The utilization of agro-industrial waste for soil amendment and liquid biofertilizer mixed bacterial antagonist in eggplant production

S Boonraeng1,* and N Punyoyai1

1 Department of Agro-industry, Faculty of Agricultural Technology
Chiangmai Rajabhat University, Thailand

* E-mail: boonraengs@gmail.com

Abstract. This research aimed to improve soil fertility with soil amendment, prepared from agro-industrial waste, and application of antagonistic bacteria for controlling bacterial wilt disease. The experiments were tested on sandy-to-sandy loam soil with low organic matter and nutrients for planting eggplants. Besides the soil improvement with spent mushroom waste and mango peel mixed kernel, the results showed that soil pH, soil organic matter, nitrogen, phosphorus, and potassium increased as available levels. Also, two species of bacterial antagonist named Bacillus subtilis and B. amyloliquefaciens were cultured and mixed in liquid biofertilizer. It was spiked for soil preparation before planting and during crop growth every week, which was affected by eggplant survival from bacterial wilt by 100% and 97% in plot and field-tested, respectively. The results from testing on sandy loam with the use of liquid biofertilizer three times/week showed that Ralstonia solanacearum in soil suppressed to the reduction of 1,000-10,000 times. The yield increased by 25.9%. The optimum harvesting time was 12-15 days of fruit growth with the antioxidant activity. Finally, this study has excellent potential to be extended for farmers who organically grown.

1. Introduction

Eggplant is one of the most important vegetables for a food processing factory, and it is usually grown in the northern part of Thailand. Although the demand has increased. But the yield has not been attained due to the eggplant had infected by bacterial wilt disease. It had caused by Ralstonia solanacearum that was low yield and productivity. Besides of R. solanacearum is a soil-borne disease and survive from day to year. That is very hard to manage by the use of chemicals on the soil cannot control. Thus, the use of bacterial antagonists is being emerged method of interest. Due to disease suppression, the antagonists have some advantages like not harmful to humans, animals, and the environment. Among the useful bacterial strains in Bacillus subtilis and B. amyloliquefaciens had been reported as effective species for the biological control eggplant pathogen.

B. amyloliquefaciens showed significantly control bacterial wilt of eggplant in glasshouse and field studies by the reduced rate of 92.1% and 73.7%, respectively [1]. However, biological control methods have been not too easy for farmers to prepared and cultured. And the production of eggplant was relevant to many factors to manage with building soil fertility, irrigation, and agricultural practices. For example, applying agricultural wastes for soil amendment directly impacts plant health and crop productivity. The degradation of organic matter in the soil can directly affect pathogens' vitality and survival by restricting available nutrients and releasing natural chemical substances with
varying inhibitory properties. Also, enhanced soil microbial communities are indirectly involved in disease suppression.

Pre-treatment for growing eggplant is the best prerequisite for effective wilt prevention by the antagonistic bacteria. That was the importance of plant colonization ability of the antagonist bacteria for bacterial wilt control [2]. Therefore, this study had selected rhizosphere bacteria that have inhibition properties for \textit{R. solanacearum}. And the antagonist bacteria were cultured with mango residues and others as biofertilizer. It had applied for eggplant production under organically grown by using spent mushroom waste as a soil amendment in sandy-to-sandy loam soil.

2. Methods
2.1. Isolation of \textit{Ralstonia solanacearum}
Eggplants had collected from infectious bacterial wilt. For a quick field diagnostic, identification of \textit{R. solanacearum} can be used by the section method. The stems were cut from a wilting plant with a blade, and the samples were placed against the inside wall of a water-filled clear conical flask. The end of the section had touched the water surface. Milky white strands containing bacteria and extracellular polysaccharide will stream from the cut ends of the xylem. Then, the prospective samples had kept for further testing as follows. Stem segments of length 10 cm from wilted plants were rinsed with sterilized distilled water containing 1% Clorox and filled in a tube. The tubes were stirred on Vortex mixture for bacterial exudation were obtained. Then a loop full of turbid suspension was streaked on 2,3,5-triphenyltetrazolium chloride media (TTC) and incubated at 35-37 °C for 2 days, reddish fluidal colonies were again streaked on TTC plates and the process was repeated till purified bacterial cultures were obtained with the homogeneity of colony morphology with pinkish center. The colony was picked up and maintained with 20% glycerol at -40 °C in the freezer.

2.2. Isolation of rhizosphere bacteria
The rhizosphere soil samples were collected from 5 sources; bamboo, forest tree, lettuce, pepper and kale. Bacillus species were isolated by using trypticase soy agar (TSA) medium. Soil samples (10 g) were suspended in 90 mL of 0.85% normal saline (pH 7.0) and shaken well at 150 rpm for one h. An appropriate dilution of this suspension was spread on TSA plate. The cultures were incubated at 37 °C for 48 h. Every single colony was assayed for morphological and Gramm’s staining, endspore.

2.3. Invitro screening for antagonistic activity
\textit{Bacillus} isolates were tested for ability to inhibit \textit{R. solanacearum} by using the paper disc method, and each bacterial were cultured on Tryptic Soy Broth (TSB) at 35 °C for 24 h. for the cell concentration \(10^{12}\) CFU/mL \textit{R. solanacearum} was cultured with tetrazolium chloride broth (TTCB) at 35 °C for 24 h. and smeared on nutrient agar (NA). The paper with 5 mm. of diameter and sterilized. Bacillus's suspensions for 20 µL with 2 \(\times 10^8\) CFU were dropped on paper discs and placed on NA plates. On the other control plates consisted \textit{R. solanacearum} only. The culture plates were incubated at 35 °C for 48 h. The zone of inhibition around paper discs was observed and recorded.

2.4. Identification of \textit{Bacillus}
Two bacillus isolates showing antagonistic activity to \textit{R. solanacearum} were identified by 16S rRNA gene sequence analysis at the National Center for Genetic Engineering and Biotechnology (BIOTEC).

2.5. PCR amplification of 16S rDNA
DNA templates for PCR amplification were prepared by using “Genomic DNA mini kit (Blood/culture cell)” (Geneaid Biotech Ltd., Taiwan). DNA coding for 16S rRNA regions was amplified by means of PCR with Taq polymerase [3–5]. A PCR product for sequencing 16S rDNA regions was prepared by using the following two primers, 20F (5'-'GAG TTT GAT CCT GGC TCA G-3'), positions 9-27 on 16S rDNA by the \textit{E. coli} numbering system [6]) and 1500R (5'-'GTT ACC TTG TTA CGA CTT-3', position 1509-1492 on 16S rDNA by the \textit{E. coli} numbering system [6]). The PCR amplification was carried out with DNA Engine Dyad® Thermal Cycler (Bio-Rad Laboratories). One hundred µL of a reaction mixture contained 15-20 ng of template DNA, 2.0 µmoles each of the
two primers, 2.5 units of Taq polymerase, 2.0 mM MgCl₂, 0.2 mM dNTP and 10 μL of 10xTaq buffer, pH 8.8, containing (NH₄)₂SO₄, which was comprised of 750 mM Tris-HCl, 200 mM (NH₄)₂SO₄ and 0.1% Tween 20. The PCR amplification was programmed to carry out an initial denaturation step at 94 °C for 3 min, 25 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and elongation at 72 °C for 2 min, followed by a final amplification step at 72°C for 3 min. The PCR product was analyzed by 0.8% (w/v) agarose gel electrophoresis and purified with a QIAquick® PCR purification kit (QIAGEN GmbH, Hilden, Germany). The purified PCR product was stored at -20 °C for further step.

2.6. Direct sequencing of 16S rDNA
Direct sequencing of the single-banded and purified PCR products (ca. 1500 bases, on 16S rDNA by the E. coli numbering system; [6]) was carried out. Sequencing of the purified PCR products was carried out with an ABI PRISM® BigDye™ Terminator Ready Reaction Cycle Sequencing Kit (version 3.1, Applied Biosystems, Foster City, California, USA). The primers 27F (5’-AGA GTT TGA TCM TGG CTC AG-3’) and 518F (5’-CCA GCA GCC GCG GTA ATA CG-3’) for partial sequencing, and additional 1492R (5’-TAC GGY TAC CTT GTT ACG ACT T-3’) and 800R (5’-TAC CAG GGT ATC TAA TCC-3’) for full-length sequencing were used for sequencing of 16S rDNA. Ten μL of a sequencing reaction mixture contained 5–20 ng of template DNAs, 2.0 μL of BigDye™ terminator ready reaction mixture, 5–20 ng of DNA template, 1.6 pmole of the sequencing primer, 1.5 μL of 5xBigDyeTM sequencing buffer and deionized water. The PCR reactions were carried out as follows: an initial denaturation step at 96°C for 30 sec, 25 cycles of denaturation at 96 °C for 10 sec, annealing at 50 °C for 5 sec and elongation at 60 °C for 4 min. Eighty μL of freshly prepared ethanol/acetate solution was added to the sequencing reaction mixture in a 1.5 mL microcentrifuge tube and mixed well with a brief vortex. The mixture was left to stand at room temperature for 15 min and centrifuged at the maximum speed of 14,500 rpm for 20 min at room temperature. The ethanol solution was immediately removed carefully from the tube with aspirator equipped with a fine tip. The resulting DNA pellets were washed by adding 250 μL of 70% ethanol to the tube, and vortexed briefly. The precipitated DNA was collected by centrifugation for 5 min at the maximum speed. The remaining ethanol was carefully removed from the tube with an aspirator equipped with a fine tip. The DNA obtained was dried in a heat box at 90 °C for 1 min, and the dried DNA was stored at either 4 °C or -20 °C. The DNA pellets were suspended in 20 μL of a terminator sequencing reagent, mixed on a vortex and spun down. The double-stranded DNA was completely separated by heating at 95 °C for 2 min and immediately placed on ice until ready to load on the instrument. The DNA sequencing was performed on an ABI PRISM® 3730XL DNA Sequence (Applied Biosystems, Foster City, California, USA).

2.7. Sequence analyses
The nucleotide sequences obtained from all primers were assembled using the Cap contig assembly program, an accessory application in BioEdit (Biological sequence alignment editor) Program. The identification of phylogenetic neighbors was initially carried out by the BLAST [7] and mega BLAST [8] programs against the database of type strains with validly published prokaryotic names [8]. The 50 sequences with the highest scores were then selected to calculate pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon server (http://www.eztaxon.org/; [8].

2.8. Biocontrol efficacy in pot trials
This experiment was conducted completely randomized design (CRD) with 5 treatments and 5 replications. The 5 treatments were used of: R. solanacearum, R. solanacearum + B. amyloliquefaciens, R. solanacearum + B. subtilis, R. solanacearum + B. amyloliquefaciens + B. subtilis, B. amyloliquefaciens + B. subtilis and a control in which there was no application. Thirty days old of 45 plants were performed for each treatment and transferred to pots with 2 kg. sterilized mixture (sandy-loam soil:compost:spent mushroom media, 3:1:1), left for 7 days. The biocontrol bacterial suspension of 50 mL (10¹⁰ CFU/mL) was poured into the soil at each pot's base. After three days of inoculation, a
50 mL of \( R. \ solanacearum \) suspension (10\(^{10}\) CFU/mL) grown in TTCB medium was poured into the soil. The eggplants were irrigated two times/day and left for wilt development for up to four weeks. Wilt exhibit was recorded at weekly intervals, and the wilting percentage was also calculated, the height was measured.

2.9. Field evaluation of eggplant for wilt disease incidence
Field treatment was carried out for six months from February to July 2019. The plot size was 54 × 20 m on sandy-loam soil.

2.10. Liquid biofertilizer production
The liquid biofertilizer was produced from fruit-based waste material obtained by Princess Food Co., Ltd. and using a commercial method practised by [9]. Three independent batches of liquid biofertilizer were produced for 200 L and mixed by two bacterial antagonists. Liquid biofertilizer was packed in 10 L plastic (PP) bottles. The samples were then delivered for dilution 100 times with water and poured into the eggplant every week.

2.11. Soil preparation
The spent mushroom media treated soil amendments, mango peel mixed kernel and broiler litters for 2 kg/m\(^2\). The microbial activator PD-1 obtained by the Land Development Department of Thailand was dissolved and sprayed on soil. Then, the soil was plowed and left for 30 days. The soil was turned over for 25 plots, each measuring 1.2 × 20 m and were laid out, with 1.0 m alley between 2 plots. Soil samples were collected for physicochemical property analysis by comparing before and after preparation.

2.12. Planting of eggplant
The experiment was conducted in a CRD, with five treatments and five replications. The treatments were the same pot experiments. Transplanting of eggplant seedlings was done four weeks after seed germination. On each plot there were two columns with 20 plants in each column. Within each column, the planting interval was 80 cm. The interval between 2 columns was 1 m. Two weeks after transplanting, application of compost was done for 100 g/plant around the stem. Sampling and data collection were carried out at weekly intervals for wilt infection, and heights were measured.

2.13. Scaling up of eggplant production
Eggplant production at farmers’ scale was conducted in three fields and each using area of 0.25 hectares. The efficacy of liquid biofertilizer on eggplant yields, quality and harvesting index, the experiments were prepared soil as the same methods at previous studies. The treatments were compared the frequencies between 1-3 times/week of application for liquid biofertilizer.

3. Results and discussion
3.1. Screening of Bacillus antagonistic potential
The wilt disease was infected on eggplant, and it could exhibit symptom in the host for 5-10 days. It was isolated and purified for antagonistic testing of Bacillus. A total of 34 Bacillus isolates were exhibited antagonistic activity for seven isolates. All of them were two isolates that high activity, as shown in Table 1. The in-vitro test showed that the lettuce soil’s isolate exhibited the highest clear zone and included the isolate from kale soil. Two strains were identified through the 16S rRNA gene sequencing followed by ABI Prism® 3730XL DNA sequence. They were \( B. \ amyloliquefaciens \) and \( B. \ subtilis \). Both strains showed 99-100% similarity with the matching isolates at the BLAST and mega BLAST database. The \( B. \ amyloliquefaciens \) was showed the highest inhibitory zone for 1.8 cm. \( B. \ amyloliquefaciens \) is the most potential microorganism that used for biocontrol of plant. It was secreted various peptides, which have shown distinct capacities to inhibit plant pathogen [1]. However, \( B. \ amyloliquefaciens \) DSBA-11 was the best among other species of \( B. \ amyloliquefaciens \) and indicated maximum inhibited the growth of \( R. \ solanacearum \) 4.91 cm [9].
The comparative bio-efficacy to control of wilt as well as growth promoting for eggplant in pot trials which was designed 5 treatments. The results (Table 2) showed that on the 1st week after inoculation of the pathogen, eggplants were infected by R. solanacearum and disease incidence of 17.78%.

### Table 2. Biocontrol efficacy in pot trials of eggplant

| Treatments               | Height (cm.) (1 day) | Height (cm.) (30 days) | Disease incidence (%) of eggplant 1 week | Disease incidence (%) of eggplant 4 weeks | R. solanacearum (CFU/g of soil) |
|--------------------------|----------------------|------------------------|------------------------------------------|------------------------------------------|----------------------------------|
| Control                  | 16.83 ± 0.35^a       | 41.03 ± 0.55^a         | 4.45 ± 3.8^a                             | 4.45 ± 3.8^a                             | 7.0 × 10^7 ± 1.41^c              |
| R. solanacearum          | 16.90 ± 0.82^b       | 21.03 ± 0.23^bc        | 17.78 ± 3.8^bc                           | 80.0 ± 1.7^d                             | 1.91 × 10^7 ± 2.12^b             |
| R. solanacearum + BL     | 17.13 ± 0.25^c       | 52.03 ± 0.68^cd        | 0.0 ± 0.0^a                             | 0.0 ± 0.0^a                              | 6.7 × 10^6 ± 4.95^b              |
| R. solanacearum + BS     | 16.87 ± 0.31^c       | 56.27 ± 0.27^cd        | 0.0 ± 0.0^a                             | 0.0 ± 0.0^a                              | 9.6 × 10^4 ± 3.54^b              |
| R. solanacearum + BL+BS  | 16.77 ± 0.51^d       | 55.83 ± 0.40^cd        | 0.0 ± 0.0^a                             | 0.0 ± 0.0^a                              | 4.4 × 10^4 ± 2.83^b              |

Note: BL = Bacillus amyloliquefaciens, BS = Bacillus subtilis

Means (a-d) with a column followed by different letter are significantly different at 95% confidence.

Also, the R. solanacearum treatment has contained the soil’s disease at the highest concentration in 1.9 × 10^7 CFU/g. The treatment with treated by two strains of Bacillus antagonists and wilt pathogen remained survival for 100% of four weeks. The wilt disease in soil was decreased by B. amyloliquefaciens and B. subtilis nearly 1,000 times. After 30 days of disease inoculation, the maximum height of eggplants for three treatments was not significant; R. solanacearum + BS and R. solanacearum + BL+BS. Bacillus spp. showed plant growth promotion expression attributes such as siderophores and IAA 10]. That was indicated the Bacillus spp. which rhizobacterial properties of their antagonistic activity and plant growth promotion.

### 3.3. Field evaluation of eggplant for wilt disease incidence

According to eggplant inoculated with R. solanacearum showed wilt symptoms after seven days of inoculation. And the results from pot trials that indicated the two strains of Bacillus were suppressed pathogen and no wilt incidence for 4 weeks. Field trials were conducted the same treatments, but soil was improved fertility due to sandy – loam soil and low organic matter. The use of spending mushroom media, mango peel mixed kernel, broiler litters for 2 kg/m² and included the microbial activator PD-1. The soil properties were better than before treating (Table 3), and especially organic matter, micronutrients were also at optimum levels.

### Table 3. Soil properties for field evaluation of eggplant for wilt disease incidence

| Soil Parameters          | Before | After | Optimum* |
|--------------------------|--------|-------|----------|
| pH                       | 5.3    | 6.9   | 6.0-7.0  |
| Bulk density (g/cm³)     | 1.72   | 1.45  | -        |
| Organic matter (%)       | 0.12   | 2.9   | 2.5-3.0  |
| Total Nitrogen (mg/kg)   | Trace  | 74    | 50-80    |
| Phosphorus (mg/kg)       | 8      | 409   | 26-42    |
The field evaluation of wilt incidence showed that at after 4 weeks of disease inoculation was 67.0%, whereas the treatments of Bacillus antagonists were exhibited below 5%. The results of *R. solanacearum* in rhizosphere soil and soil were indicated that suppression for 10,000-1,000 times, respectively. The factor was associated with wilt controlling that was soil preparation by using organic materials and microorganism. The saprophytic microorganism that was obtained from spent mushroom media. This method was the same Bokashi technique, were introduced as the best performer in the suppression of *R. solanacearum* [11]. The application of *Bacillus amyloliquefaciens* strain S20 alone could control eggplant wilt with an efficacy of 25.3% during a 40 days experiment. If strain S20 was used with organic fertilizer, the control efficacy against eggplant wilt reached as high as 70.7% [12].

The production results at farmers’ scale showed that the soil for planting contained *R. solanacearum* 2.5 × 10³ CFU/g. So, the method for soil preparation was the same method for field evaluation. Then the use of two strains of Bacillus antagonists mixed with liquid biofertilizer for the ratio of 1:100. The experiments were conducted with four treatments and five replications in 0.25 hectares. They were a controlled treatment (no use of pathogen and bacterial antagonists), liquid biofertilizer and antagonist bacteria for 1, 2 and 3 times/week. The results shown in Table 5 indicated that disease incidence of control treatment for 13.84%, whereas other treatments bacterial wilts were reduced with the survival rate to 97%. The height of eggplants with the Bacillus sp. was better for high yield by an average of 0.74 kg/plant/week that yield was increased about 25%. However, the frequencies of liquid biofertilizer mixing Bacillus antagonists were not significant for the height and growth rate of fruit.

**Table 4. Field evaluation of eggplant for wilt disease incidence**

| Treatments                      | Height (cm.) | Disease incidence (%) at 4 weeks | Rhizosphere soil (CFU/g) | Soil (CFU/g) |
|---------------------------------|--------------|---------------------------------|--------------------------|--------------|
| Control                         |              |                                 |                          |              |
| *R. solanacearum*               |              |                                 |                          |              |
| *R. solanacearum*+BL            |              |                                 |                          |              |
| *R. solanacearum*+BS            |              |                                 |                          |              |
| *R. solanacearum*+BL+BS         |              |                                 |                          |              |
|                                 | 15.73 ± 1.54 | 80.25 ± 1.84b                   | 15.0 ± 2.8b              | 4.0 × 10^2×4.84b |
|                                 | 16.12 ± 1.37 | 58.54 ± 1.39b                   | 67.0 ± 4.2c              | 3.9 × 10^2×6.38a |
|                                 | 15.89 ± 1.61 | 125.62 ± 0.61ab                 | 3.3 ± 1.1a               | 2.9 × 10^2×3.00b |
|                                 | 15.65 ± 1.72 | 130.35 ± 0.45ab                 | 3.2 ± 1.2a               | 6.6 × 10^2×0.66b |
|                                 | 16.05 ± 1.58 | 128.80 ± 2.70a                  | 3.0 ± 1.3a               | 3.4 × 10^2×2.40b |

Means (a-c) with a column followed by different letter are significantly different at 95% confidence

**Table 5. Scaling production up of farmers’ field**

| Treatments         | Height (cm.) | Disease incidence (%) at 4 weeks | Growth rate of fruit (cm/day) | Yields (Kg/plant /week) |
|--------------------|--------------|---------------------------------|------------------------------|-------------------------|
| Control            |              |                                 |                              |                         |
| LF 1 time/week     | 28.00 ± 7.54 | 121.17 ± 14.57                  | 13.84 ± 2.33b               | 2.84 ± 0.23b            |
| LF 2 times/week    | 32.67 ± 4.93 | 132.50 ± 17.18                  | 2.11 ± 0.64a                | 0.69 ± 0.15             |
| LF 3 times/week    | 33.17 ± 6.91 | 135.00 ± 13.16                  | 2.42 ± 1.02a                | 0.73 ± 0.16             |
| LF 4 times/week    | 29.33 ± 5.43 | 133.84 ± 19.02                  | 2.32 ± 0.95a                | 0.71 ± 0.15             |

ns = not significant at 95% confidence
Means (a-b) with a column followed by different letter are significantly different at 95% confidence

So, farmers could use the antagonists at a minimum 1 time/week due to colonization, formation of biofilm by Bacillus strains on root surface may provide an additional advantage for the prevention of wilt pathogen [2]. The eggplant production under field condition for harvesting time can be

Reference from the manual of eggplant production, Princess Food Co., Ltd., Chiang Mai, Thailand
investigated of anthocyanin, antioxidant capacity and total phenolic acid content. The optimum was 12-15 days of fruit growth for highly bioactive ingredients.

4. Conclusion
Eggplant production was succeeded by the use of agro-industrial wastes for soil amendments and soil fertility improvement. And the application Bacillus antagonists that indicated wilt disease suppression by the reduced rate 1,000-10,000 time and wilt incidence was exhibited 3%. The most effective biocontrol technique was inoculated Bacillus antagonists before planting for seven days, and it was used as liquid biofertilizer for eggplant every week. So, this method was suited for organically grown eggplant.

References
[1] Sakthivel K, Manigundan K, Gautam R K, Singh P K, Nakkeeran S and Sharma S K 2019 Bacillus spp. for suppression of eggplant bacterial wilt pathogen in Andaman Islands: Isolation and characterization Indian J. Exp. Biol. 57 131–7
[2] Achari G A and Ramesh R 2019 Colonization of eggplant by endophytic bacteria antagonistic to Ralstonia solanacearum, the bacterial wilt pathogen Proceedings of the National Academy of Sciences, India Section B: Biological Sciences vol 89 pp 585–93
[3] Kawasaki H, Hoshino Y, Hirata A and Yamasato K 1993 Is intracytoplasmic membrane structure a generic criterion? It does not coincide with phylogenetic interrelationships among phototrophic purple nonsulfur bacteria Arch. Microbiol. 160 358–62
[4] Yamada Y, Katsura K, Kawasaki H, Widyastuti Y, Saono S, Seki T, Uchimura T and Komagata K 2000 Asaia bogorensis gen. nov., sp. nov., an unusual acetic acid bacterium in the α-Proteobacteria Int. J. Syst. Evol. Microbiol. 50 823–9
[5] Katsura K, Kawasaki H, Potacharoen W, Saono S, Seki T, Yamada Y, Uchimura T and Komagata K 2001 Asaia siamensis sp. nov., an acetic acid bacterium in the α-Proteobacteria Int. J. Syst. Evol. Microbiol. 51 559–65
[6] Brosius J, Dull T J, Sleeter D D and Noller H F 1981 Gene organization and primary structure of a ribosomal RNA operon from Escherichia coli J. Mol. Biol. 148 107–27
[7] Altschul S F, Madden T L, Schaffer A A, Zhang J, Zhang Z, Miller W and Lipman D J 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs Nucleic Acids Res. 25 3389–402
[8] Chun J, Lee J H, Jung Y, Kim M, Kim S, Kim B K and Lim Y W 2007 EzTaxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences Int. J. Syst. Evol. Microbiol. 57 2259–61
[9] Boonraeng S, 2016 Quality and Food Safety of Minimally Processed Organic Vegetables. Faculty of Agricultural Technology Chiang Mai Rajabhat University 325 pp.
[10] Singh D, Yadav K D, Chaudhary G, Rana V S and Sharma R K 2016 Potential of Bacillus amyloliquefaciens for biocontrol of bacterial wilt of tomato incited by Ralstonia solanacearum J. Plant Pathol. Microbiol. 7 1–6
[11] Lwin M and Ranamukhaarachchi S L 2006 Development of biological control of Ralstonia solanacearum through antagonistic microbial populations Annu. Rev. Earth Planet. Sci. 8 657–60
[12] Chen D, Liu X, Li C, Tian W, Shen Q and Shen B 2014 Isolation of Bacillus amyloliquefaciens S20 and its application in control of eggplant bacterial wilt J. Environ. Manage. 137 120–7