAGGGTC
ATTTGCCACTGGGAAACTTGAGTGCAGAAGAGGAGA
GTGGAATTCCACCTGTAAAATGCGAAATATATATGA
GATGAGTACCGAACACCAATGGCGAACGCCTCTCTC
TGGTCTGACACTGACACTCAAGAGCGAAAGCATGGG
GCAGCGAACAGATATTACTATACCCTGGTAGTCCAC
ACCGATAAACAATAACTGCTAGGGTGTCTATTG

3. Vijayapur isolate 2

The Microbe was found to be most *Bacillus cereus* strain AMB_17, Sequence ID (Accession no.): JX971533. The next closest homologue was found to be *Bacillus cereus* strain H25, Sequence ID: MH045979.

>_16SRDNAF

TCAACACGCTATACTGAAGGTTTTAGTGTACGGGTG
CCCAACACGTGGGTAACCTGCCCATAAGACTGGGAT
AACTCCGGGAAACCGGGGCTAATACCGGATAACATT
TTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCG
GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCT
AGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGC
GTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCG
TAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTT
GAATAAGCTGGCACCTTGACGGTACCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA
CGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGT
AAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGA
AAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAAC
TGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATT
CCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGG
AACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAAC
TGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAA

4. Vijayapur isolate 8

The Microbe was found to be most *Bacillus pumilis* strain SCSGAB0102, Sequence ID (Accession no.): JX315311. The next closest homologue was found to be *Bacillus pumilis* strain AIMST 1.A.sub3L, Sequence ID: JF819677.

>_16SRDNAF

TGCAGTCGAGCGGACAGGCGGGAGATTTGCGTCTCT
CTATGTTAGCGACGGACGGACGAGTGACACGTGTAT
CACCTGCCTGCCAGACTGGGAGGATTCCTACTCGCC
GGAGCTAATACCGGATAGTTCCTTGTACCGGGGGAA
GGGGGGAAGAAAGACAGCTCCCACTGTTGCTTACGG
ATGGACCCGATTAGCATTATCTAGGTGGAGAGGCTC
CGGCTCACCAAGGCGACGATGCGGATCCGACCTGAA
AGGGTGATCCTGCACACTGGGACTGACCACACTCCT
AACTCCTACGGGAGGGGGGAATTGGAATCTTGGCAA
TGGACTGATCCTGACGGACAACGCCGCATGAGTGAT
GAAGGTTTTCGGATCACTTTTTCTGTTGTTAGGAATA
ACCCCGCTGAGAACTGCTTGCACCTTGACTGCACCT
AACCAGAAAGCCACCTATAACTACGTGCCAGCAGCC
GCGGTAATACGGAAGTGGCAAGCGTTAATCGGAATT
ATTGGGCGTAAAGGGCTCGCATGCGGATTCTTAATG
CTGATGTGAAAGCCCCCGGCTCAACACTGGAGGGTC
ATTTGCCACTGGGAAACTTGAGTGCAGAAGAGGAGA
GTGGAATTCCACCTGTAAAATGCGAAATATATATGA
GATGAGTACCGAACACCAATGGCGAACGCCTCTCTC
TGGTCTGACACTGACACTCAAGAGCGAAAGCATGGG
GCAGCGAACAGATATTACTATACCCTGGTAGTCCAC
ACCGATAAACAATAACTGCTAGGGTGTCTATTG

5. Belagavi isolate 8

The Microbe was found to be most *Pseudomonas aeruginosa* strain CCUG 70744, Sequence ID (Accession no.): CP023255. The next closest homologue was found to be *Pseudomonas aeruginosa* strain AR_0446, Sequence ID: CP029660.

>_16SRDNAF

AGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCA
GCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCT
GGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAAT
ACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTT
CGGACCTCACGCTATCAGATGAGCCTAGGTCGGATT
AGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG
ATCCGTAACTGGTCTGAGAGGATGATCAGTCACACT
GGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGGACAATGGGCGAAAGCCT
GATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCG
GATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGT
AAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAA
TAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTA
ATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAA
AACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAA
TTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAA
GGAACACCAGTGGCGAAGGCGACCACCTGGACTGA
TACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAGACG
ATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTG
GCGCAGCT

6. Belagavi isolate 9

The Microbe was found to be most *Pseudomonas aeruginosa* strain Dut-lxm0725, Sequence ID (Accession no.): MF100795. The next closest homologue was found to be

Pseudomonas aeruginosa strain CNSG21, Sequence ID: KY962356.

>**_16SRDNAF**
GCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATT
CAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGC
CTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTA
ATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATC
TTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGA
TTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGA
CGATCCGTAACTGGTCTGAGAGGATGATCAGTCACA
CTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCT
GATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCG
GATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGT
AAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAA
TAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTA
ATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAA
AACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAA
TTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAA
GGAACACCAGTGGCGAAGGCGACCACCTGGACTGA
TACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAGACG
ATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGT

7. Haveri isolate 3

The Microbe was found to be most *Pseudomonas aeruginosa* strain FB3, Sequence ID (Accession no.): HQ658764. The next closest homologue was found to be *Pseudomonas aeruginosa* strain RA5, Sequence ID: MH160762.

>**_16SRDNAF**
ACGCTCGTAGCGGATGAAGTGGAGCTTGCTCCTGGA
TTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCT
GCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGC
TAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGA
TCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCG
GATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGC
GACGATCCGTAACTGGTCTGAGAGGATGATCAGTCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGGACAATGGGCGAAAG
CCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCT
TCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGC
AGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACA
GAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCG
GTAATACGAAGGGTGCAAGCGTTAATCGGAATTACT
GGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGG
ATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATC
CAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTG
GAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAG
GAAGGAACACCAGTGGCGAAGGCGACCACCTGGAC
TGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
CGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTA
GTGGCGCAGC

8. Belagavi isolate 4

The Microbe was found to be most *Pseudomonas aeruginosa* strain DM11, Sequence ID (Accession no.): KT229744. The next closest homologue was found to be *Pseudomonas aeruginosa* strainPA002, Sequence ID: KP728981.

>**_16SRDNAF**
CAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTC
AGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCC
TGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAA
TACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCT
TCGGACCTCACGCTATCAGATGAGCCTAGGTCGGAT
TAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGAC
GATCCGTAACTGGTCTGAGAGGATGATCAGTCACAC
TGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGGACAATGGGCGAAAGCCT
GATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCG
GATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGT
AAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAA
TAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTA
ATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAA
AACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAA
TTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAA
GGAGCACCAGTGGCGAAGGCGACCACCTGGACTGA
TACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG
ATGTCGACTAGCCGTTGGGATCCTTGAGATCTTA

9. Bagalkote isolate 5

The Microbe was found to be most *Pseudomonas putida* strain Sp16, Sequence ID (Accession no.): KF767887. The next closest homologue was found to be *Pseudomonas putida* strain CG29, Sequence ID: KF782801.

>**_16SRDNAF**
AGCCGTAGCGGATGACGGGAGCTTGCTCCTGGATTC
AGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCC
TGGTAGTGGGGGACAACGTTTCGAAACGGACGCTAA
TACCGCATACGTCCTGAGGGAGAAAGCGGGGGATCT
TCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGAT
TAGCTAGTTGGTGAGGTAATGGCTCACCTAGGCGAC
GATCCGTAACTGGTCTGAGAGGATGATCAGTCACAC
TGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGGACAATGGGCGAAAGCCT
GATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCG
GATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGT
AAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAA
TAAGCACCGGCTAACTTTGTGCCAGCAGCCGCGGTA
ATACAAAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCGCGTAGGTGGTTCATTAAGTTGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAA
AACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAA
TTTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGA
AGGAACACCAGTGGCGAAGGCGACCACCTGGACTG
ATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTA

10. Vijayapur isolate 3

The Microbe was found to be most *Pseudomonas aeruginosa* strain AR_0357, Sequence ID (Accession no.): CP027166. The next closest homologue was found to be *Pseudomonas aeruginosa* strain AR_0446, Sequence ID: CP029660.

>**_16SRDNAF**
GCAGCTCGAGCGGATGAAGGGAGCTTGCTCCTGGAT
TCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTG
CCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
AATACCGCATACGTCCTGAGGGAGAAAGTGGGGGAT
CTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG

ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCG
ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCAC
ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGGACAATGGGCGAAAGC
CTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTT
CGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCA
GTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAG
AATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGG
TAATACGAAGGGTGCAAGCGTTAATCGGAATTACTG
GGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGA
TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCC
AAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGG
AATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGG
AAGGAACACCAGTGGCGAAGGCGACCACCTGGACT
GATACTGACACTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
CGATGTCGACTAGCCGTTGGGA

Table 1: *In vitro* evaluation of *Bacillus* spp. against the growth of *Erwinia carotovora* subsp. *Carotovora*

Treatments	Isolates	Mean diameter of inhibition zone (mm)
T_1	Vijayapur isolate 2	15.33 (4.04) *
T_2	Vijayapur isolate 4	14.33 (3.92)
T_3	Vijayapur isolate 5	14.00 (3.87)
T_4	Vijayapur isolate 7	16.00 (4.12)
T_5	Vijayapur isolate 8	14.67 (3.96)
T_6	Haveri isolate 5	12.00 (3.61)
T_7	Dharwad isolate 1	14.00 (3.87)
T_8	Belagavi isolate 12	15.00 (4.00)
T_9	Belagavi isolate 13	16.67 (4.20)
T_{10}	Bagalkote isolate 15	13.00 (3.74)
T_{11}	Belagavi isolate 16	14.00 (3.87)
T_{12}	Belagavi isolate 17	13.67 (3.83)
S. Em.±		0.059
C.D. at 1%		0.235

* - √ x+1 transformed values

Table 2: *In vitro* evaluation of *Pseudomonas* spp. against the growth of *Erwinia carotovora* subsp. *carotovora*

Treatments	Isolates	Mean diameter of inhibition zone (mm)
T_1	Vijayapur isolate 3	12.17 (3.63) *
T_2	Dharwad isolate 2	12.00 (3.61)
T_3	Haveri isolate 3	15.00 (4.00)
T_4	Belagavi isolate 4	14.00 (3.87)
T_5	Belagavi isolate 8	18.00 (4.36)
T_6	Belagavi isolate 9	16.00 (4.12)
T_7	Bagalkote isolate 5	13.00 (3.74)
S.Em. ±		0.030
C. D. at 1%		0.128

* - √ x+1 transformed values

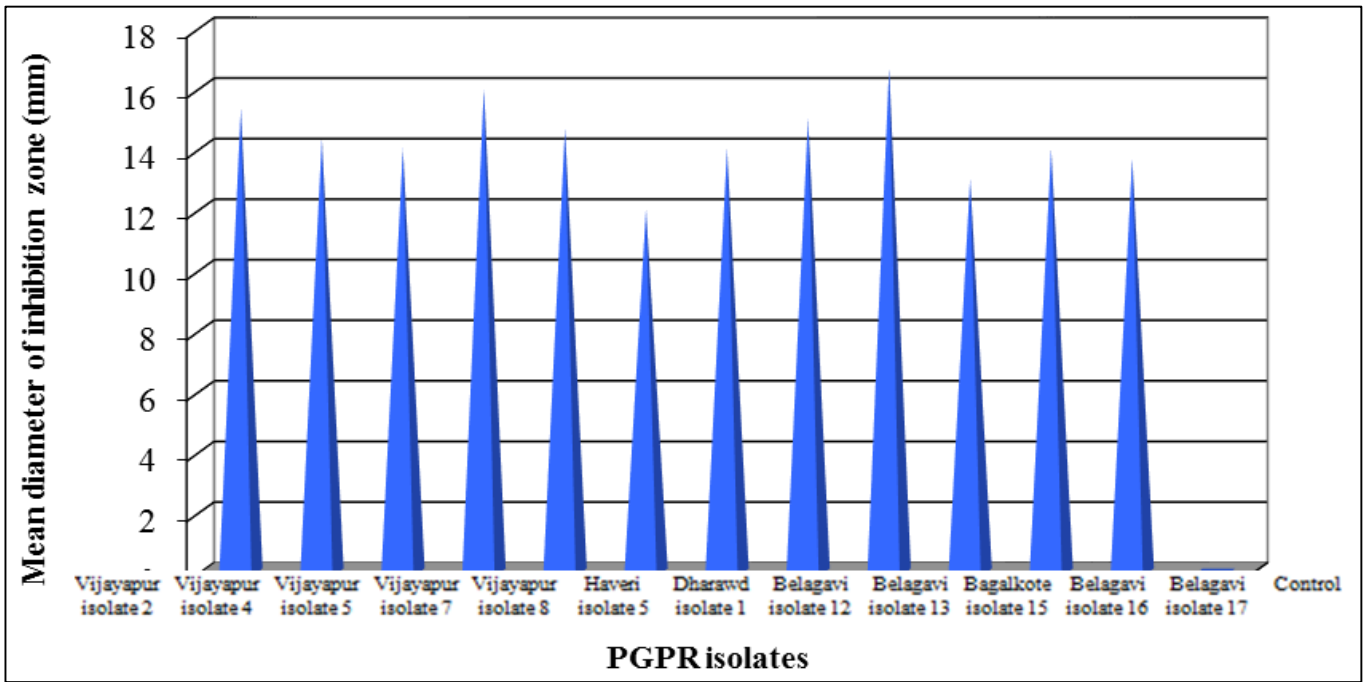


Fig 1: *In vitro* evaluation of *Bacillus* spp. against the growth of *Erwinia carotovora* subsp. *carotovora*

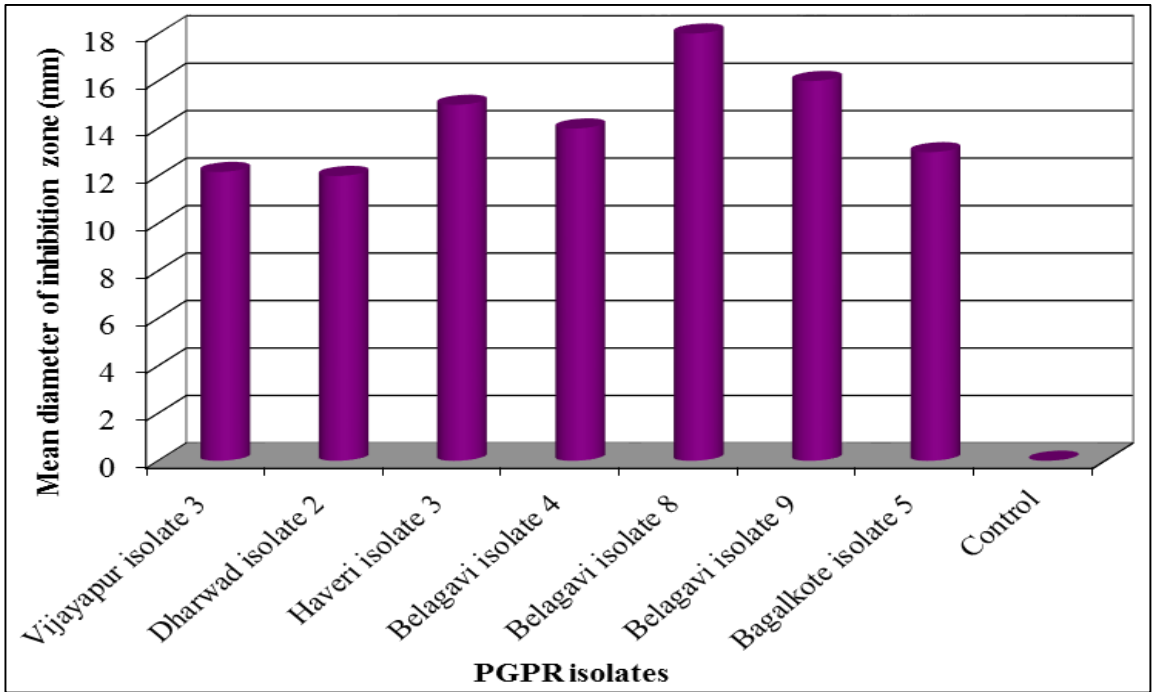
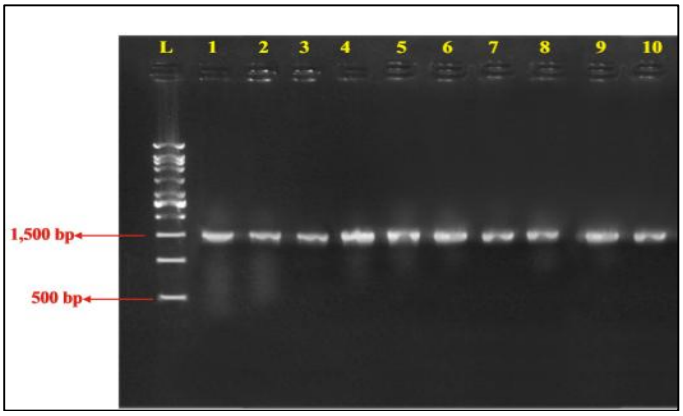


Fig 2: *In vitro* evaluation of *Pseudomonas* spp. against the growth of *Erwinia carotovora* subsp. *carotovora*



L - 100 bp ladder 1) Belagavi isolate 13 2)Vijayapura isolate 12 3)Vijayapura isolate 7 4) Belagavi isolate 12 5) Belagavi isolate 8 6) Belagavi isolate 9 7) Haveri isolate 3 8) Belagavi isolate 4 9) Vijayapura isolate 3 10) Dharwad isolate 2

Plate 1: PCR amplification of effective PGPRs

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