ISSN 0971-930X
Journal of Biological Control
Published by Society for Biocontrol Advancement (Copyright 2000 SBA)

*Journal of Biological Control*, 26 (1): 62-69, 2012

**Research Article**

# Post harvest fruit bioassay of phylloplane, pomoplane and endophytic microbes against chilli anthracnose pathogen, *Colletotrichum capsici* (Syd.) E. J. Butler & Bisby

**B. RAMANUJAM*, VINAYA HEMANNAVAR, HONNUR BASHA and R. RANGESHWARAN**

*National Bureau of Agriculturally Important Insects, HA Farm post, Bellary Road, Hebbal, Bangalore 560024, Karnataka, India.*
*Corresponding author E-mail*: bonamramanujam58@gmail.com

**ABSTRACT**: Two hundred and fifty eight phylloplane/pomoplane/endophytic bacterial isolates from chilli leaves/fruits and one hundred pomoplane yeast isolates from vegetable/fruits were screened against *Colletotrichum capsici* by fruit bioassay (post harvest) method. Among the pomoplane bacterial isolates tested, *Bacillus tequilensis* (PMB-185) gave highest reduction (67.84%) of lesion development, where as among the phylloplane bacterial isolates, PHB-25 exhibited highest (48.65%) suppression of lesion caused by *C. capsici*. Among the endophytes tested, *B. megaterium* (ENB-86) produced the highest suppression of lesion (59.66%) and rhizospheric bacterium *Pseudomonas putida* (PBA-5) showed 50.68% suppression. Six bacteria exhibiting significant suppression (50.29 to 67.84%) were identified by 16s rDNA analysis and all of them belonged to *Bacillus* spp. including *B. tequilensis* (PMB-185), *B. pumilus* (PMB-183), two *B. subtilis* (PMB-123 and ENB-24) and two *B. megaterium* (PMB-53 and ENB-86). Among the yeast isolates tested, the maximum reduction (72.16%) of lesion development was observed with the yeast isolate, *Hanseniaspora uvarum* (Y-73) which was the highest among all the antagonists tested. The results indicated that spraying of *H. uvarum* (Y-73) or *B. tequilensis* (PMB-185) on freshly harvested chilli fruits reduced post harvest fruit damage by *C. capsici* in chilli.

**KEY WORDS**: Chilli anthracnose, *Colletotrichum capsici*, Fruit bioassay, *Pichia guilliermondii, Bacillus* species.

(Article chronicle: Received: 19-9-2011 Revised: 27-12-2011 Accepted: 11-1-2012)

## INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most important spice/vegetable/cash crop grown in India belonging to solanaceae. It is an essential ingredient of Indian cuisine and used both as green and ripe fruit to impart pungency and flavour to the food. India accounts for 25% of the world's total production of chilli. Anthracnose or ripe fruit rot caused by *Colletotrichum capsici* (Syd.) E. J. Butler & Bisby is a serious problem limiting the profitable cultivation and seed production throughout the major chilli growing regions of India. Thind and Jhooty (1985) reported that anthracnose of chilli caused losses of 66-84 per cent. Vinaya *et al.,* (2009) surveyed the major diseases that afflict chilli in Karnataka and found that *C. capsici* was the most predominant fungi encountered (71.24%). Apart from anthracnose, *Colletotrichum* species also cause dieback in plants which can devastate the crop (Than *et al*. 2008). During storage, *C. capsici* cause severe damage to fruits in the form of anthracnose lesions thus reducing it's marketability (Manandhar *et al*., 1995). Although many fungicides like *Maneb, Carbendazim, Triazole* etc., are available for the management of fruit rot, their continuous and non-discriminatory use is known to cause undesirable effects such as residual toxicity, resistance development, environmental pollution and health hazards to humans and animals (Ngullie *et al*., 2010). The antagonistic organisms offer great potential for safe and effective management of diseases of vegetable crops without any adverse effect on the environment.

The present study was taken up to screen natural bacterial and yeast microflora from chilli phylloplane, fruit surface (pomoplane) and endophytic (tissue of leaves/ fruits) including those from other vegetables/fruits for their antagonistic effect against *C. capsici*. Harvested fresh chilli fruits that are most susceptible were used in the bioassay.

## MATERIALS AND METHODS

### Isolation of bacteria and yeast microflora from chillies/ vegetables/fruits

Sixty six leaf samples, 100 green fruit samples and 88 ripe fruit samples of chillies were collected from fifty two different chilli cultivars/ varieties from Bangalore, Raichur, Dharwad, Gadag, Haveri, Gulbarga and Yadgiri

districts of Karnataka, Mahbubnagar, Guntur, Khammam and Anantpur districts of Andhra Pradesh and Idukki district of Kerala for isolation of phylloplane/pomoplane/ endophytic bacterial isolates. Seventy two samples of other fruits (grapes, oranges, sapota, banana, pear, pomegranate, apples, custard apple, guava and sweet lime), vegetables (capsicum, cluster beans, sweet potato, green pea pods and cucumber) and leaves (mango and cashew) were collected from Bangalore district for isolation of pomoplane yeasts.

Isolation of phylloplane bacteria from chilli was carried out by plating leaf washings on nutrient agar (NA) medium (Ramanujam, 2008). One gram leaves from each sample were cut into discs of 6-mm diameter, transferred to 100-ml sterile water blank and stirred for 20 min using magnetic stirrer. From these washings, dilutions of $10^{-3}$, $10^{-4}$ and $10^{-5}$ were prepared and one ml aliquots of these dilutions were plated on NA by spread plate technique. The plates were incubated for 48hr at 30°C in a BOD. The bacterial colonies obtained on the Petri plates were purified and maintained on NA slants in a refrigerator. For isolation of pomoplane bacteria, 100g of fruits from each sample were used and isolated as described above.

Endophytic bacteria from chilli leaves/fruit tissues were isolated according to the procedure described by McInroy and Kloepper (1995). Chilli leaf/fruit sample was surface sterilized with 20% $H_2O_2$ (v/v) and washed four times with 0.02M potassium phosphate buffer (pH 7.0). The sample was macerated in 9 ml potassium phosphate buffer (0.02M, pH 7.0) and diluted to $10^{-2}$, $10^{-3}$,$10^{-4}$ and $10^{-5}$ concentrations. One ml of aliquots of each of these dilutions were plated on tryptic soya agar (TSA) medium. The plates were incubated for 4 days at 25ºC in a BOD. The bacterial colonies obtained thereby on the Petri plates were purified and maintained on TSA slants in a refrigerator.

For isolation of yeasts, 100g of the sample was suspended in 100 ml sterile distilled water and shaken vigorously for a few minutes. Serial dilutions ($10^{-2}$, $10^{-3}$ and $10^{-4}$) of the sample suspension were made in sterile distilled water. An aliquot of 1 ml of each dilution was plated on yeast extract peptone dextrose agar (YPDA) medium containing $10g^{-l}$ yeast extract, $20g^{-l}$ peptone, $20g^{-l}$ dextrose and $20g^{-l}$ agar and the cultured plates were incubated at 25ºC for 48-72hr (Chanchaichaovivat *et al*., 2007). The yeast isolates were maintained on nutrient yeast dextrose agar (NYDA) slants containing $8g^{-l}$ nutrient broth, $5g^{-l}$ yeast extract, $10g^{-l}$ glucose, and $20g^{-l}$ agar.

**Preparation of bacteria/yeast inocula**

A loopfull of bacterium/yeast was inoculated into 100ml of nutrient broth (NB) and nutrient yeast dextrose broth (NYDB) respectively and incubated in a rotary shaker for 48hr at 30ºC temperature for bacteria and 25ºC for yeast. Cell suspension was prepared by centrifuging 48hr broth culture at 5000 rpm for 15min and the pellet obtained was mixed in 50ml sterile water containing 0.1% carboxy methyl cellulose (CMC) as sticker and 0.1% Tween-80 as dispersing agent.

**Preparation of pathogen inoculum**

Virulent isolate of *C. capsici* (Cc-1) was isolated from anthracnose infected chilli fruit sample collected from IIHR Bangalore and grown on potato dextrose agar (PDA) at 25ºC for 15 days. The spore suspension was prepared by flooding the culture plate with sterile water and gently scraping with sterile inoculation needle. The suspension was filtered through muslin cloth and spore concentration was adjusted to $2x10^6$spores/ml using a haemocytometer.

**Fruit bioassay method**

Ripe chilli (susceptible variety, Byadagi) without any wound or scar on the surface were used for the study. Fruits were washed thoroughly with tap water and surface sterilized with 1% (v/v) sodium hypochlorite for five minutes followed by 70% ethanol for one minute and then rinsed twice with sterile distilled water. The surface sterilized fruits (15 fruits/isolate) were spray inoculated with bacterial/yeast cell suspension of $2x10^8$ cells/ml uniformly on fruit surface and allowed to dry for 2 hours. The surface sterlized fruits sprayed with sterile water served as check. After drying, 10µl of spore suspension of *C. capsici* at $2x10^6$spores/ml was injected into each fruit at the center using a sterile hypodermic needle (modified from Montri *et al*., 2009). The inoculated fruits were incubated in moist chamber at 28ºC temperature and 70% RH for development of anthracnose lesion. The lesion length was recorded on the treated and un-treated fruits after nine days of incubation. Data generated from the experiment was statistically analysed for reduction in the lesion length, if any, by one-way analysis of variance (ANOVA).

**Identification of bacteria and yeasts**

The comparitive 16S rDNA sequence was used for identification of promising bacterial antagonists and the ITS–region sequences for yeast identifiaction. Universal primer B16SF (5'AGAGTTTGATCCTGGCTCAG 3') and B16SR (5' CGGTGTGTACAAGACCC 3') (Schreiner *et al*., 2010) were used for the amplication of bacterial 16S rDNA region. For amplification of yeast ITS – region, primers YITS-1F (5'TCCGTAGGTGAACCTGCGG3') and YITS-2R (5'TCCTCCGCTTATTGATATGC 3') were used (Hierro *et al.,* 2004). The sequences were aligned and compared with NCBI database using BLAST search tool for identity establishment.

## RESULTS AND DISCUSSION

Two hundred and fifty eight bacterial isolates comprising of one hundred and fifty five phylloplane/pomoplane isolates from chilli leaves/fruits, ninety six endophytic bacterial isolates from chilli leaf/fruit tissues were isolated.

One hundred yeast isolates were obtained from different fruits/vegetables. Three rhizosphere isolates of *Bacillus subtilis* and four rhizosphere isolates of *Pseudomonas* sp., from NBAII culture collection were also used for the study. The bacterial and yeast antagonists showing more than 50% suppression of lesion development by *C. capsici* were identified through molecular characterization.

Among the twenty nine phylloplane bacteria tested for suppression of *C. capsici* by fruit bioassay method, the highest suppression (48.65%) of lesion development was shown by PHB-25 and the lowest (8.16 %) by PHB-30. However, six of the isolates did not show any inhibitory effect. Among seven rhizospheric bacterial culture collection of NBAII isolate PBA5 (*Pseudomonas putida*) showed highest (50.68%) lesion suppression (Table 1). Among one hundred and twenty six pomoplane bacteria tested, the isolate PMB-185 (*Bacillus tequilensis*) gave highest (67.84%) lesion suppression and the lowest (7.49%) by PMB-225 (Table 2). Seventeen pomoplane isolates did not show any suppression of *C. capsici*. Among the ninty six endophytic bacterial isolates, ENB-86 *(B. megaterium)* gave highest inhibition of lesion (59.66%) and ENB-53 showed the lowest (3.48%) (Table 3). Thirteen of the endophytic bacterial isolates showed no inhibition. The potential of microbial antagonists to control post-harvest diseases was initially demonstrated by an avocado phylloplane isolate of *B. subtilis* (ATCC55466/B246) (Korsten *et al.,* 1988, 1993, 1995). It was suggested by Korsten and De Jager (1995) that several modes of action may be involved in the biocontrol activity of *B. subtilis* including antibiosis, competitive exclusion and nutrient competition.

**Table 1: Suppression of *Colletotrichum capsici* by phylloplane and NBAII bacterial isolates**

| Sl. No | Phylloplane isolates | Lesion length suppression (%) | Sl. No. | NBAII isolates | Lesion length suppression (%) |
|---|---|---|---|---|---|
| 1 | PHB–22 | 34.63 (26.04) | 1 | PBA-5 | 50.68 (45.39) |
| 2 | PHB–25 | 48.65 (44.22) | 2 | PBA–14(1) | 43.38 (41.19) |
| 3 | PHB–28 | 25.68 (30.44) | 3 | PBA–8A | 6.36 (14.60) |
| 4 | PHB–29 | 35.14 (36.35) | 4 | PBA–14 | 13.63 (21.67) |
| 5 | PHB–30 | 8.16 (16.59) | 5 | S–7 | 19.05 (25.87) |
| 6 | PHB–35 | 37.78 (37.92) | 6 | S–9 | 46.75 (43.13) |
| 7 | PHB–36 | 27.78 (31.80) | 7 | S–14 | 12.38 (20.60) |
| 8 | PHB–38 | 19.23 (26.01) | CD (*P*=0.01) | | 6.41 |
| 9 | PHB–55 | 12.36 (20.58) | | | |
| 10 | PHB–56 | 12.36 (20.58) | | | |
| 11 | PHB–57 | 39.33 (38.84) | | | |
| 12 | PHB–58 | 28.09 (32.0) | | | |
| 13 | PHB–59 | 31.46 (34.11) | | | |
| 14 | PHB–79 | 18.75 (25.65) | | | |
| 15 | PHB–139 | 33.21(35.19) | | | |
| 16 | PHB–140 | 26.57(31.02) | | | |
| 17 | PHB–145 | 17.34(24.60) | | | |
| 18 | PHB–146 | 18.08(25.16) | | | |
| 19 | PHB–148 | 20.30(26.78) | | | |
| 20 | PHB–149 | 25.09(30.06) | | | |
| 21 | PHB–150 | 28.78(32.44) | | | |
| 22 | PHB–151 | 29.52(32.91) | | | |
| 23 | PHB–154 | 28.41(32.21) | | | |
| | CD (*P*=0.01) | 1.11 | | | |

Figures in parentheses are angular transformed values. Isolates showing no inhibition are not shown

**Table 2: Suppression of *Colletotrichum capsici* by pomoplane bacterial isolates**

| Sl. No. | Pomoplane isolates | Lesion length suppression (%) | Sl. No | Pomoplane isolates | Lesion length suppression (%) | Sl. No | Pomoplane isolates | Lesion length suppression (%) | Sl. No. | Pomoplane isolates | Lesion length suppression (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | PMB-40 | 23.08 (28.71) | 28 | PMB-135 | 25.83(30.54) | 55 | PMB -173 | 32.86(34.97) | 82 | PMB -204 | 11.88(20.16) |
| 2 | PMB-44 | 31.06 (33.87) | 29 | PMB-136 | 26.57(31.02) | 56 | PMB -174 | 35.71(36.69) | 83 | PMB -205 | 12.55(20.74) |
| 3 | PMB-50 | 15.08 (22.85) | 30 | PMB-137 | 34.00 (35.66) | 57 | PMB -176 | 48.57(44.18) | 84 | PMB -206 | 15.33(23.05) |
| 4 | PMB-53 | 54.33 (47.48) | 31 | PMB -138 | 28.04(31.97) | 58 | PMB -177 | 36.84(37.37) | 85 | PMB-207 | 17.13(24.44) |
| 5 | PMB-54 | 16.22 (23.75) | 32 | PMB -141 | 29.15(32.67) | 59 | PMB -180 | 24.56(29.70) | 86 | PMB-208 | 38.95(38.61) |
| 6 | PMB-83 | 18.75 (25.65) | 33 | PMB -142 | 29.52(32.91) | 60 | PHB-181 | 15.20(22.94) | 87 | PMB-209 | 11.19(19.54) |
| 7 | PMB-93 | 12.50 (20.70) | 34 | PMB -143 | 26.57(31.02) | 61 | PMB -182 | 48.57(44.18) | 88 | PMB -210 | 31.49(34.13) |
| 8 | PMB-95 | 32.26 (34.61) | 35 | PMB -144 | 30.26(33.37) | 62 | PMB -183 | 50.29(45.16) | 89 | PMB-211 | 21.10(27.34) |
| 9 | PMB-97 | 49.68 (44.81) | 36 | PMB -147 | 19.56(26.24) | 63 | PMB-184 | 28.65(32.36) | 90 | PMB-212 | 49.07(44.05) |
| 10 | PMB-98 | 48.71 (44.26) | 37 | PMB -152 | 30.63(33.60) | 64 | PMB-185 | 67.84(55.45) | 91 | PMB-213 | 31.20(33.95) |
| 11 | PMB-100 | 29.03 (32.60) | 38 | PMB -153 | 29.89(33.14) | 65 | PMB -186 | 22.22(28.12) | 92 | PMB -214 | 15.33(23.05) |
| 12 | PMB-111 | 24.00 (29.33) | 39 | PMB -155 | 26.94(31.26) | 66 | PMB-187 | 46.20(42.82) | 93 | PMB-215 | 43.04(36.74) |
| 13 | PMB-112 | 48.00 (43.85) | 40 | PMB -156 | 30.00(33.21) | 67 | PMB -189 | 11.11(19.47) | 94 | PMB -216 | 17.13(24.44) |
| 14 | PMB-119 | 30.10 (33.27) | 41 | PMB -157 | 19.19(25.98) | 68 | PMB -190 | 48.54(44.16) | 95 | PMB -217 | 11.19(19.54) |
| 15 | PMB-120 | 48.24 (43.99) | 42 | PMB -158 | 30.00(33.21) | 69 | PMB-191 | 11.70(20.00) | 96 | PMB -218 | 17.40(24.65) |
| 16 | PMB-121 | 41.52 (40.11) | 43 | PMB -159 | 8.57(17.02) | 70 | PMB -192 | 8.15(16.58) | 97 | PMB -219 | 24.67(29.78) |
| 17 | PMB-122 | 19.00 (25.84) | 44 | PMB -160 | 16.67(24.09) | 71 | PMB-193 | 49.86(44.92) | 98 | PMB -220 | 25.33(30.21) |
| 18 | PMB-123 | 61.69 (51.76) | 45 | PMB -161 | 21.43(27.57) | 72 | PMB-194 | 13.81(21.81) | 99 | PMB -221 | 47.47(43.08) |
| 19 | PMB-125 | 11.69 (19.99) | 46 | PMB -162 | 14.39(22.29) | 73 | PMB-195 | 11.44(19.76) | 100 | PMB -222 | 33.92(35.62) |
| 20 | PMB-127 | 42.39 (40.62) | 47 | PMB -163 | 19.05(25.87) | 74 | PMB -196 | 11.88(20.16) | 101 | PMB -223 | 20.70(27.06) |
| 21 | PMB-128 | 21.34 (27.51) | 48 | PMB -164 | 38.10(38.11) | 75 | PMB-197 | 48.07(43.89) | 102 | PMB -224 | 39.65(39.02) |
| 22 | PMB-129 | 31.28 (34.00) | 49 | PMB -165 | 40.48(39.51) | 76 | PMB -198 | 29.28(32.76) | 103 | PMB -225 | 7.49(15.88) |
| 23 | PMB-130 | 12.18(20.42) | 50 | PMB -166 | 26.57(31.02) | 77 | PMB-199 | 17.13(24.44) | 104 | PMB -226 | 38.33(38.25) |
| 24 | PMB-131 | 21.03(27.29) | 51 | PMB -167 | 26.19(30.78) | 78 | PMB -200 | 37.98(38.04) | 105 | PMB -227 | 37.00(37.46) |
| 25 | PMB-132 | 17.34(24.60) | 52 | PMB -168 | 16.67(24.09) | 79 | PMB-201 | 37.98(38.04) | 106 | PMB -228 | 41.63(40.18) |
| 26 | PMB-133 | 21.03(27.29) | 53 | PMB -169 | 42.86(40.89) | 80 | PMB -202 | 24.35(29.56) | 107 | PMB -229 | 36.56(37.20) |
| 27 | PMB-134 | 21.40(27.55) | 54 | PMB -171 | 20.95(27.24) | 81 | PMB-203 | 13.81(21.81) | 108 | PMB -230 | 33.92(35.62) |
| | | | | | | | | | 109 | PMB -231 | 8.49(16.94) |
| | CD ($P$=0.01) | 1.99 | | | | | | | | | |

Figures in parentheses are angular transformed values. Isolates showing no inhibition are not shown

**Table 3: Suppression of *Colletotrichum capsici* by endophytic bacteria**

| Sl. No. | | Lesion length suppression (%) | Sl. No | | Lesion length suppression (%) | Sl. No | | Lesion length suppression (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | ENB-4 | 37.50 (37.76) | 29 | ENB-69 | 15.78 (23.40) | 57 | ENB-99 | 36.56(37.20) |
| 2 | ENB-14 | 42.70 (40.80) | 30 | ENB-70 | 15.78 (23.40) | 58 | ENB-100 | 38.99(38.64) |
| 3 | ENB-17 | 6.81 (15.12) | 31 | ENB-71 | 36.84 (37.37) | 59 | ENB-101 | 25.33(30.21) |
| 4 | ENB-24 | 55.41 (48.10) | 32 | ENB-72 | 36.84(37.37) | 60 | ENB-102 | 18.94(25.79) |
| 5 | ENB-26 | 33.33 (35.26) | 33 | ENB-73 | 31.57 (34.18) | 61 | ENB-103 | 43.61(41.32) |
| 6 | ENB-27 | 38.97 (38.62) | 34 | ENB-74 | 20.88(27.19) | 62 | ENB-104 | 31.72(34.27) |
| 7 | ENB-28 | 41.91 (40.34) | 35 | ENB-75 | 25.27(30.17) | 63 | ENB-105 | 19.78(26.40) |
| 8 | ENB-30 | 40.69 (39.63) | 36 | ENB-76 | 44.73(41.97) | 64 | ENB-106 | 25.27(30.17) |
| 9 | ENB-31 | 20.54 (26.95) | 37 | ENB-77 | 27.11(31.42) | 65 | ENB-107 | 30.04(33.23) |
| 10 | ENB-40 | 6.25 (14.47) | 38 | ENB-78 | 25.27(30.17) | 66 | ENB-108 | 33.70(35.48) |
| 11 | ENB-41 | 41.75 (40.25) | 39 | ENB-79 | 29.28(32.76) | 67 | ENB-109 | 34.80(36.15) |
| 12 | ENB45 | 42.70 (40.80) | 40 | ENB-80 | 28.65(32.36) | 68 | ENB-110 | 36.63(37.24) |
| 13 | ENB-50 | 40.54 (39.54) | 41 | ENB-81 | 26.37(30.89) | 69 | ENB-111 | 26.01(30.66) |
| 14 | ENB-51 | 27.77 (31.80) | 42 | ENB-82 | 23.08(28.71) | 70 | ENB-112 | 29.67(33.00) |
| 15 | ENB-52 | 27.78 (31.80) | 43 | ENB-83 | 26.37(30.89) | 71 | ENB-113 | 30.40(33.46) |
| 16 | ENB-53 | 3.48 (10.75) | 44 | ENB-84 | 21.98(27.95) | 72 | ENB-114 | 18.68(25.60) |
| 17 | ENB-54 | 38.37 (38.27) | 45 | ENB-85 | 58.66 (49.98) | 73 | ENB-115 | 16.48(23.95) |
| 18 | ENB-55 | 17.44 (24.68) | 46 | ENB-86 | 59.66 (50.57) | 74 | ENB-116 | 28.21(32.08) |
| 19 | ENB-56 | 31.57 (34.18) | 47 | ENB-89 | 32.00 (34.45) | 75 | ENB-117 | 22.71(28.46) |
| 20 | ENB-57 | 30.23 (33.35) | 48 | ENB-90 | 29.30 (32.77) | 76 | ENB-118 | 21.61(27.70) |
| 21 | ENB-58 | 40.69 (39.63) | 49 | ENB-91 | 20.51(26.92) | 77 | ENB-119 | 30.04(33.23) |
| 22 | ENB-59 | 31.39 (34.07) | 50 | ENB-92 | 32.23(34.59) | 78 | ENB-120 | 29.30(32.77) |
| 23 | ENB-62 | 27.90 (31.88) | 51 | ENB-93 | 20.88(27.19) | 79 | ENB-121 | 24.54(29.69) |
| 24 | ENB-63 | 6.97 (15.30) | 52 | ENB-94 | 19.78(26.40) | 80 | ENB-122 | 28.57(32.31) |
| 25 | ENB-64 | 10.99(19.36) | 53 | ENB-95 | 21.98(27.95) | 81 | ENB-123 | 27.11(31.37) |
| 26 | ENB-65 | 36.84(37.37) | 54 | ENB-96 | 23.08(28.71) | 82 | ENB-124 | 14.65(22.50) |
| 27 | ENB-66 | 36.84 (37.37) | 55 | ENB-97 | 29.30(32.77) | 83 | ENB-125 | 12.45(20.66) |
| 28 | ENB-67 | 10.52 (18.91) | 56 | ENB-98 | 42.51(40.69) | CD (p=0.01) | | 5.22 |

Figures in parentheses are angular transformed values. Isolates showing no inhibition are not shown

Seven bacteria exhibiting significant suppression (50.29 to 67.84%) were identified by 16s rDNA analysis and six of them belonged to *Bacillus* spp. and one to *Pseudomonas* spp. *B. tequilensis* (PMB-185) gave maximum (67.84%) suppression among all the bacterial isolates tested and the next best was *B. subtilis* (PMB-123 isolate) which showed 61.69% suppression (Fig. 1)**.** The isolate ENB-24 identified as *B. subtilis* showed 55.41% suppression. Isolates PMB-53 and ENB-86 were identified as *B. megaterium* and showed 54.33 and 59.66 % suppression respectively. The isolate PMB-183 were identified as *B. pumilus* showed 50.29% suppression and *P. putida* (PBA-5) showed 50.68% suppression of *C.capsici* (Table 5). Xue-qing *et al***.,** 2004 reported control of capsicum anthrancnose by endophytic *Bacillus subtilis* isolates BS-1 and BS-2 under greenhouse conditions. Ramamoorthy and Samiyappan (2001) reported that *Pseudomonas fluorescens* isolate pf1 effectively inhibited the mycelial growth of *C. capsici* under *in vitro* and decreased the fruit rot incidence in chilli under

greenhouse conditions. Havenga *et al*., (1999) showed that *B*. *subtilis* multiplied rapidly four hours after inoculation onto avocado fruit surfaces and accumulate in fruit depressions and around germinating conidia. Kloepper *et al*., (2004) has reported the biocontrol potential of *B*. *pumilus* isolates in controlling pathogens like *Cercospora beticola, Peronospora tabacina, Erwinia tracheiphila* etc., in both lab studies and field trials. In the present study among the bacteria tested maximum inhibition was observed only with *Bacillus* species. One endophytic bacterial isolate ENB-85 which showed 58.66% suppression of *C. capsici* was identified as *Staphylococcus sciuri*, which is reported to be human pathogen and hence further studies with this isolate was discontinued (Stepanovic *et al*., 2005).

The yeast isolate Y–73 which showed maximum suppression was identified as *Hanseniaspora uvarum* by ITS sequencing followed by *Pichia guilliermondii* (Y–12) which showed 64.29% supression (Table 4 &

**Table 4: Suppression of *Colletotrichum capsici* by yeast isolates**

| Sl. No. | Yeast isolates | Lesion length suppression (%) | Sl. No | Yeast isolates | Lesion length suppression (%) | Sl. No | Yeast isolates | Lesion length suppression (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | Y-1 | 46.67 (43.09) | 29 | Y-41 | 13.24 (21.33) | 57 | Y-83 | 20.88(27.19) |
| 2 | Y-2 | 56.67 (48.83) | 30 | Y-44 | 17.65 (26.31) | 58 | Y-84 | 18.32(25.34) |
| 3 | Y-3 | 26.67 (31.09) | 31 | Y-45 | 12.87 (21.02) | 59 | Y-85 | 52.38(46.36) |
| 4 | Y-4 | 19.12 (25.93) | 32 | Y-46 | 19.49 (26.19) | 60 | Y-86 | 13.55(21.59) |
| 5 | Y-5 | 62.50 (52.24) | 33 | Y-51 | 9.45(17.90) | 61 | Y-88 | 22.71(28.46) |
| 6 | Y-6 | 47.57 (43.60) | 34 | Y-52 | 5.12(13.07) | 62 | Y-90 | 19.78(26.40) |
| 7 | Y-7 | 18.37 (25.37) | 35 | Y-53 | 8.27(16.71) | 63 | Y-93 | 3.30(10.46) |
| 8 | Y-8 | 45.45 (42.39) | 36 | Y-54 | 9.06(17.51) | 64 | Y-94 | 11.36(19.69) |
| 9 | Y-9 | 15.45 (23.14) | 37 | Y-59 | 12.25(20.48) | 65 | Y-99 | 27.11(31.37) |
| 10 | Y-10 | 8.33 (16.77) | 38 | Y-60 | 16.12(23.67) | 66 | Y-103 | 10.99(19.36) |
| 11 | Y-11 | 16.33 (23.83) | 39 | Y-61 | 18.32(25.34) | 67 | MPI-6 | 23.33 (28.88) |
| 12 | Y-12 | 64.29 (53.30) | 40 | Y-63 | 18.32(25.34) | 68 | MPI-5 | 6.67 (14.96) |
| 13 | Y-14 | 47.06 (43.31) | 41 | Y-64 | 13.55(21.59) | 69 | MPI-11 | 34.38(35.89) |
| 14 | Y-15 | 28.13 (32.03) | 42 | Y-65 | 12.09(20.34) | 70 | JPI-1 | 24.24(29.49) |
| 15 | Y-16 | 31.11 (33.90) | 43 | Y-66 | 13.55(21.59) | CD ($P$=0.01) | | 2.99 |
| 16 | Y-17 | 59.22 (50.31) | 44 | Y-67 | 20.88(27.19) | | | |
| 17 | Y-18 | 54.55 (47.61) | 45 | Y-68 | 13.55(21.59) | | | |
| 18 | Y-19 | 59.38 (50.40) | 46 | Y-69 | 12.09(20.34) | | | |
| 19 | Y-20 | 49.52 (44.72) | 47 | Y-70 | 47.99(43.84) | | | |
| 20 | Y-23 | 27.08 (31.35) | 48 | Y-71 | 12.09(20.34) | | | |
| 21 | Y-24 | 42.86 (40.89) | 49 | Y-73 | 72.16(58.15) | | | |
| 22 | Y-25 | 41.90(40.33) | 50 | Y-74 | 16.12(23.67) | | | |
| 23 | Y-30 | 3.31 (10.48) | 51 | Y-75 | 20.88(27.19) | | | |
| 24 | Y-31 | 38.97(38.62) | 52 | Y-76 | 1.10(6.02) | | | |
| 25 | Y-33 | 22.06(28.01) | 53 | Y-77 | 23.44(28.95) | | | |
| 26 | Y-36 | 33.09 (35.11) | 54 | Y-80 | 47.25(43.42) | | | |
| 27 | Y-37 | 4.78 (12.62) | 55 | Y-81 | 7.33(15.59) | | | |
| 28 | Y-39 | 28.31 (32.14) | 56 | Y-82 | 25.27(30.17) | | | |

Figures in parentheses are angular transformed values. Isolates showing no inhibition are not shown

**Table 5: Promising bacterial/yeast antagonists identified based on fruit bioassay**

| Sl.No | Isolates | Isolate No. | Accession number | Source/Location | % reduction in the lesion length |
|---|---|---|---|---|---|
| Bacterial isolates | | | | | |
| 1 | *Bacillus tequilensis* | PHB-185 | JQ229967 | Green fruits (Hybrid 005) Guntur district, A.P. | 67.84 |
| 2 | *Bacillus subtilis* | PMB-123 | JN167993 | Green & Ripe chilli fruits (Arka-Haritha variety), Bangalore. | 61.69 |
| 3 | *Bacillus subtilis* | ENB-24 | JN167994 | Chilli leaves (ACS-06-1 variety) Bangalore. | 55.41 |
| 4 | *Bacillus megaterium* | PMB-53 | JN167995 | Green and ripe fruits (Byadagi variety), Bangalore. | 54.33 |
| 5 | *Bacillus megaterium* | ENB-86 | JQ247579 | Chilli leaves (Namdhari variety) Malur, Kolar district. | 59.66 |
| 6 | *Pseudomonas putida* | PBA-5 | HM439953 | NBAII culture collection | 50.68 |
| 7 | *Bacillus pumilus* | PMB -183 | JQ229968 | Green fruits (Bhadhra variety) Guntur district, A.P. | 50.29 |
| Yeast isolates | | | | | |
| 1 | *Hanseniaspora uvarum* | Y-73 | JQ247580 | Grapes (Bangalore blue) Bangalore. | 72.16 |
| 2 | *Pichia guillermondii* | Y-12 | HQ448930 | Green pea pods. Bangalore. | 64.29 |

Percent reduction calculated based on inoculated control fruits

Fig. 1). The lowest (1.10%) was exhibited by the isolate Y-76. Thirty yeast isolates showed no inhibition. Liu *et al*., (2010) reported that combination of *Hanseniaspora uvarum* and ammonium molybdate effectively controlled the gray mold of grape caused by *Botrytis cinerea* in laboratory fruit bioassays. Chanchaichaovivat *et al*., (2007) reported *P. guilliermondii* strain R13 reduced anthracnose disease to 6.7% under *in vivo* condition. Six yeast isolates also showed >50% reduction in lesion length of *C. capsici*. They were identified as *Kodamaea* (*Pichia*) *ohmeri* (Y–5), *Candida orthopsilosis* (Y–18 & Y–19), *Trichosporon asahii* (Y–2 &Y–17) and *Lodderomyces elongisporus* (Y–85). These isolates exhibited 52.38 to 62.50% reduction in lesion length. But, these are reported to be human pathogens (Taj *et al*., 2006; Tavanti *et al*. 2007; Chowdhary *et al*., 2004; Shawn *et al*., 2008) and hence further studies with these isolates were discontinued.

The present study has shown that seven bacterial isolates (*B. tequilensis* PHB-185, *B. subtilis* PMB-123 and ENB-24, *B. megaterium* PMB-53 and ENB-86, *B. pumilus* PMB –183, *P. putida* PBA-5) and two yeast isolates (*H. uvarum* Y-73 and *P. guilliermondii* Y-12) were found effective in reducing anthracnose lesion caused by *C. capsici* by more than 50%. These promising antagonists can be further used for chilli anthracnose disease management under field conditions as well as during storage and drying (post-harvest).

## REFERENCES

Chanchaichaovivat A, Ruenwongsa P, Panijpan B. 2007. Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*). *Biol Control*. **42**: 326–335.

Chowdhary A, Ahmad S, Khan ZU, Doval DC, Randhawa HS. 2004. *Trichosporon asahii* as an emerging etiologic agent of sisseminated trichosporonosis: a case report and an update. *Ind J Med Microbiol*. **22**: 16–22.

Havenga W, De Jager ES, Korsten L. 1999. Factors affecting biocontrol efficacy of *Bacillus subtilis* against *Colletotrichum gloeosporioides*. *Sou Afr Avo Gros' Asso Yearbook*. 1999*,* **22**: 12–20.

Hierro N, Gonzalez A, Mas A, Guillamo JM. 2004. New PCR-based methods for yeast identification. *J Appl insert Microbiol*. **97**: 792–80.

Kloepper JW, Ryu CM, Zhang S. 2004. Inducedsystemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*. **94**: 1259–66.

Korsten L, Bezuidenhout JJ Kotzé JM. 1988. Biological control of avocado postharvest diseases. *Sou Afr Avo Gros' Asso Yearbook*. **11**: 75.

Korsten L, De Jager, E S. 1995. Mode of action of *Bacillus subtilis* for control of avocado post-harvest pathogens. *Sou Afr Avo Gros' Asso Yearbook*. **18**: 124–130.

Korsten L, De Jager ES, De Villiers EE, Lourens A, Kotze JM, Wehner FC. 1995. Evaluation of bacterial epiphytes isolated from avocado leaf and fruit surfaces for biocontrol of avocado post-harvest diseases. *Plant Dis.* **79**: 1149–1156.

Korsten L, De Villiers EE, Rowell A, Kotze JM. 1993. Post-harvest biological control of avocado fruit diseases. *Sou Afr Avo Gros' Asso Yearbook*. **16**: 65–69.

Liu HM, Guo JH, Luo L, Liu P, Wang BQ, Cheng YJ, Deng BX, Long CA. 2010. Improvement of *Hanseniaspora uvarum* biocontrol activity against gray mold by the addition of ammonium molybdate and the possible mechanisms involved. *Crop Prot.* **29**: 277–282.

Manandhar JB, Hartman GL, Wang TC. 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Dis*. **79**: 380–383.

McInroy JA, Kloepper JW. 1995. Population dynamics of endophytic bacteria in field-grown sweet corn and cotton. *Can J Microbiol*. **41:** 895–901.

Montri P, Taylor PWJ, Mongkolporn O. 2009. Pathotypes of *Colletotrichum capsici,* the Causal Agent of Chili Anthracnose,in Thailand. *Plant Dis.* **93**: 17–20.

Ngullie M, Daiho L, Upadhyay DN. 2010. Biological management of fruit rot in the world's hottest chilli (*Capsicum chinense* Jacq.). *J Pl Prot Res.* **50**: 269–273.

Ramamoorthy V, Samiyappan R. 2001. Induction of defense related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici*. *J Mycol Pl Pathol.* **31**: 146–155.

Ramanujam B. 2008. Isolation, identification and evaluation of biocontrol agents. In: Eapen SJ, Kumar A, Anandaraj M. (Eds.). *Plant Pathogens and their Biocontrol agents Diagnostics and Characterization*: Indian Institute of Spices Research. p. 207–224.

Schreiner K, Hagn A, Kyselkova M, Moenne LY, Welzl G, Munch JC, Schloter M. 2010. Comparison of Barley Succession and Take-All Disease as Environmental Factors Shaping the Rhizobacterial Community during Take-All Decline. *Appl Environ Microbiol*. **76**: 4703–4712.

Stepanovic S, Dakic I, Morrison D, Hauschild T, Jezek P, Petras P, Marte A, Vukovic D, Shittu A, Devriese LA. 2005. Identification and Characterization of Clinical Isolates of Members of the *Staphylococcus sciuri* Group. *J Clin Microbiol*. **43**: 956–958.

Shawn R. Lockhart, Shawn A. Messer, Michael A. Pfaller and Daniel J. Diekema. 2008. *Lodderomyces elongisporus* Masquerading as *Candida parapsilosis* as a Cause of Bloodstream Infections. *J Clin Microbiol.* **46**: 374–376**.**

Taj ASJ, Doiphode SH, Han XY. 2006. *Kodamaea* (*Pichia*) *ohmeri* fungaemia in a premature neonate. *J Med Microbiol*. **55**: 237–239.

Tavanti A, Lambert A, Hensgens M, Ghelardi E, Campa M, Senes S. 2007. Genotyping of *Candida orthopsilosis* Clinical Isolates by Amplification Fragment Length Polymorphism Reveals Genetic Diversity among Independent Isolates and Strain Maintenance within Patients. *J Clin Microbiol*. **45**: 1455–1462.

Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O Taylor, PWJ. 2008. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. *Plant Pathol*. **57**: 562–572.

Thind TS, Jhooty JS. 1985. Relative prevalence of fungal diseases of chilli fruits in Punjab. *Ind J Mycol Plant Pathol*. **15**: 305–307.

Vinaya H, Rao M SL, Yashoda H, Mohankumar HD. 2009. Status of seed borne incidence of anthracnose of chilli in northern Karnataka and evaluation of seed health testing methods for the detection of *Colletotrichum capsici*. *Kar J Agri Sci*. **22**: 807–809.

Xue-qing CAI, Hong HE, Yi-yuan YE, Fang-ping HU. 2004. Effect of endophytic bacteria BS-1 and BS-2 (*Bacillus subtilis*) on the control of capsicum anthrancnose and active oxygen metabolism in capsicum fruits. *J Fug Agri Uni*. **1**: 102–105.