CHAPTER 1

INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 Introduction

Plants are associated with different types of microorganisms. These microorganisms interact with their host plants by deploying different mechanisms as plants offer a wide diversity of habitats such as the phyllosphere, the rhizosphere and the endosphere.

Endophytes are defined as "a microorganism associated with living plant tissues that produces no apparent indication of its presence in the plant and seems not to cause harm to the host" [1, 2]. The endophytes employ different mechanism to gain entry into the plant tissues especially in the roots. The most common mode of entry of endophytic bacteria into plant tissues is through primary and lateral root cracks. Of course, bacteria residing in the rhizosphere might also have the potential to enter and colonize the plant roots. This micro-ecosystem has been widely known as one of the primary sources for endophytic colonization [3, 4]. Endophytes also enter the plants through the stomata, particularly on leaves and young stems. Endophytic bacteria facilitate the acquisition of essential nutrients such as nitrogen, phosphorus, iron etc., and also modulate level of different hormones including auxins, cytokinin and gibberellins in a host plant [4, 5, and 6]. The bacterial endophytes are also known to activate host plant defense against biotic and abiotic stress [7, 8]. Bacterial endophytes have also been studied for their potential to inhibit phytopathogens [8]. Although new mechanisms used by some bacterial endophyte in reducing the effects of pathogens are been deciphered, current knowledge about endophytes, pathogen, and plant interaction dynamics is still not fully understood. There are several pertinent queries about endophytes still remain to be solved, some of these are as follows:

[1] The exact role of different endophytes in plant and their host range. Whether the host decides the endophyte, or the endophyte decides the host.
[2] The impact of an endophyte on other endophytes and the phytopathogens.
[3] Does a host carry endophytes from generation to generation to protect them from pathogen counterparts?
[4] How does a host plant maintain particular load of an endophyte?

Ralstonia solanacearum is a Gram-negative, soil-borne bacterium which causes lethal bacterial wilt disease in more than 450 plant species from 54 different botanical families of monocot as well as dicot plants, which includes many important crops such as tomato, potato, brinjal, chili etc [9-13]. The bacterium colonizes the internal vascular tissue of plants before colonizing the entire plant.Different approaches including crop rotation, use of pesticides/chemicals, plant breeding, field sanitation, use of tolerant cultivars have been practiced but all these are not found adequate to efficiently manage the disease [8]. Use of *R. solanacearum* antagonistic microbes as bio-control agent can be an alternative approach to fight the disease [14-17]. Our laboratory has isolated and characterized a *R. solanacearum* strain F1C1 from wilted chili plant from Tezpur, Assam, India [18]. The laboratory has developed virulence assays to study F1C1 strain in controlled environment. These assays are very easy, less time consuming as the whole process from seed germination to completion of virulence assay takes around 15 to 20 days. Due to the soil free nature, and controlled environment, the interference of other microbes during the time of infection in these assays is negligible [19-22]. During the development of these assays, we were fascinated to find out what kind of microbes, especially bacterial endophytes are present inside these seedlings and if they have any antagonistic activity against F1C1 or not.

In this study, we isolated different bacterial endophytes from healthy fresh grown tomato seedlings. We tried to find out the role of these endophytic bacteria against F1C1. Few of the isolates were found to be antagonistic against F1C1. All the bacterial isolates were characterized. We also studied the effect of the antagonistic isolates against F1C1 in tomato seedlings. One of the isolates Characterized as *Pseudomonas putida* N4T was rigorously studied for its plant colonization. N4T was also found to be colonizing inside brinjal seedlings and it reduced the disease caused by F1C1 in the brinjal seedlings, the colonization of N4T was studied in grown up plants also. Also, the genome sequence of N4T was analyzed and a comparative phylogenomics study was carried out.

Objectives of this study

Objective I: **Isolation & characterization of *R. solanacearum* F1C1 antagonistic bacteria from tomato seedlings and study their effect in control of bacterial wilt in tomato seedlings**

- Isolation of bacterial endophytes by surface sterilization, crushing and plating and study antagonistic activity against F1C1 by agar well diffusion and cross-streaking method
- Characterization of the isolates for twitching motility, cellulase assay and identification by 16S rDNA sequencing
- Interaction of the five F1C1 antagonistic isolates among each other
- Standardization and study the bio-control efficacy of all the antagonistic isolates individually and collectively using root inoculation and leaf inoculation method
- Study the effect of the isolates in tomato seedlings

Objective II: Characterization of *Pseudomonas putida* N4T and study its effect in control of bacterial wilt in grown-up tomato plants and in brinjal seedlings

- Tagging of N4T with GFP reporter and studied the colonization in brinjal seedlings and grown up tomato plants by visualizing under fluorescence microscope
- Study the efficacy of N4T in control of grown up tomato plants
- study the efficacy of N4T in protection of brinjal seedlings from F1C1 infection
- Study the Hypersensitivity response of N4T in tobacco plant
- Study the role of *gacA* gene in N4T against C10 and F1C1 by creating insertion mutation and studying the mutant against C10 and F1C1

Objective III: Genome sequence analysis and comparative phylogenomics of *Pseudomonas putida* N4T

- Sequencing of N4T genome by Illumina platform and annotation using different tool
- *In silico* mining of possible secondary metabolite biosynthesis gene clusters using antismash6

- Study the possible plant growth promoting traits and Plant colonization and virulence factors using Plabase tool
- Comparative phylogenomics of N4T with five other *Pseudomonas* strains using EDGAR tool

Review of the literature

Plants are associated with plethora of microbes which resides in different parts of their host plants and share different kind of relationships with host plants. Although the interaction between host plant and associated microbes are studied in recent years albeit to unravel dynamics of these interactions and to understand the factors involved in plant-microbe association more research is required. Although most of the microbes associated with plants are found to be non-pathogenic to the host, there are different plant pathogens that cause disease to host plants. The interaction dynamics between the host with the pathogen, the pathogen with the native non-pathogenic microbes and the pathogen- non-pathogen-host-environment is a matter of research.

Among other, endophytic microbes, known for their colonization potential inside plants' internal tissues,without pathogenic consequences merit special mention. Endophytes are microorganisms that successfully colonize the internal tissue of plants without showing any symptoms [23, 24]. Endophytes influence plants through internal interactions, by colonizing and living within the belowground (roots) or aboveground plant tissues (stem and leaves), forming the plant microbial endosphere. Although plant-endophyte interaction has been studied by many researchers recently, many conceptual aspects related to the nature of endophytes, their role in plant health, mode and mechanisms of entry and localization etc. are yet not clear. For instance, although endophytes are known to be harmless to the host, a recent study by Brader et al. (2017) illustrated that endophytes can also be defined in terms of their ecological niche and not only the function they perform in the host. The study further revealed that some species of endophytes can either be pathogenic or beneficial depending on host [24]. The majority of the endophyte does not show any harmful effects on a few plant species, however, when tested on other plants, they may be pathogenic. The pathogenicity characteristic of endophytes can be based on a number of biotic interactions and environmental factors. For example, fluorescent *Pseudomonads*, known to be beneficial to most plants, established to be pathogenic to the leather leaf plant under special conditions [26]. Simultaneously it is a matter of dispute that if a plant pathogen becomes avirulent, will it be called as endophyte! Another intriguing observation is that few endophytes which do not usually cause disease to the host plants have been found to be opportunistic pathogen under certain conditions.

Bacterial endophytes are known to exhibit various activities in host plant including plant growth promotion, seedling emergence and also it has been reported by various researchers that bacterial endophytes provide resistance against plant pathogen and environmental stress [4-8]. Bacterial endophytes have the ability to synthesize plant hormones including indole-3-acetic acid, secret siderophores and solubilize phosphate [5, 6, 27, 28, 29]. It has been reported that several bacterial endophytes reside in the same niche similar to plant pathogens, which might help them to be suitable bio-control agents. Many of the bacterial endophytes get associated with the host plant during the different developmental stage of the host plants through environment by different mode, but detailed investigation is required to know different mechanisms that bacteria used to get entry to host. It might be plant chooses some of the endophytes and they become essential part of the host and are carried from generation to generation to provide different benefits to the host. Although the role of bacterial endophytes in plant health and against plant diseases are well reported, to have in depth knowledge of the intricacies of interaction between plant, endophyte, pathogen and environment more research is required.

There are several reports regarding bacterial endophytes exhibiting antagonistic activity against phytopathogens and a few of them has been demonstrated to control the disease caused by these pathogens in their hosts. However, most of these studies are limited to laboratory condition. Under field condition some of the endophytes were not found to be efficient in suppression of the disease caused by the pathogens. *R. solanacearum* is a Gram-negative, soil-borne bacterium which causes lethal bacterial wilt disease in more than 450 plant species from 54 different botanical families, which includes many important crops such as tomato, potato, brinjal, chili, etc. [9-13]. *R. solanacearum* is considered as one of the most devastating phytopathogen due to its wide host range, aggressive nature of the disease, high genetic diversity and adaptability in different environment [30, 31]. In addition to its genotypic variation and severity, this bacterium has an astounding capacity to survive in the soil for many years and forms latent infections within indigenous weeds, resulting in a big challenge in the eradication of this bacterium [32]. To control the disease, several methods like crop rotation, use of pesticides/chemicals, plant breeding, field sanitation, use of tolerant cultivars has been practiced but all these approaches have been found to be not adequate to efficiently control the disease. Use of microbes having antagonistic activity against *R. solanacearum* as bio-control agent can be an alternative approach to fight the disease. A range of recent studies have showed that plant associated antagonistic bacteria has potential bio-control effect against the *R. solanacearum* infection in laboratory and field conditions [7, 8, 14, 15].

R. solanacearum F1C1 strain was isolated from wilted chili plant, collected from nearby field of Tezpur, Assam, Indian [18]. Different methods were developed to study the pathogenicity of F1C1 in seedlings stages of tomato and brinjal [19-22]. The seedlings used in these studies are six to seven days old, freshly grown and have two cotyledon leaves. The seedlings are germinated in a controlled environment to support gnotobiotic condition and the pathogenicity assays are performed in hydroponic condition. These assays are very easy, less time consuming as the whole process from seed germination to completion of pathogenicity assay takes around 15 to 20 days. Due to the soil free nature-controlled environment, the interference of other microbes during the time of infection in these assays is negligible. In recent studies scientist have used these assays to study bio-control potential of bacteria against *R. solanacearum* [33, 34].

Table 1.1: List of some bio-control agents used against phytopathogen causing bacterial wilt dis

Biocontrol agents	Phytopathogen	Bibliography
Pseudomonas fluorescens PICF7 *Paenibacillus* K165 *Serratia marcescens* UPM39B3	*Verticillium dahliae*	[35-41]
Bacillus subtilis Lu144 *Bacillus amyloliquefaciens* Bg-C31 *Pseudomonas fluorescens* EB69 *Bacillus amyloliquefaciens* BZ6-1 *Streptomyces virginiae* Y30 and E36 *Bacillus amyloliquefaciens* SQR-7 and SQR-101 *B. methylotrophicus* SQR-29 *Ralstonia pickettii* QL-A6 *Pseudomonas monteilii* *Glomus fasciculatum* *Brevibacillus brevis* L-25 *Streptomyces roche* L-9 *Bacillus amyloliquefaciens* *Bacillus sp.* (RCh6) *Pseudomonas mallei* (RBG4) *Trichoderma viride* *B. subtilis* *Azotobacter chroococcum* *Glomus fasciculatum* *P. fluorescens* *B. amyloliquefaciens* QL-5, QL-18 *Acinetobacter sp.* Xa6, *Enterobacter sp.* Xy3 *B. vallismortis* ExTN-1 *Glomus mossease* *Streptomyces* sp. NEAU-HV9	*Ralstonia solanacearum*	[33,34, 41-52]

During the development of the above-mentioned assays, we were captivated to find out what kind of microbes, especially bacterial endophytes are present inside these seedlings and if they have any antagonistic activity against F1C1 or not. In this Ph.D. thesis we have studied endophytic bacteria isolated from freshly grown healthy tomato seedlings. We found that tomato seedling contains antagonistic bacteria against F1C1. All the bacterial isolates were characterized, and the five potential antagonistic bacteria were studied in vivo to find out their effect against F1C1 pathogenicity. All the five antagonistic isolates were able to reduce the disease and appearance of disease symptoms also delayed. One antagonistic isolates viz, N4T belongs to *Pseudomonas putida* was studied rigorously for its colonization inside tomato seedlings and grown up plant, also it was observed that N4T can dwell inside brinjal seedlings and it also exhibited potential to control the disease caused by F1C1. The whole genome sequence analysis and comparative phylogenomics of N4T was also carried out in this research study. The whole genome sequence analysis confirmed that indeed the N4T strains belongs to *P. putida* species. Further, different genes associated with plant colonization and plant growth promotion were predicted using *in sillico* tool.

Bibliography

[1] Montesinos,E. Plant-associated microorganisms: a view from the scope of microbiology. *Int. Microbiol*,6:221-223, 2003.

[2] Strobel, G. The emergence of endophytic microbes and their biological promise. *Journal of Fungi*, *4*(2): 57, 2018.

[3] Kandel, S.L., Joubert, P.M., Doty, S.L. Bacterial Endophyte Colonization and Distribution within Plants. *Microorganisms*, 5(4):77, 2017.

[4] Santoyo, G., *et al.* Plant growth-promoting bacterial endophytes. *Microbiological Research*, 182:92-99, 2016

[5] Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., Dowling, D.N. Bacterial endophytes: recent developments and applications. *FEMS Microbiol. Lett.*, 278:1–9, 2008.

[6] Rosenblueth, M., Romero, E.M. Bacterial endophytes and their interactions with hosts. Mol. Plant Microbe Interact., 19:827–837,2006.

[7] Walters, D.R., Ratse, J., Havis, N.D. Controlling crop diseases using induced resistance: challenges for the future. *J. Exp. Bot.*, 64:1263–1280, 2013.

[8] Ciampi-Panno, L., Fernandez, C., Bustamante, P., Andrade, N., Ojeda, S.,Conteras, A. Biological control of bacterial wilt of potatoes caused by *Pseudomonas solanacearum*. *Am. Potato J.* 66:315–332, 1989.

[9] Genin, S. 2010. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacaerum*. *New Phytologist,*187:920-8, 2010.

[10] Hayward, A. Biology and epidemiology of bacterial wilt caused by Pseudomonas solanacearum. *Annual review of phytopathology,* 29(1): 65-87, 1991.

[11] Elphinstone, J. The current bacterial wilt situation: A global overview, Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex, C. *Allen, P. Prior, and AC Hayward, eds. American Phytopathological Society, St. Paul, M,* (9-28), 2005.

[12] Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., and Prior, P. Ralstonia solanacearum strains from Martinique (French West Indies) exhibiting a new pathogenic potential. *Applied and environmental microbiology*, 73(21):6790-6801, 2007.

[13] Genin, S., Boucher, C. Lessons learned from the genome analysis of *Ralstonia solanacearum. Annual Review Phytopathology,* 42:107-34, 2004.

[14] Kumar, R. *Studying virulence functions of Ralstonia solanacearum, the Causal Agent of Bacterial Wilt in Plants*. PhD Thesis, Department of Molecular Biology and Biotechnology, Tezpur University, India, 2014.

[15] McLaughlin, R.J.,Sequeira, L.,Weingartner, D.P. Biocontrol of bacterial wilt of potato with an avirulent strain of *Pseudomonas solanacearum*: Interactions with root-knot nematodes. *Am. Potato J.,* 67:93–107, 1990

[16] Toyota, K., Kimura, M. Suppresision of *Ralstonia solanacearum* in soil following colonization by other strains of *R. solanacearum*. *Soil Sci. Plant Nutr.,* 46:449–459, 2000

[17] Viaene, T.,Langendries, S.,Beirinckx, S.,Maes, M.,Goormachtig, S. *Streptomyces* as a plant's best friend? *FEMS Microbiol. Ecol.,* 92, 2016.DOI:10.1093/femsec/fiw119.

[18] Dias, M.P., Bastos, M.S., Xavier, V.B., Cassel, E., Astarita, L.V.,Santarémm, E.R. Plant growth and resistance promoted by *Streptomyces* spp. in tomato. *Plant Physiol. Biochem.*, 118:479–493, 2017.

[19] Kumar, R., Barman, A., Jha, G., & Ray, S. K. (2013). Identification and establishment of genomic identity of *Ralstonia solanacearum* isolated from a wilted chilli plant at Tezpur, North East India. *Current Science*, 1571-1578.

[20] Kumar, R., Barman, A., Phukan, T., Kabyashree, K., Singh, N., Jha, G., . . . Ray, S. K. *Ralstonia solanacearum* virulence in tomato seedlings inoculated by leaf clipping. *Plant Pathology, 66*(5):835-841, 2017.

[21] Singh, N., Phukan, T., Sharma, P., Kabyashree, K., Barman, A., Kumar, R., . . . Ray, S. An innovative root inoculation method to study *Ralstonia solanacearum* pathogenicity in tomato seedlings. *Phytopathology, 108*(4):436-442, 2018. DOI: 10.1094/PHYTO-08-17-0291-R

[22] Phukan, T., Kabyashree, K., Singh, R., Sharma, P. L., Singh, N., Barman, A., . . . Ray, S. K. (2019). *Ralstonia solanacearum* virulence in eggplant seedlings by the leaf-clip inoculation. *Phytopathology Research, 1*(1):1-11, 2019.

[23] Singh, N., Kumar, R., & Ray, S. K. An innovative approach to study *Ralstonia solanacearum* pathogenicity in 6 to 7 days old tomato seedlings by root dip inoculation. *Bio-protocol, 8*(21), 2018.DOI: 10.21769/BioProtoc.3065

[24] Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.-J., Sessitsch, A. Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annu Rev Phytopathol,* 55:61-83, 2017.

[25] Fadiji, A.E., Babalola, O.O. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. *Front BioengBiotechnol*, 8:467, 2020.

[26] Kloepper, J.W., McInroy, J.A., Liu, K., Hu, C-H. Symptoms of fern distortion syndrome resulting from inoculation with opportunistic endophytic fluorescent *Pseudomonas* spp. *PLOS ONE*, 8:e5853,2013.

[27] Gaiero, J.R., McCall, C.A., Thompson, K.A., Day, N.J., Best, A.S., Dunfield, K.E. Inside the root microbiome: Bacterial root endophytes and plant growth promotion. *Am. J. Bot.*, 100:1738–1750, 2013. DOI: 10.3732/ajb.1200572

[28] Lebeis, S.L. The potential for give and take in plant-microbiome relationships. *Front. Plant Sci.*, 5:287, 2014. DOI: 10.3389/fpls.2014.00287.

[29] Bastian, F., Cohen, A., Piccoli, P., Luna, V., Baraldi, R., Bottini, R. Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillumseropedicae* in chemically-defined culture media. *Plant Growth Regul.*, 24:7–11,1998. DOI: 10.1023/A:1005964031159.

[30] Hayward, A.C. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Ann. Rev. Phytopathol.*,29, 65–87, 1991.

[31] Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., ... & Foster, G. D. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular plant pathology*, *13*(6), 614-629, 2012.

[32] Wenneker, M., Verdel, M., Groeneveld, R., *et al. Ralstonia (Pseudomonas) solanacearum* Race 3 (Biovar 2) in Surface Water and Natural Weed Hosts: First Report on Stinging Nettle (Urtica dioica). *European Journal of Plant Pathology*, 105:307–315,1999. https://doi.org/10.1023/A:1008795417575.

[33] Agarwal, H., Dowarah, B., Baruah, P. M., Bordoloi, K. S., Krishnatreya, D. B., &Agarwala, N. Endophytes from Gnetumgnemon L. can protect seedlings against the infection of phytopathogenic bacterium *Ralstonia solanacearum* as well as promote plant growth in tomato. *Microbiologicalresearch*, *238*, 126503, 2020.

[34] Ling, L., Han, X., Li, X., Zhang, X., Wang, H., Zhang, L., Cao, P., Wu, Y., Wang, X., Zhao, J., Xiang, W. A *Streptomyces* sp. NEAU-HV9: Isolation, Identification, and Potential as a Biocontrol Agent against *Ralstonia Solanacearum* of Tomato Plants. *Microorganisms*,8(3):351, 2020.

[35] Dowarah, B., Agarwal, H., Krishnatreya, D. B., Sharma, P. L., Kalita, N., &Agarwala, N. Evaluation of seed associated endophytic bacteria from tolerant

chilli cv. Firingi Jolokia for their biocontrol potential against bacterial wilt disease. *Microbiological Research*, *248*, 126751, 2021.

[36] Mercado-Blanco, J., Rodríguez-Jurado, D., Hervás, A., Jiménez-Diaz, R.M. Suppression of Verticillium wilt in olive planting stocks by root-associated fluorescent *Pseudomonas* spp. Biol. Control 30, 474–486, 2004.

[37] Schilirò, E., Ferrara, M., Nigro, F., Mercado-Blanco, J. Genetic responses induced in olive roots upon colonization by the biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7. *PLoS One* 7, e48646, 2012.

[38] Cabanás, C.G.L., Schilirò, E., Valverde-Corredor, A., Mercado-Blanco, J. The biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7 induces systemic defense responses in aerial tissues upon colonization of olive roots. *Front. Microbiol.* 5, 427, 2014.

[39] Tjamos, E.C., Tsitsigiannis, D.I., Tjamos, S.E., Antoniou, P.P., Katinakis, P. Selection and screening of endorhizosphere bacteria from solarized soils as biocontrol agents against *Verticillium dahliae* of solanaceous hosts. *Eur. J. Plant Pathol*. 110, 35–44, 2004.

[40] Tjamos, S.E., Flemetakis, E., Paplomatas, E.J., Katinakis, P. Induction of resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the biocontrol agent K-165 and athogenesis-related proteins gene expression. *Mol. Plant Microbe Interact*. 18, 555–561, 2005.

[41] Ting, A.S.Y., Meon, S., Kadir, J., Radu, S., Singh, G. Endophytic microorganisms as potential growth- promoters of banana. *Biocontrol* 53, 541–553, 2008.

[42] Wang, X., Liang, G. Control efficacy of an endophytic *Bacillus amyloliquefaciens* strain BZ6-1 against peanut bacterial Wilt *Ralstonia solanacearum*. *Biomed. Res. Int*. 2014, 465435, 2014.

[43] Yuan, S., L. Wang, K. Wu, J. Shi, M. Wang, X. Yang, Q. Shen, and B. Shen. Evaluation of *Bacillus*-fortified organic fertilizer for controlling tobacco bacterial wilt in greenhouse and field experiments. *Appl. Soil Ecol*. 75:86–94, 2014

[44] Wei, Z., J. Huang, S. Tan, X. Mei, Q. Shen, and Y. Xu. The congeneric strain *Ralstonia pickettii* QL-A6 of *Ralstonia solanacearum* as an effective biocontrol agent for bacterial wilt of tomato. *Biol. Control* 65:278–285, 2013.

[45] Singh, R., S.K. Soni, and A. Kalra. Synergy between *Glomus fasciculatum* and beneficial *Pseudomonas* in reducing root diseases and improving yield and forskolin content in *Coleus forskohlii* Brig. under organic field condition. *Mycorrhiza* 23:35–44, 2013.

[46] Liu, Y., J. Shi, Y. Feng, X. Yang, X. Li, and Q. Shen. Tobacco bacterial wilt can be biologically controlled by the application of antagonistic strains in combination with organic fertilizer. *Biol. Fertil. Soils* 49:447–464, 2013.

[47] Ding, C., Q. Shen, R. Zhang, and W. Chen. Evaluation of rhizosphere bacteria and derived bio-organic fertilizers as potential biocontrol agents against bacterial wilt (*Ralstonia solanacearum*) of potato. Plant Soil 366:453–466, 2013.

[48] Ramesh, R., and G.S. Phadke. 2012. Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by *Ralstonia solanacearum*. *Crop Prot.* 37:35–41.

[49] Wei, Z., X. Yang, S. Yin, Q. Shen, W. Ran, and Y. Xu. Efficacy of Bacillus-fortified organic fertilizer in controlling bacterial wilt of tomato in the field. *Appl. Soil. Ecol.* 48:152, 2011.

[50] Hu, H.Q., X.S. Li, and H. He. Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of capsicum bacterial wilt. *Biol. Control* 54:359–365, 2010.

[51] Xue, Q.-Y., Y. Chen, S.-M. Li, L.-F. Chen, G.-C. Ding, D.-W. Guo, and J.-H. Guo. Evaluation of the strains of Acinetobacter and Enterobacter as potential biocontrol agents against Ralstonia wilt of tomato. *Biol. Control* 48:252–258, 2009.

[52] Thanh, D.T., L.T.T. Tarn, N.T. Hanh, N.H. Tuyen, S.Y. Lee, and K.-S. Park. Biological control of soilborne diseases on tomato, potato and black pepper by

selected PGPR in the greenhouse and field in Vietnam. *Plant Pathol. J*. 25:263–269,2009.

[53] Taiwo, L.B., D.T. Adebayo, O.S. Adebayo, and J.A. Adediran. Compost and *Glomus mosseae* for management of bacterial and *Fusarium* wilts of tomato. *IJVS*, 13:49–6, 2007.