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REGULAR ARTICLE

EFFICACY OF ANTAGONIST STRAINS OF *BACILLUS MEGATERIUM*, *ENTEROBACTER CLOACAE*, *PICHIA GUILLIERMONDII* AND *CANDIDA ETHANOLICA* AGAINST BACTERIAL WILT DISEASE OF TOMATO

Mai Thanh Nguyen[1*], Senaratne L. Ranamukhaarachchi[1], David B. Hannaway[2]

[1]*Asian Institute of Technology, Thailand*
[2]*Oregon State University, USA*

SUMMARY

Bacterial wilt disease (BWD) caused by *Ralstonia solanacearum* is a serious and widespread disease in tomato. The persistence and its wide host range often restrict the effectiveness of cultural and chemical control measures. Four antagonists: *Bacillus megaterium*, *Enterobacter cloacae, Pichia guillermondii* and *Candida ethanolica* were tested and found significantly antagonistic to *R. solanacearum* in *in vivo* conditions with unsterilized soils and hence possess potential to control BWD in tomato. *Enterobacter cloacae, Pichia guillermondii* and *Candida ethanolica* also increased growth and fruit yield compared to the control. Disease severity varied with antagonist and time of application; compared with the control, disease severity was reduced 41.6-77.1% when antagonists were applied one week prior to transplanting tomato seedlings. These results can be used as a potential tool to control BWD in tomato commercially as an eco-friendly manner and provide encouragement for continued research on biological control of bacterial wilt disease by antagonists.

Key words: Antagonists, Bacterial wilt, Bio-control, *Ralstonia solanacearum*, Soil-borne pathogen, Systemic disease, Tomato

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*Corresponding Author, Email: maithanh0676@yahoo.com

1. Introduction

Bacterial wilt disease (BWD) caused by *Ralstonia solanacearum* is one of the most serious soil-borne diseases in tropical South-East Asia (Burgess *et al*., 2008) limiting the production of capsicum and tomato and other commercial solanaceous crops. The damage leads to large losses of yield and income, and disease control is difficult (Hartman, 1993; Doan and Nguyen, 2005). Although disease resistant varieties are available, their resistance is often incomplete (Wydra *et al*., 2005). Cultural practices have been found to be ineffective due to high variability of the pathogen (Sequeira, 1993). Chemical control may not be suitable due to high cost and environmental risks associated with application. Biological control would be highly preferred for disease control in the future. According to Timur *et al.* (2006), a number of soil bacteria and plant growth promoting rhizobacteria (PGPR) are currently being investigated for their role in the control of BW pathogen, however, none is currently available commercially and effective for the biological control, and hence it is yet to be determined. Various actinomycetes and bacteria, such as *Bacillus mesentericus, B. megaterium, B. subtilis, and B. mycoides* have been reported as active biological control agents against *R. solanacearum* (Shekhawat *et al*., 1993; Doan and Nguyen, 2006). In contrast, Shekhawat *et al*. (1992) showed that isolates of *B. subtilis* were not effective against BWd pathogen in potato under glasshouse and field conditions. *Pseudomonas fluorescens* was found to be

effective in reducing BW incidence in both glasshouse and field conditions. This follows earlier work (Suslow, 1982; Wellwer, 1988) leading to the suggestion that fluorescent *Pseudomonades* be evaluated as a possible bio-control agent for the BW pathogen. Elphinstone and Aley (1992) found a decline of *R. solanacearum* in the maize rhizosphere due to an increased population of *P. cepacia* which was antagonistic *in vitro* to *R. solanacearum*. Additionally, antagonists *Pseudomonas fluorescens* PfG32, and *Stenotrophmonas maltophilia* were reported to have suppressive effects on *R. solanacearum* (Karden Mulya *et al*., 1996 and Messiha *et al*., 2007). Research on *Candida ethanolica* showed promise for the control of the BW pathogen (Myint Lwin, 2006; Lwin and Ranamukhaarachchi, 2006).

Nguyen and Ranamukhaarachchi (2010) isolated 73 suspected antagonists from tomato and capsicum grown in BW disease-infested soils and initially evaluated and screened *in vitro*; eight prospective candidates were subsequently evaluated in greenhouse pots using sterilized soils. From the *in vivo* study, three antagonists: *Bacillus megaterium*, *Enterobacter cloacae, Pichia guillermondii* isolated from soils (Nguyen and Ranamukhaarachchi, 2010) and *Candida ethanolica,* a yeast isolated from Effective Microorganism solution (Lwin and Ranamukhaarachchi, 2006) identified by using 16S rDNA and 26S rDNA sequencing (Nguyen and Ranamukhaarachchi, 2010) gave encouraging results in the suppression of BW pathogen. Since microbial populations are diverse and success in field conditions often is affected by interactions with other pathogenic and/or non-pathogenic microorganisms, it was necessary to re-evaluate all prospective antagonists *in vivo* in soils where solanaceous crops are grown. Therefore, the current study was conducted to evaluate the four antagonists, namely *Bacillus megaterium*, *Enterobacter cloacae, Pichia guilliermondii* and *Candida ethanolica*, for their ability to disease suppression in the presence of diverse microbial populations that exist in natural cultivated soils under non-sterilized conditions in order to determine their potential for future use as effective biological agents for bacterial wilt disease control.

2. Material and Methods

Isolation of the pathogen and potential antagonists

Bacterial wilt infected plant parts of tomato and soils were collected from the Duc Trong district in Lam Dong Province, Vietnam and the Banglen district in Nakhon Pathom Province, Thailand. The BW pathogen, *R. solanacearum*, was isolated from infected plant parts using TZC medium (Kelman, 1954). Pathogenicity was confirmed by inoculating tomato seedlings using the root dipping method (Winstead and Kelman, 1952).

Strains of *Bacillus megaterium*, *Enterobacter cloacae* and *Pichia guillermondii* isolated from soils (Nguyen and Ranamukhaarachchi, 2010) and *Candida ethanolica* isolated from Effective Microorganism (EM) solution (Lwin and Ranamukhaarachchi, 2006) were used for the current study. All isolates were identified by BIOTEC Culture Collection (BCC), Thailand Science Park using 16S rDNA sequencing (Marmur, 1961; Brosius *et al*., 1981; Kawasaki *et al*., 1993; Yamada *et al*., 2000; Katsura *et al*., 2001; Anon, 2007; Anon, 2009) and 26S rDNA sequencing (Manitis *et al*., 1982; Kurtzman and Robnett, 1998; Anon, 2009). Furthermore, all isolates were studied with microscopic structure, staining, cultural and biochemical procedures adopted for the identification of unknown microbial organisms (Fahy and Hayward, 1983; Cappuccino and Sherman, 1996).

Evaluation of potential bio-control agents

Three pot experiments were conducted from September 2008 to June 2009 in the Agricultural Systems Research Farm greenhouse at the Asian Institute of Technology to evaluate the four screened antagonists (Table 1) against *R. solanacearum*. Two control treatments were included: one with only the pathogen and the other without pathogen added. A susceptible tomato variety of Thailand, Sida, was selected for the study. The three experiments were: 1) simultaneous inoculation of tomato seedlings with both antagonists and pathogen, 2) inoculation of antagonists to

soils one week prior to pathogen inoculation, and 3) inoculation of antagonists to soils one week after pathogen inoculation. Experiments were conducted using a completely randomized design with three replications.

In the first pot experiment where both the antagonists and the pathogen were introduced simultaneously, the suspensions of equal concentrations of each antagonist (10^8 CFU ml^{-1}) and pathogen (10^8 CFU ml^{-1}) were mixed separately with equal volumes of pathogen of the same concentration in separate screw cap bottles, and allowed to interact for one hour in a mechanical shaker. Colony forming unit (CFU) concentration was determined by the dilution planting method with sucrose peptone agar medium (SPA).

Following mixing, roots of 20-day-old tomato seedlings raised in a greenhouse nursery were inoculated for 5 minutes using root dip technique. Two seedlings were transplanted into each pot which contained 8 kg of unsterilized potting mixture composed of 90% top soil from the upper 10 cm of a tomato and capsicum growing site and 10% compost. Plants were watered to field capacity each morning and afternoon with micro sprinklers. Prior to transplanting, seedlings were given a nutrient solution prepared by dissolving 3 g of NPK (15-15-15) mixture in one liter of water starting 10 days after germination. A 20-20-15 NPK mixture (2 g/pot) was applied 10 days after transplanting (DAT), and 2 g/pot of a 20-0-20 mixture was applied at 25 and 40 DAT. Disease incidence was recorded beginning at 7 days after inoculation using a 1-5 scale as suggested by Kelman and Person (1961) in which 1 = no visible symptoms, 2 = 1-25% of plants showing wilt symptoms, 3 = 26-50%, 4 = 51-75%, and 5 = greater than 75% of plants displaying wilt symptoms.

In the second experiment, antagonists were introduced to the soil one week prior to pathogen inoculation to facilitate their establishment. Two, twenty-day old tomato seedlings were transplanted into each pot one-week after introducing the pathogen. To inoculate soils, 20 ml of the mixture of antagonist and pathogen suspensions were used (10^8 CFU ml^{-1}). Watering and fertilization were as described in the first experiment. Disease incidence was recorded from the seventh day onwards using the same above-described 1-5 scoring method.

In the third experiment, the pathogen was introduced into the soil 1 week prior to introduction of seedlings, using 15 ml of its suspension (10^8CFU ml^{-1}). Two, twenty-day-old tomato seedlings were transplanted into each pot, and maintained with watering and fertilization as described for the first and second experiments. When the plants died due to BW disease, another set of tomato seedlings was transplanted into the same pots and maintained in the same manner. Once the second set of plants also died from BW disease, the pots were considered to be pathogen-infested soils (Lwin and Ranamukhaarachchi, 2006). Antagonists were then inoculated into the pots and allowed to interact with the pathogen for one week. At the end of the one week period, two twenty-day-old tomato seedlings were transplanted into each pot. Plants were watered and fertilized as previously described. Weeding was performed by hand. Disease incidence was monitored and recorded from the seventh day onward following transplanting using the above-described 1-5 scale. Plant height, biomass and fruit weight also were recorded.

All three experiments were repeated immediately after completing each study. Prior to repeating each study, the soil in pots was removed and pots were thoroughly washed with running water obtained from the normal water supply at the farm. Fresh soil was obtained from the same site and the same procedures were used as explained previously for experiments 1, 2 and 3.

Data Analysis

Analysis of variance was performed for the completely randomized design for plant height, biomass and fruit weight per pot. Mean separations were performed using Fisher's Protected Least Significant Difference (LSD) method (Steel and Torrie, 1980). Disease severity data were gathered using the 1-5 scale of Kelman and Person

(1961), and subjected to standard ranking methods.

3. Results

Disease Severity

In pot experiment 1, there was a 12.5-50.0% reduction in the severity of BW when tomato seedlings were inoculated simultaneously with the pathogen and selected antagonists (Table 1). In the control, where there was only the pathogen, all tomato seedlings were infected by *R. solanacearum* and died within 2 weeks after planting. Disease severity was high in all antagonist treated plants. The lowest disease severity was found with *E. cloacae* and *C. ethanolica* treated plants. Results were similar in the repeated experiment. Pots inoculated with *C. ethanolica* and *E. cloacae* had disease severity of 18.8%, and 27%, respectively.

In pot experiment 2, all antagonists reduced disease severity 49.0-53.3% compared to the control (Table 1). *B. megaterium*, *E. cloacae,* and *P. guillermondii* treated plants had a disease severity of 25-26%, with *C. ethanolica* exhibiting the lowest severity (21.7%). In the repeated experiment, the disease severity in *C. ethanolica, B. megaterium*, *P. guillermondii,* and *E. cloacae* treated plants was 41-46%.

In pot experiment 3, E. cloacae treated plants exhibited the lowest disease severity (16.7%); *B. megaterium* and C. *ethanolica* treated plants had moderate severity (25%), and *P. guillermondii* treated plants had somewhat higher values (31.3%), compared with 82.1% for the control. In the repeated experiment, *B. megaterium*, *P. guillermondii* and *C. ethanolica* treatments resulted in 31-35% disease incidence, while *E. cloacae* had the lowest (12.5%) compared to the control (89.6%)(Table-1).

Table 1. Effect of bio-control agents on the severity of tomato bacterial wilt disease

Treatments	Disease severity (%)					
	Experiment 1 Antagonist & pathogen applied simultaneuosly		Experiment 2 Antagonist established one week before pathogen		Experiment 3 Antagonist established one week after pathogen	
	First crop	Second Crop	First crop	Second Crop	First crop	Second Crop
Control +/	100 (5)*/	100 (4)	75.0 (4)	87.5 (5)	82.1 (5)	89.6 (4)
B. megaterium	81.3 (3)	75.0 (3)	25.0 (2)	43.8 (2)	25.0 (2)	31.3 (2)
E. cloacae	50.0 (1)	27.1 (2)	25.0 (2)	45.9 (4)	16.7 (1)	12.5 (1)
P. guillermondii	87.5 (4)	75.0 (3)	26.0 (3)	45.8 (3)	31.3 (4)	35.4 (3)
C. ethanolica	52.9 (2)	18.8 (1)	21.7 (1)	41.7 (1)	25.3 (3)	35.4 (3)

+/ Control included only *R. solanacearum* without antagonists;

*/ Values within parenthesis indicate tha ranked performance of antagonists, with 1 being the highest disease suppression (lowest disease incidence) and increasing numbers showing increasing disease incidence;

Growth and Yield of Tomato

The growth and yield data showed that infected plants that survived were able to continue to grow and produce yield. These parameters were compared with healthy plants with no symptoms of BW disease. In the first experiment, only a very few plants in a few treatments survived because of high BW disease incidence. Therefore, first experiment data are not presented. Results from the other two experiments are presented in subsequent sections.

Plant Height

In experiment 2, plant height reflected the suppressive effects of the pathogen, ability of antagonists to reduce such negative effects on the inoculated plants, and the tomato plants' ability to continue to grow in the presence of the pathogen (Table 2). Control plants grown without pathogen had satisfactory growth. In the presence of the pathogen, however, there was a significant growth reduction. When the

antagonists were established before the pathogen, plant height did not differ significantly. Plants exposed to pathogen alone had reduced height. In the repeated study, *E. cloacae*, *C. ethanolica* and *P. guillermondii* treated plants were taller; between 83.1 and 91.7 cm, *B. megaterium* treated pots were shorter, averaging 76.2 cm compared with 81.8 cm for the control.

Table 2. Effect of bio-control agents on tomato plant height and biomass when established at one week before pathogen in Experiment 2

Treatments	Plant height, cm		Biomass, g/plant	
	First crop	Second Crop	First crop	Second Crop
Control +/	76.8 ± 8.4 */	81.8 ± 9.8	43..1 ± 8.8	39.9 ± 6.8
Control ++/	56.8 ± 33.7	64.3 ± 14.6	25.2 ± 15.4	29.0 ± 16.8
B. megaterium	76.0 ± 8.0	76.2 ± 15.5	44.5 ± 11.5	37.6 ± 5.5
E. cloacae	80.1 ± 12.0	91.7 ± 8.5	61.4 ± 9.7	47.0 ± 2.4
P. guillermondii	78.5 ± 11.8	83.1 ± 6.8	46.7 ± 8.3	49.4 ± 4.9
C. ethanolica	73.6 ± 30.8	84.4 ± 4.9	44.0 ± 5.2	47.6 ± 4.0
LSD (5%)	ns	10.2	9.8	7.8
SE	2.9	1.8	1.95	1.4

+/ Control without *R. solanacearum* and antagonists;
++/ Control with *R. solanacearum* without antagonists;
*/ Standard deviation;

In experiment 3, where antagonists were established after the pathogen, results were similar to those of experiment 2, but overall height was slightly reduced (Table 3). In the repeated experiment, the result remained unchanged, except for antagonist *P. guillermondii*, where plant height was reduced but was insignificantly different from the control without pathogen (72.8 cm).

Table3. Effect of bio-control agents on tomato plant height and biomass when established at one week after pathogen in Experiment 3

Treatment	Plant height, cm		Biomass, g/plant	
	First crop	Second Crop	First crop	Second Crop
Control +/	73.0 ± 3.4 */	72.8 ± 9.0	35.0 ± 3.4	34.7 ± 6.9
Control ++/	47.3 ± 27.3	48.3 ± 2.9	23.0 ± 9.1	18.7 ± 10.8
B. megaterium	73.3 ± 8.2	73.4 ± 5.3	35.0 ± 4.6	27.8 ± 7.6
E. cloacae	78.6 ± 6.0	72.6 ± 4.2	51.6 ± 7.9	35.0 ± 7.0
P. guillermondii	80.3 ± 7.1	69.6 ± 5.4	36.8 ± 9.1	39.2 ± 3.4
C. ethanolica	74.2 ± 2.0	72.4 ± 8.6	35.4 ± 3.0	38.8 ± 5.9
LSD (5%)	11.7	12.7	6.4	6.9
SE	2.2	2.1	1.4	1.4

+/ Control without *R. solanacearum* and antagonists;
++/ Control with *R. solanacearum* without antagonists;
*/ Standard deviation;

Biomass

In experiment 2, biomass increased significantly (61 g/plant) for *E. cloacae* treated plants. In the repeated study, biomass was highest in *P. guillermondii* (49.4 g/plant), but was not significantly different from *E. cloacae* (47.0 g/plant) and *C. ethanolica* (47.6 g/plant) (Table 2). The lowest biomass was observed in treatments that had the pathogen alone (29 g/plant). Other treatments, including plants in the control without pathogen, had intermediate biomass values (39.9 g/plant).

In experiment 3, biomass values were lower than those of experiment 2 (Table 3). With slight differences, the same trends were

observed in the repeated study, with antagonists reducing pathogen effects.

Fruit Weight per Plant

In experiment 2, fruit weight averaged 281.2 g/plant in the absence of pathogen and antagonists, and 103.6 g/plant in the presence of pathogen alone (Table 4). Plants from treatments with antagonists had fruit weights in the range of 430.8 to 477.1 g/plant, but differences were not significantly different from the two control treatments (P=0.05). In the repeated experiment, fruit weight per plant was lowest for plants that were inoculated with only the pathogen (71.2 g/plant), and 167.6 g/plant in the absence of both pathogen and antagonists. The highest yield was observed for the *P. guillermondii* treatment (252.7 g/plant), but was not significantly greater than *C. ethanolica* treated plants (228.7 g/plant) (Table 3). *E. cloacae* had moderate yields (209.6 g/plant), while *B. megaterium* had the lowest yields (167.0 g/plant) nearly identical to the 167.6 g/plant for the control (plants grown in the absence of both the pathogen and antagonists).

Table 4. Effect of bio-control agents on tomato yield

Treatment	Fruit weight, g/plant			
	Experiment 2 Antagonist established one week before pathogen		Experiment 3 Antagonist established one week after pathogen	
	First crop	Second Crop	First crop	Second Crop
Control +/	281.2 ± 153.6*/	167.6 ± 44.8	130.0 ± 38.0	93.4 ± 39.8
Control ++/	103.6 ± 10.9	71.2 ± 43.1	76.0 ± 27.0	37.0 ± 33.4
B. megaterium	462.5 ± 181.2	167.0 ± 11.5	214.4 ± 39.0	150.2 ± 12.1
E. cloacae	477.1 ± 234.0	209.6 ± 37.8	228.6 ± 85.1	171.4 ± 19.2
P. guillermondii	434.5 ± 128.2	252.7 ± 54.1	229.4 ± 35.0	245.9 ± 44.4
C. ethanolica	430.8 ± 60.5	228.7 ± 45.1	253.7 ± 63.7	201.8 ± 79.4
LSD (5%)	104.5	39.5	48.3	41.5
SE	10.0	26.5	11.0	11.0

+/ Control without *R. solanacearum* and antagonists;
++/ Control with *R. solanacearum* without antagonists;
*/ Standard deviation;

In experiment 3, where the antagonists were introduced to pathogen infected soils, *C. ethanolica* had the highest fruit weight, but the mean value was not significantly higher than other antagonist treatments (Table 4). The two control treatments had the highest fruit yield. In the repeated study, the lowest fruit weight was in pots in the absence of antagonists (37 g/plant) while healthy plants without pathogen yielded 93.4 g/plant. Insignificantly higher yields were observed for the *P. guillermondii*, *C. ethanolica* and *E. cloacae* treated pots. Importantly, all antagonist treated plants had higher fruit yields than plants growing in the presence of pathogen alone.

4. Discussion

Biological control using antagonistic microorganisms has been pursued for nearly four decades to develop sustainable control of BW (Baker and Cook, 1974; Shekhawat *et al*., 1992, 1993; Doan and Nguyen, 2003; Lwin and Ranamukhaarachchi, 2006). The current study was a continuation of a series of studies reported by Lwin and Ranamukhaarachchi (2006), which suggested a high potential for the suppression of BW disease pathogen using antagonists as biological control agents.

Selected antagonists performed differently in *in vivo* studies depending on application timing. Isolates of *E. cloacae* showed good suppression of the BW pathogen in 3 pot experiments (Table 1). Similar results also were observed with *B. megaterium*. *C. ethanolica* gave the best suppression of the pathogen in pot experiments 1 and 2. Although ranking showed slight inferiority of *C. ethanolica* compared to *E. cloacae* in the third experiment, there was further lowering of

disease severity (25.3%-35.4%) when tomato seedlings were transplanted one week after introducing the antagonist into soils highly infested with the pathogen. *P. guillermondii* also showed excellent performance against the pathogen when the antagonist was introduced prior to the pathogen and tomato seedlings were transplanted one week later (pot experiments 2 and 3).

The results demonstrated the varied performance of different antagonists against *R. solanacearum* depending upon the time of application. Shekhawat *et al*. (1992) also reported that although *in vitro* inhibition studies may select microbial antagonists against *R. solanacearum*, such studies may not reflect the potential of those antagonists to perform similarly under field conditions. In their later studies it was found that isolates of *B. subtilis* were effective against BW in potato *in vitro*, but were not effective under either glasshouse or field conditions (Shekhawat *et al*., 1993). Lwin and Ranamukhaarachchi (2006) reported that *C. ethanolica* suppressed bacterial wilt pathogen in both *in vitro* and *in vivo*, but there was variability in the response.

Disease severity was high when both antagonist and pathogen were introduced simultaneously by the root dipping of tomato seedlings (current pot experiment 1) compared to when the antagonist was introduced earlier or later to the pathogen (as in current pot experiments 2 and 3). The reason for increased disease severity in pot experiment 1 appeared to be the opportunity available for the pathogen to easily invade the root cells in the root dipping method through wounds caused on roots during uprooting of seedlings. Even though antagonists could suppress the pathogen, this suppression must occur before infection begins, outside of root cells. According to Sequeira (1993), *R. solanacearum* lives in the highly protective environment of the xylem vessels, but when confronted with competition outside of the root, it does poorly. Once the pathogen has entered the roots of tomato seedlings it has the opportunity for disease development and antagonists in the solution could not suppress those disease organisms that had entered the root. This resulted in very high disease incidence in experiment 1.

Disease suppression by antagonists increased when they were introduced one week before or one week after inoculating with the pathogen and by providing one week for their interaction before transplanting tomato seedlings. Simultaneous introduction resulted in the lowest disease suppression. In *in vitro* studies, the antagonists showed suppressive effects 2-3 days following inoculation. Providing one week for the pathogen and antagonist to interact in experiments 2 and 3 resulted in reduced disease severity. Therefore, multiplication of antagonists and/or time requirement for the commencement of antagonistic activities necessitates a minimum of a few days in soil prior to the pathogen's attack. This is supported by the work of Hartman *et al*. (1993) who reported that a *P. cepacia* cell suspension applied to soil 7 days before inoculation with *P. solanacearum* reduced the occurrence of BW disease by 65%. In the current study, plants treated with *B. megaterium, P. guillermondii*, *E. cloacae* and *C. ethanolica* one week before introducing pathogen had a lower disease incidence, with the presence of three antagonists *P. guillermondii*, *E. cloacae* and *C. ethanolica* associated with greater fruit yield, biomass and plant height compared to control plants.

Higher disease severity in the repeated experiments may have been due to higher temperature conditions. In the first experiment average temperatures were 20-31^{0}C, compared with 24-35^{0}C during the repeated experiments. According to Martin and French (1985), high temperatures promote bacterial wilt development, while bacterial populations are reduced in cold soils.

Hosts influence disease development in several ways, which includes the relative susceptibility of host to disease, the host's age, vigor, stage of growth, and composition of plant populations (Maylor, 1993). The reverse also can be true, and hence the difference in the suppression could be anticipated.

Three isolated antagonists *P. guillermondii*, *E. cloacae* and *C. ethanolica* were the most promising under unsterilized conditions. They were effective not only in reducing wilt disease incidence, but also in increasing tomato fruit yield from 78 g/plant to 196 g/plant compared to the control. This is consistent with Berga *et al*. (2001) who observed yield decrease due to an increase in bacterial wilt incidence. Kloepper *et al*. (1980) also reported the beneficial attributes of antagonists and their direct inhibitory effects on pathogen with enhancing plant growth. Wydra and Semrau (2005) reported comparable reduction in *R. solanacearum* wilt disease and a yield increase associated with *Bacillus* spp. and fluorescent *Pseudomonas*.

These results provided encouragement for continuing research on biological control of bacterial wilt by antagonistic isolates on tomato under different field conditions, and evaluating these antagonists for their potential usefulness in reducing BWD in other Solanaceous crops.

Conclusions

All four screened bio-control agents, *B. megaterium*, *P. guillermondii*, *E. cloacae* and *C. ethanolica*, showed effectiveness in reducing the severity of bacterial wilt disease caused by *R. solanacearum* in unsterilized soils with normal microbial populations. The disease suppression varied with antagonist and time of application. The findings suggest that these antagonists can be used to suppress BW in field conditions and their antagonistic activities can be enhanced by the application of antagonists to infested soils one week prior to transplanting tomato seedlings.

E. cloacae is not a primary human pathogen, however, some strains are considered to be an important cause of nosocomial infections (Rogéria Keller *et al*., 1998). Additionally, some strains of *P. guilliermondii* have been isolated from humans and have been determined to be opportunistic pathogens (Hurley *et al*., 1987). The potential for human pathogenicity as a result of the variation within strains of *E. cloacae* and *P. guilliermondii* is poorly understood. Strains of these species occur in a wide variety of habitats, including soils, flowers, fruit surfaces, and they exhibit considerable heterogeneity (Jang and Nishijima, 1990; Keger-van Rij, 1984; Last and Price, 1969). Therefore, rigorous toxicological testing is needed to establish whether strains of *E. cloacae* and *P. guilliermondii* pose a human health risk.

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