OPEN ACCESS Plant Pathology Journal

ISSN 1812-5387
DOI: 10.3923/ppj.2017.

Research Article
Effectiveness and Mechanism of Endophytic *Bacillus* in Suppressing Wilt Intensity of Banana Seedling Inoculated by Blood Disease Bacterium

Hadiwiyono and Salim Widono

Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A Kentingan, 57126 Surakarta, Indonesia

Abstract

Background and Objective: The main constraint faced by the banana farmers in Indonesia is bacterial wilt caused by Blood Disease Bacterium (BDB). Biological control of the disease using endophytic *Bacillus* is promising to develop. This study aimed at reporting the experimental study of the effectiveness and mechanism of some endophytic *Bacillus* of banana in suppressing wilt intensity of banana seedling artificially inoculated by BDB. **Materials and Methods:** The study was conducted in greenhouse, using 10 isolates of endophytic *Bacillus* of banana and banana seedlings produced by tissue culture acclimated for 3 months. A unit of treatment consisted of three banana seedlings arranged by completely randomized design with three replications. Each seedling was planted on 1 kg of soil media infested by endophytic *Bacillus* and BDB at 10^8 CFU g^{-1}. The pathogen inoculation was done on day 7 after *Bacillus* infestation. Some capability analyses of the isolates of producing growth regulator, enzymatic and chemical compounds were also done *in vitro* for supporting the study of antagonistic mechanism. **Results:** The results showed that some isolates of the endophytic *Bacillus* significantly ($p<0.05$) suppressed the infection rate and wilt intensity. The *Bacillus* reduced disease intensity by 51.44-24.07%. The antagonistic isolates reduced infection rate from 0.36 to 0.03-0.09 unit day^{-1}. The significance was also showed by the Area Under the Disease Progres Curve (AUDPC) variable. The isolates produced Indole Acetic Acid (IAA) and grown positively on chitin and pectin medium. The volatile and nonvolatile compounds produced by the isolates inhibited growth of the pathogen significantly ($p<0.05$). **Conclusion:** Some isolates of endophytic *Bacillus* are promising as biological control agents of bacterial wilt of banana caused by BDB as they reduce wilt intensity effectively and they produce IAA, chitinase, pectinase, volatile and nonvolatile compound that are presumably involved in the antagonism mechanism.

Key words: Chitinase, pectinase, volatile compound, nonvolatile compound, antagonism mechanism

Received: Accepted: Published:

Citation: Hadiwiyono and Salim Widono, 2017. Effectiveness and mechanism of endophytic *Bacillus* in suppressing wilt intensity of banana seedling inoculated by blood disease bacterium. Plant Pathol. J., CC: CC-CC.

Corresponding Author: Hadiwiyono, Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A Kentingan, 57126 Surakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Blood bacterial wilt is the most important disease of banana in Indonesia[1,3]. The disease is caused by Blood Disease Bacterium (BDB) that formerly was proposed by Gaumann[2] as *Pseudomonas celebensis*. The pathogen causes yellowing, wilting and eventually death of banana. The pathogen infects all stages of banana growth both vegetative and generative. On the fruiting stage, the diseased banana produces inedible fruits due to the fruit to be browning, broken off growing, maturing and eventually roting[1,2,4]. Recently BDB has been categorized as an invasive species because of the rapid spread from Celebes Island where the disease was firstly found by Gaumann[2] to other islands of Indonesia. It has spread in all of provinces of Indonesia and Malaysia[4]. In the fields, it had been reported that the disease incidence and crop losses could reach 100%[4,5].

Blood Disease Bacterium (BDB) is still difficult to control. Therefore, its control development technology needs to be conducted. The development of technology for disease control leading to support sustainable agriculture is a concern of the most experts for the current and futures. Biological control has been considered among the most promising technologies for sustainable agriculture. It reduces the reliance on synthetic pesticides, minimizes the negative impact on the environment and improves workers safety while at the same time maintaining the economic viability of crop production[6-8]. One of the interesting biological control agents is endophytic *Bacillus*, because it is one of more environmentally friendly alternatives, which is able to colonize an ecological niche similar to that of vascular wilt pathogens such as BDB[9]. Endophytic *Bacillus* lives in associating with various plants. It promotes growth and induces resistance of plants to some pathogens[9-14]. This research aims to study of the effectiveness and mechanism of endophytic *Bacillus* in suppressing wilt intensity on banana seedling artificially inoculated by BDB. The result of evaluation on some antagonism mechanism of some effective isolates to BDB is also presented.

MATERIALS AND METHODS

***In vivo* test:** An experiment to evaluate the capability of endophytic *Bacillus* of suppressing wilt intensity of blood disease was done on banana seedling inoculated artificially. The main materials in this study were BDB isolates. The inoculums of BDB were obtained by culture of the pathogen in CPG broth medium that were incubated for 4 days. The *Bacillus* isolates were cultured in nutrient broth and then were incubated for 3 days. A unit treatment consisted of 3 banana seedlings inoculated by BDB suspension with density at10^8 CFU g^{-1} of soil media. The medium consisted of soil and compost with composition 1:1 in volume. Each seedling was planted on polybag filled by 1 kg of the medium. The treatments included infestation of 10 isolates of endophytic *Bacillus* in the medium with the density at 10^8 CFU g^{-1} of soil media done 7 days before pathogen inoculation. The treatments were arranged by completely randomized design with three replications. The variables observed were wilt intensity, infection rate and area under the disease progress curve (AUDPC)[15-17].

Wilt intensity was assessed by the Eq. 1:

$$IP\ (\%) = \frac{\sum(n \times v)}{N \times Z} \times 100 \quad (1)$$

where, IP = Wilt intensity, n = Number of seedling with certain score, v = Wilting, Z = Higher score and N = Number of seedling as a treatment unit, where as for scoring used categories: 0 = No wilting leaf, 1 = One-two wilting leaf/leaves, 2 = Three-four wilting leaves, 3 = Five-six wilting leaves and 4 = All of leaves are wilting[15].

Infection rate was assessed by using the Eq. 2[16]:

$$r = \frac{2.3}{\Delta t}\left(\log\frac{1}{1-Xt!} + \log\frac{1}{1-X0!}\right) \quad (2)$$

where, r = Infection rate (unit per week), t = Interval of observation, X0 = Wilt intensity in proportion and Xt = Wilt intensity in proportion at t.

Assessment of AUDPC was done by the Eq. 3[16,17]:

$$AUDPC = \sum_{i:1}^{n} \frac{Xi + 1 + Xl}{2}\left(t_{i+1-ti}\right) \quad (3)$$

where, AUDPC = Area under the disease progress curve, Xi = Wilt intensity at ti; ti = Time of observation and n = Sum of observation.

Indole acetic acid assay: The capability test of *Bacillus* of producing Indole Acetic Acid (IAA) was done as follows: First, standard solution of IAA was made by mixing synthesis IAA with distilled water at 0.0, 0.2 and etc. until 2.0 ppm. After that 3 mL IAA solution was taken and to each of the solution 1 mL Salkowski's solvent was added and then homogenized. The

absorbance value of IAA solutions was assessed by spectrophotometer at λ530 nm. The second, pure culture of *Bacillus* isolate was sub-cultured on NA medium and incubated for 48 h. The bacterial suspension was made 10 mL using McFarland standard solution[18] with cell density at 10^8 CFU mL^{-1}. Approximately 3 mL of bacterial suspension was removed into 30 mL LB liquid solution (Luria Bertani) + tryptophan. Each treatment was replicated 3 times and incubated at 28°C in shaker at 1500 rpm for 6 min by which supernatant and pellet were separated. Analysis of IAA content used calorimeter method[19]. Approximately 2 mL supernatant and 1 mL Salkowski's reagent were mixed[20-21]. The samples were allowed for 60 min before assessing the absorbance value using spectrophotometer at λ530 nm. Regression formulation was substituted by absorbance value of sample.

Chitinase and pectinase assay: The capability test of *Bacillus* of producing chitinase and pectinase was based on the activity zone of pectin lyase produced by *Bacillus* on pectin medium. Filter papers of diameter of 0.5 cm dyed into *Bacillus* suspension at 108 CFU mL^{-1} were placed symmetrically on the CPG agar medium containing 0.5% chitin or pectin in Petri dish of diameter of 9 cm. The observation of chitin and pectin lyse activation was based on the clear zone on the pectin medium after incubating for 72 h.

Assay of volatile compound in culture filtrate of *Bacillus*: This test was done by culturing *Bacillus* on NA and BDB on CPG agar medium in the cover of Petri dish. *Bacillus* cultures incubated for 2 days were covered by BDB cultures in cover Petri dish by positioning upside down than the couple dishes were silted by transparent plastic isolator. Growth of the BDB was observed on the 9th day of incubation. The observation was done by harvesting bacterial culture using 10 mL sterile water. Bacterial cell density was observed by assessing absorbance value of bacterial suspension using spectrophotometer at λ530 nm. The absorbance values were converted to colony forming unit using a standard formulation.

Assay nonvolatile compound in culture filtrate of *Bacillus*: The bacteria were culture in NB incubated for 4 days. Amount of 3 mL *Bacillus* culture filtrate at 10^8 CFU mL^{-1} was centrifuged at 5 rpm for 20 min. The supernatant resulted from the centrifugation was sterilized by autoclave at 121°C and 1 atm for an hour and then it was placed under UV for 12 h. Approximately 0.1 mL of BDB suspension at 10^8CFU mL^{-1} were culture on the CPG agar medium using Petri dish of diameter of 9 cm. Filter papers of diameter of 0.5 cm dyed into *Bacillus* culture filtrate were placed on the agar media in symmetric position. The cultures were incubated for 72 h. The capability observation of inhibiting BDB growth was based on inhibition zone indicated by clear zone around the filter papers.

Statistical analysis: The statistical analysis of quantitative data was conducted using the SPSS 21.0 statistical software (SPSS Inc, Chicago, IL, USA). The data of disease variables were subjected to F-test and the discrimination of the variation among treatments was performed using the Duncan's Multiple Range Test (DMRT) at 5% probability. Where as assays data such as the *Bacillus* ability to produce phytohormone, chitinase, pectinase and inhibitor compounds were subjected to descriptive analysis.

RESULTS

***In vivo* test:** The results showed that all isolates of endophytic *Bacillus* could effectively suppress blood disease intensity on the banana seedlings artificially inoculated by BDB. The isolates could suppress wilt intensity, infection rate and AUDPC (Table 1). The greatest effective one was isolate B18 suppressing wilt intensity at 74.51% (94.44-24.07%), whereas the smallest one was isolate B01 suppressing wilt intensity at 45.00% (944.44-51.85%).

Assay of enzyme and inhibitor compounds: Results of the current researches show that all of *Bacillus* isolates can produce phytohormone of Indole Acetic Acid (IAA), enzyme of chitinase and pectinase. The capability of *Bacillus* to produce IAA was showed by the results of IAA assay from

Table 1: Endophytic *Bacillus* capability of suppressing wilt intensity, infection rate and AUDPC on seedling of banana artificially inoculated by BDB

Isolates of *Bacillus*	Wilt intensity (%)*	Infection rate (unit $days^{-1}$)	AUDPC*
No treatment	94.44±0.00^{e}	0.36^{b}	1375.00±116.67^{b}
Carrier agent	92.59±3.21^{e}	0.33^{b}	1500.00±63.10^{b}
B01	51.85±3.21^{d}	0.09^{a}	652.78±63.10^{a}
B02	42.59±3.21^{c}	0.07^{a}	533.33±75.00^{a}
B03	40.74±3.21^{c}	0.07^{a}	452.78±04.81^{a}
B04	42.59±6.42^{c}	0.07^{a}	577.77±84.30^{a}
B09	25.93±3.21^{a}	0.04^{a}	327.78±48.83^{a}
B15	29.63±2.62^{ab}	0.04^{a}	488.89±48.83^{a}
B17	37.04±11.5^{bc}	0.06^{a}	488.89±179.96^{a}
B18	24.07±3.21^{a}	0.03^{a}	300.00±62.55^{a}
B20	35.19±6.42^{bc}	0.05^{a}	466.67±76.38^{a}
B22	31.48±3.21^{ab}	0.05^{a}	527.78±61.43^{a}

*Averages followed by the same letter are non-significantly different based on DMRT at 5% level and the values following the averages are standard error of mean

Table 2: Capability of endophytic *Bacillus* of producing phytohormone, enzyme, volatile and nonvolatile compounds

		Enzymes		Inhibitor compounds	
Bacillus isolates	IAA (ppm)	Chitinase	Pectinase	Volatile $\times 10^8$ CFU mL^{-1}	Nonvolatile* diameter of clear zone (cm)
B01	9.66	+	+	1.50	0.91^{ef}
B02	6.85	+	+	1.65	0.85^{ef}
B03	9.58	+	+	1.37	0.90^{ef}
B04	6.15	+	+	1.48	0.99^{d}
B09	9.62	+	+	1.92	0.85^{ef}
B15	6.59	+	+	1.98	1.29^{c}
B17	6.76	+	+	1.80	0.89^{e}
B18	5.59	+	+	1.25	1.84^{a}
B20	8.59	+	+	2.02	0.80^{f}
B22	6.87	+	+	1.84	1.45^{b}
No treatment	0.00	-	-	2.89	0.00^{g}
Sterile water	0.00	-	-	2.95	0.00^{g}

+ = Produce pectinase activity zone, - = No activity zone, *Averages followed by the same letter are non-significantly different based on DMRT at 5% level

5.59-9.66 ppm where no IAA was in the media with no treatment and treated with sterilized water. All of isolates showed the growth well on both chitin and pectin medium. It means that the *Bacillus* could use the chitin and pectin as carbon source due to the ability to produce the enzyme of chitinase and pectinase. All of the isolates also produced volatile and nonvolatile inhibitor compounds showed by decreasing growth of BDB on the treated medium (Table 2).

DISCUSSION

The current research results showed that the endophytic *Bacillus* had effective capability of suppressing wilt intensity of banana artificially inoculated by blood disease bacteria. The results supported the previous research indicating that endophytic *Bacillus* were effectively reduced wilt intensity of blood disease on plantlets and *in vitro* test of the bacteria that could inhibit the growth of banana wilt pathogens[10,11]. The result was linear to different pathogens according to previous researches of Ji *et al*.[13], Nagendran *et al*.[22] and Nawangsih *et al*.[23], Ji *et al*.[13] did a screening on antagonistic activities against *Ralstonia solanacearum in vitro* and four isolates showed a remarkable inhibitory effect. The evaluation of the antagonistic strains against bacterial wilt of mulberry indicated that the strain Lu144 effectively reduced disease incidence. Nagendran *et al*.[22] reported that rice plots treated with endophytic *Bacillus* had a significantly ($p<0.05$) lower intensity of bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* compared to untreated control plots by which also recorded a higher grain and straw yield. Whereas, Nawangsih *et al*.[23] did a greenhouse test on six selected of endophytic bacteria to *R. solanacearum* by which they obtained BL10 isolates (similar *Bacillus amyloliquefaciens* strain JK-SD002) as promising bio-control agents. At six weeks after transplanting, plants treated with BL10 isolate showed a significantly ($p<0.05$) lower disease incidence (43.33%) than those of control (83%).

Many factors may affect the capability of bio-control agent of decreasing disease intensity. The capability of biological control agent of producing some compounds may be involved in antagonistic mechanism. Seemingly, the inhibitor compounds are involved in antagonism mechanism and reducing wilt intensity of blood disease of banana. Hadiwiyono and Widono[10] showed that some isolates of *Bacillus* formed a clear zone inhibition on the dual culture with BDB and *Fusarium oxysporum* f. sp. *cubense* by then it was suspected that the bacteria could produce inhibitor compounds. Based on the current evident it was proved that endophytic *Bacillus* could produce volatile and nonvolatile compounds inhibiting growth of BDB. Toxic compounds produced by biological control agent have also been reported as an important mechanism in biological control of plant diseases. Arrebola *et al*.[24], Islam *et al*.[25] and Yuan *et al*.[26] reported that some isolates of *Bacillus* could produce some volatile compounds inhibiting pathogen growth. Ryu *et al*.[27,28] discovered that bacterial-produced Volatile Organic Compounds (VOCs) triggered plant growth enhancement and induced systemic resistance constitutes a novel mechanism for rhizobacteria-plant interaction. Of the PGPR tested, two of seven strains (*B. subtilis* GB03 and *B. amyloliquefaciens* IN937a) elicited constitutively growth promotion and induced systemic resistance of Arabidopsis seedlings, suggesting that synthesis of bioactive VOCs is a strain-specific phenomenon.

According to Reddy[14] *Bacillus* can produce some secondary metabolites such mycobactin, bacitracin and zwittermicin being useful in inhibiting bacterial pathogen. Other researchers reported that inhibiting capability of *Bacillus*

was caused by the existence of toxic chemical compound of acetoin[29], bacilycin[30], fengycin and bacillomycin[31]. Some biosynthetic genes of antimicrobial compounds produced by *Bacillus* have been identified; *srfAA* (surfactin), *bacA* (bacylisin), *fenD* (fengycin), *bmyB* (bacillomycin), *spaS* (subtilin) and *ituC* (iturin)[32].

Possibly, phytohormone could also be involved in suppressing disease intensity of blood disease due to the ability to produce Indole Acetic Acid (IAA). The compound has been also reported that it could promote growth[33-35] and induce resistance to plant pathogens[27,36-38]. The IAA is important for vigor of the plant and most frequently it is used to explain the mechanism of plant growth promoting microbes. The IAA will take role in regulating the plant growth to be healthy plant. By IAA the healthy plant will be more resistant or tolerant to pathogen infection because of the role in cell proliferation, elongation and enlargement[39,40]. Some researchers however reported that IAA could promote plant disease development or induced susceptibility[40,41]. Wang *et al.*[41] added that signaling IAA pathway could be repressed using salicylic acid.

This research proves that all of *Bacillus* isolates produce chitinase and pectinase enzyme. The results are the same as those of the previous researches of Huang *et al.*[42], Saha *et al.*[43], Zhao *et al.*[44] and Kim and Chung[45]. The role of chitinase in antagonism to pathogenic fungi has been explained well. Chitinase can cause enzymatic dissolution of cell walls leading to loss of fungal protoplasm. It is one of the main antagonistic mechanisms involved in the activity of bio control agents[46,47]. All of the isolates can produce chitinase, a degrader of the fungal cell wall. It is an indication that cell wall degradation may be one of the mechanisms of antagonism exhibited by the endophytic *Bacillus* isolates. To pathogenic bacteria however both chitinase and pectinase are still poorly understood. Possibly, the role is indirect that the capability of the endophytic *Bacillus* of producing chitinase and pectinase together with IAA is helpful to pathogen in proliferation and colonization of the antagonistic bacteria. It has been reported that pectinase is one of the determinants of induced systemic resistant[48,49].

CONCLUSION

All isolates of endophytic *Bacillus* tested, were capable of suppressing wilt intensity, infection rate and AUDPC. The isolate of B18 was the most effective in suppressing wilt intensity from 24.07 to 94.40%. All of isolates could produce IAA, chitinase, pectinase, volatile compound and non volatile compound that were presumably involved in the antagonism mechanism of *Bacillus*.

SIGNIFICANCE STATEMENTS

- From this study it was proved that endophytic *Bacillus* could produce some compounds that were possibly involved in biological control mechanism
- This study discovers that endogenous endophytic *Bacillus* is promising as a biological control agent for control of bacterial wilt

ACKNOWLEDGMENTS

The current research was funded by Maintenance Research Group of PNBP-Universitas Sebelas Maret (UNS), in Budgeting Year 2016 with Contract Number: 632/UN27.21/LT/2016. This research was also supported effectively by Antoni Derry Pradana Putra, a student of Department of Agrotechnology, Universitas Sebelas Maret.

REFERENCES

1. Hadiwiyono, 2011. Blood bacterial wilt disease of banana: The distribution of pathogen in infected plant, symptoms and potentiality of diseased tissues as source of infective inoculums. Nusantara Biosci., 3: 112-117.
2. Gaumann, E., 1921. Onderzoekingen over de bloedziekte der bananen op celebes, I & II. Mededeelingen van het Instituut voor Plantenziekten, Vol. 50, Ruygrok & Co., Dutch East Indies.
3. Supriadi, 2005. Present status of blood disease in Indonesia. Proceedings of the 3rd International Bacterial Wilt Symposium, February 4-8, 2002, South Africa.
4. CABI., 2016. Blood disease bacterium (blood disease bacterium of banana). Invasive Species Compendium, Centre for Agriculture and Biosciences International (CABI), UK.
5. Buddenhagen, I., 2009. Blood bacterial wilt of banana: history, field biology and solution. Proceedings of the International Symposium on Recent Advances in Banana Crop Protection for Sustainable Production and Improved Livelihoods, September 10-14, 2007, White River, South Africa.
6. Bale, J.S., J.C. van Lenteren and F. Bigler, 2008. Biological control and sustainable food production. Philos. Trans. R. Soc. London B: Biol. Sci., 363: 761-776.
7. Tracy, E.F., 2015. The promise of biological control for sustainable agriculture: A stakeholder-based analysis. J. Sci. Policy Governance, Vol. 5, No. 1.
8. Sharma, A., V.D. Diwevidi, S. Singh, K.K. Pawar and M. Jerman *et al.*, 2013. Biological control and its important in agriculture. Int. J. Biotechnol. Bioeng. Res., 4: 175-180.
9. Eljounaidi, K., S.K. Lee and H. Bae, 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases-Review and future prospects. Biol. Control, 103: 62-68.

10. Hadiwiyono and S. Widono, 2012. Endophytic *Bacillus*: the potentiality of antagonism to wilt pathogen and promoting growth to micro-plantlet of banana *in vitro*. Biomirror, 3: 1-4.
11. Hadiwiyono and S. Widono, 2013. Vigor of plantlet from microplantlet treated by filtrate and cell suspension of some isolates of bacillus and resistance to banana wilt pathogen after acclimatization. Esci J. Plant Pathol., 2: 70-75.
12. Liu, B., H. Qiao, L. Huang, H. Buchenauer, Q. Han, Z. Kang and Y. Gong, 2009. Biological control of take-all in wheat by endophytic *Bacillus subtilis* E1R-j and potential mode of action. Biol. Control, 49: 277-285.
13. Ji, X., G. Lu, Y. Gai, C. Zheng and Z. Mu, 2008. Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. FEMS Microbiol. Ecol., 65: 565-573.
14. Reddy, P.P., 2014. Plant Growth Promoting Rhizobacteria for Horticultural Crop Protection. Springer, New Delhi, India, ISBN-13: 9788132219736, Pages: 310.
15. Hadiwiyono, S. Subandiyah, C. Sumardiyono, J. Widada and M. Fegan, 2007. Effect of altitude and wounding on blood disease progress of plantain. Jurnal Hama dan Penyakit Tumbuhan Tropika, 7: 111-116.
16. Van der Plank, J.E., 1963. Plant Diseases: Epidemics and Control. 1st Edn., Academic Press, New York, USA., ISBN: 0127114505, Pages: 349.
17. Simko, I. and H.P. Piepho, 2012. The area under the disease progress stairs: Calculation, advantage and application. Phytopathology, 102: 381-389.
18. Bressan, W. and M.T. Borges, 2004. Delivery methods for introducing endophytic bacteria into maize. BioControl, 49: 315-322.
19. Bric, J.M., R.M. Bostock and S.E. Silverstone, 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied Environ. Microbiol., 57: 535-538.
20. Gordon, S.A. and R.P. Weber, 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol., 26: 192-195.
21. Zahir, Z.A., S.A. Abbas, M. Khalid and M. Arshad, 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. Pak. J. Biol. Sci., 3: 289-291.
22. Nagendran, K., G. Karthikeyan, M.F. Peeran, M. Raveendran, K. Prabakar and T. Raguchander, 2013. Management of bacterial leaf blight disease in rice with endophytic bacteria. World Applied Sci. J., 28: 2229-2241.
23. Nawangsih, A.A., I. Damayanti, S. Wiyono and J.G. Kartika, 2011. Selection and characterization of endophytic bacteria as biocontrol agents of tomato bacterial wilt disease. HAYATI J. Biosci., 18: 66-70.
24. Arrebola, E., D. Sivakumar and L. Korsten, 2010. Effect of volatile compounds produced by *Bacillus* strains on postharvest decay in citrus. Biol. Control, 53: 122-128.
25. Islam, M.R., Y.T. Jeong, Y.S. Lee and C.H. Song, 2012. Isolation and identification of antifungal compounds from *Bacillus subtilis* C9 inhibiting the growth of plant pathogenic fungi. Mycobiology, 40: 59-66.
26. Yuan, J., W. Raza, Q. Shen and Q. Huang, 2012. Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. *cubense*. Applied Environ. Microbiol., 78: 5942-5944.
27. Ryu, C.M., M.A. Farag, C.H. Hu, M.S. Reddy, H.X. Wei, P.W. Pare and J.W. Kloepper, 2003. Bacterial volatiles promote growth in *Arabidopsis*. Proc. Natl. Acad. Sci. USA., 100: 4927-4932.
28. Ryu, C.M., M.A. Farag, C.H. Hu, M.S. Reddy, J.W. Kloepper and P.W. Pare, 2004. Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol., 134: 1017-1026.
29. Compant, S., B. Duffy, J. Nowak, C. Clement and E.A. Barka, 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. Applied Environ. Microbiol., 71: 4951-4959.
30. Rajavel, M., A. Mitra and B. Gopal, 2009. Role of *Bacillus subtilis* BacB in the synthesis of bacilysin. J. Biol. Chem., 284: 31882-31892.
31. Ramarathnam, R., S. Bo, Y. Chen, W.G.D. Fernando, G. Xuewen and T. de Kievit, 2007. Molecular and biochemical detection of fengycin-and bacillomycin D-producing *Bacillus* spp., antagonistic to fungal pathogens of canola and wheat. Can. J. Microbiol., 53: 901-911.
32. Mora, I., J. Cabrefiga and E. Montesinos, 2011. Antimicrobial peptide genes in *Bacillus* strains from plant environments. Int. Microbiol., 14: 213-223.
33. Etesami, H., H.A. Alikhani and H.M. Hosseini, 2015. Indole-3-Acetic Acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. Methods X, 2: 72-78.
34. Tabatabaei, S., P. Ehsanzadeh, H. Etesami, H.A. Alikhani and B.R. Glick, 2016. Indole-3-Acetic Acid (IAA) producing *Pseudomonas* isolates inhibit seed germination and α-amylase activity in durum wheat (*Triticum turgidum* L.). Span. J. Agric. Res., Vol. 14, No. 1.
35. Mohite, B., 2013. Isolation and characterization of Indole Acetic Acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J. Soil Sci. Plant Nutr., 13: 638-649.
36. Ding, X., Y. Cao, L. Huang, J. Zhao, C. Xu, X. Li and S. Wang, 2008. Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansion expression and promotes salicylate-and jasmonate-independent basal immunity in rice. Plant Cell, 20: 228-240.
37. Denance, N., A. Sanchez-Vallet, D. Goffner and A. Molina, 2013. Disease resistance or growth: The role of plant hormones in balancing immune responses and fitness costs. Front. Plant Sci., Vol. 4. 10.3389/fpls.2013.00155.

38. Akhtar, M.S. and Z.A. Siddiqui, 2010. Role of Plant Growth Promoting Rhizobacteria in Biocontrol of Plant Diseases and Sustainable Agriculture. In: Plant Growth and Health Promoting Bacteria, Maheshwari, D.K. (Ed.). Springer-Verlag, Berlin, Heidelberg, pp: 157-196.
39. Yu, T., J. Chen, H. Lu and X. Zheng, 2009. Indole-3-acetic acid improves postharvest biological control of blue mold rot of apple by *Cryptococcus laurentii*. Phytopathology, 99: 258-264.
40. Gonzalez-Lamothe, R., M. El Oirdi, N. Brisson and K. Bouarab, 2012. The conjugated auxin indole-3-acetic acid-aspartic acid promotes plant disease development. Plant Cell, 24: 762-777.
41. Wang, D., K. Pajerowska-Mukhtar, A.H. Culler and X. Dong, 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr. Biol., 17: 1784-1790.
42. Huang, C.J., T.K. Wang, S.C. Chung and C.Y. Chen, 2005. Identification of an antifungal chitinase from a potential biocontrol agent, *Bacillus cereus* 28-29. J. Biochem. Mol. Biol., 38: 82-88.
43. Saha, D., G.D. Purkayastha, A. Ghosh, M. Isha and A. Saha, 2012. Isolation and characterization of two new *Bacillus subtilis* strains from the rhizosphere of eggplant as potential biocontrol agents. J. Plant Pathol., 94: 109-118.
44. Zhao, L., Y. Xu, X.H. Lai, C. Shan, Z. Deng and Y. Ji, 2015. Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. Braz. J. Microbiol., 46: 977-989.
45. Kim, P.I. and K.C. Chung, 2004. Production of an antifungal protein for control of *Colletotrichum lagenarium* by *Bacillus amyloliquefaciens* MET0908. FEMS Microbiol. Lett., 234: 177-183.
46. Sharma, N., K.P. Sharma, R.K. Gaur and V.K. Gupta, 2011. Role of chitinase in plant defense. Asian J. Biochem., 6: 29-37.
47. Ma, B.C., W.L. Tang, L.Y. Ma, L.L. Li and L.B. Zhang *et al*., 2009. The role of chitinase gene expression in the defense of harvested banana against anthracnose disease. J. Am. Soc. Hortic. Sci., 134: 379-386.
48. Nakaho, K. and C. Allen, 2009. A pectinase-deficient *Ralstonia solanacearum* strain induces reduced and delayed structural defences in tomato xylem. J. Phytopathol., 157: 228-234.
49. Peng, X., X. Xie, Z. Bai and H. Zhang, 2006. Purification and induced resistance of pectinases from *Penicillium oxalicum* BZH-2002. Chin. J. Applied Environ. Biol., 12: 750-753.