Biochemical Characterizations of Selected Indigenous Endophytic Bacteria Potential as Growth Promoters and Biocontrol Agents on Tomato

Y Yanti¹, H Hamid¹, Reflin¹, Yaherwanidi¹

¹Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Padang, West Sumatra, Indonesia 25163

Corresponding author’s email address: mira23@agr.unand.ac.id; yy.anthie79@gmail.com

Abstract. Nine indigenous endophytic bacteria strains showing the best ability to promote growth and control tomato pathogens had been screened in our previous research. The strains' biochemical properties, such as nutrition and other traits, must be characterized to design the best formulations for the strains' biochemical properties. This study aimed to describe the biochemical characteristics of the selected indigenous endophytic bacteria. The variables observed were utilization of carbon sources (glucose, fructose, sucrose, lactose, glycerol, and olive oil), nitrogen sources (peptone, yeast extract, urea, NH₄Cl, NH₄SO₄, and NH₄NO₃), and citrate, hydrolytic activities (urease, triple sugar iron, starch hydrolysis, gelatin hydrolysis, chitinase, cellulase, protease, lipase, and catalase), oxidative/fermentative assay, salt tolerance, and growth ability at 4°C and 44°C. This study showed that all the endophytic bacteria strains characterized had various biochemical characteristics. All strains showed the different ability to utilize nitrogen and carbon source. Some strains survived to grow at 4°C except Bacillus cereus AGBE 1.2 TL. All strains tolerate growth in 4% NaCl concentrations, while some strains can tolerate up to 6%. This result can be used for further studies to develop the most suitable formulations for each strain to get the best results of the growth-promoting and biocontrol activities of the indigenous endophytic bacteria strains.

Keywords: Biochemical characterization, endophytic bacteria, nutrient source

1. Introduction

The chemicals used over a long period to control bacterial diseases transmitted through the soil can result in severe environmental contamination [1]. Research on cleaner and safer technological alternatives to humans and the environment has received widespread attention recently. Research conducted shows that microorganism's use does not endanger the environment; therefore, it is safe for food and human health [2].

Naturally, plants can be associated with microorganisms in various ways. Endophytes are microorganisms inside the internal tissue of living plants [3]. According to the widely used definition, endophytic bacteria colonize the host tissue internally, sometimes in high numbers, without damaging the host or raising plant disease symptoms [4]. Some endophytic bacterial species have beneficial effects on plant growth and development by producing growth-promoting substances and/or by fixing nitrogen from the atmosphere and defense against pathogens through disease suppression with regulators encouraging plant growth and antibiotic production [5, 6]. Endophytic bacteria can benefit the host plant
generally by producing phytohormone, solubilizer phosphate, compounds such as flavonoids and antibiotics, or competence of invasion sites that suppress phytopathogens [7].

They are considering the benefits of intensive agriculture in the present time. The negative impact of chemical fertilizers and pesticides against the environment and usage of Plant Growth-Promoting Rhizobacteria (PGPR) as biofertilizers is one of the most promising biotechnologies used to increase the primary production, eliminating the need for chemical fertilizers [8]. Commercial application of PGPR to control soil-borne diseases depends on the development of commercial formulations in which bacteria can survive for a considerable length of time, the development of a suitable method of application to control pathogen establishment and disease development, and assessment of their efficacy under field conditions [9].

Our previous study had screened nine endophytic bacterial isolates showing the best performance in plant growth-promoting activities [10]. Those isolates also had the potential as biocontrol agents of Ralstonia and Fusarium wilt. Those isolates had been identified as Bacillus cereus AGBE 1.2 TL, B. toyonensis AGB E 2.1 TL, B. cereus SLB E 1.1 SN, B. cereus SLB E 1.1 AP, B. pseudomycoides SLB E 3.1 AP, B. cereus SLB E 1.1 BB, B. cereus SLB E 2.1 BB, B. thuringiensis SLB E 2.3 BB and B. cereus SLB E 3.1 BB.

To develop a successful formulation for the endophytic bacteria strains, the study of their biochemical characteristics and nutrient source utilization is necessary to increase the formula's efficacy. This study aimed to characterize the selected indigenous endophytic bacteria's biochemical characteristics for further studies to develop the most suitable formulations for each strain and get the best results of the indigenous's growth-promoting and biocontrol activities endophytic strains.

2. Materials and Methods

2.1. Biochemical Characterization

The biochemical tests consisting of several enzymatic and physiological activities, or differential growth tests conducted using standard methods [11, 12]. Citrate utilization was determined on Simmons citrate agar, while the Oxidative/Fermentative (OF) assay was performed using OF media. Urease activity assay conducted using urea broth medium and phenol red as an indicator. Starch Hydrolysis Activity was tested using starch agar, and Gelatin Hydrolysis Activity was tested using gelatin agar. Meanwhile, Catalase activity was tested by dropping 3% H2O2 to the colony.

Chitinase activity was tested on the agar plates by the method described by Chernin et al. [13]. The semi-minimal medium composed of synthetic media and nutrient broth (3:1) was supplemented with 0.2% colloidal chitin and solidified with 1.5% agar for 48 h. The cellulase activity was tested on M9 medium [14] supplemented with yeast extract (0.12% w/v) and carboxyl-methylcellulose (CMC) (1% w/v) [15]. Isolates surrounded by a clear halo after eight days of incubation at 28°C were considered positive for cellulase production.

Protease activity was detected in 3% (wt/vol) powdered milk-agar plates, according to Walsh et al. [16]. Lipases were tested on basal medium (bacto peptone 10 g l⁻¹, NaCl 5 g l⁻¹, CaCl2·H2O 0.1 g l⁻¹, and agar 9 g l⁻¹) supplemented with Tween 80 (1% w/v) [17]. After seven days of incubation at 26°C, opaque zones formed around colonies were considered lipolytic activity.

2.2. Salt Tolerance Assay

Growth tolerance under saline condition/salt (NaCl) tolerance was tested by growing the strains on Nutrient Agar added with NaCl (final concentrations 2%, 4%, 6%, 8%, 10%) and incubated for five days [18].

2.3. Carbon Source Utilization

Carbon source utilization was tested by the growth of the strains on bacto agar added with each carbon source (1%w/w of glucose, fructose, sucrose, lactose, glycerol, and olive oil) incubated for 48 h.
2.4. Nitrogen Source Utilization
Nitrogen source utilization was tested by the growth of the strains on bacto agar added with each carbon source (1%v/w of peptone, yeast extract, urea, NH₄Cl, NH₄SO₄, and NH₃NO₃) incubated for 48 h.

3. Results and Discussion

3.1. Result
Based on the Simmon citrate agar assay, six strains showed positive results (Table 1). All the isolates showed the ability to grow under aerobic (oxidative) and anaerobic (fermentative) conditions. Biochemical characterization indicated that the endophytic bacteria showed different characteristics (Table 1). Protease and catalase enzyme productions were detected in all strains. All the isolates had no urea degradation ability, indicated by urease production and ammonium ions’ release into the medium. Five strains (B. cereus AGB 1.2 TL, B. toyonensis AGB E 2.1 TL, B. cereus SLB E 1.1 SN, B. cereus SLB E 1.1 AP, and B. pseudomycoides SLB E 3.1 AP) could hydrolyze starch. Three strains (B. cereus AGBE 1.2 TL, B. cereus SLB E 1.1 SN, and B. cereus SLB E 1.1 BB) had gelatin hydrolysis ability. Three strains (B. cereus AGBE 1.2 TL B. cereus SLB E 1.1 AP, B. pseudomycoides SLB E 3.1 AP, and B. cereus SLB E 3.1 BB) had cellulase activity. Meanwhile, only B. toyonensis AGB E 2.1 and B. pseudomycoides SLB E 3.1 AP showing no activity on chitinase hydrolyzing.

The salt tolerance of the endophytic bacteria strains was determined by NA supplemented culture growth with 2–10% (w/v) NaCl. The results showed that all the endophytic bacteria could grow on NaCl plus 2–4% (w/v) NaCl, while some strains could tolerate and grow on NaCl medium plus 6% (w/v) NaCl. The best strains with the highest NaCl tolerance (6%) were Bacillus cereus AGBE 1.2 TL, Bacillus toyonensis AGB E 2.1 TL, Bacillus cereus SLB E 1.1 BB, Bacillus thuringiensis SLB E 2.3 BB, and Bacillus cereus SLB E 3.1 BB (Table 2).

The carbon and nitrogen source utilization assay (Table 3 and 4) was performed to determine the strains' growth only with sole nutrition source. The information about nutrition source utilization of the endophytic bacteria strains is necessary for developing the strain formulations. The formulations will act as the nutrition source of the strains before used for commercial biocontrol agents. All the strains were shown to have various patterns of both carbon and nitrogen source utilization. Still, all the isolates grew on the sole carbon source of glucose and fructose and a single nitrogen source of peptone and yeast extract. However, not all strains could use a mineral source of nitrogen from urea and ammonia salt.

Table 1. Citrate Utilization, Triple Sugar Iron, Oxidative/Fermentative and Hydrolytic Activities of the Endophytic Bacteria Strains

| Isolates             | Citrate utilization | Oxidative/Fermentative | Hydrolytic Activities | Urease | Starch Hydrolysis | Gelatin Hydrolysis | Chitinase | Cellulase | Protease | Lipase | Catalase |
|----------------------|---------------------|------------------------|-----------------------|--------|-------------------|--------------------|------------|-----------|----------|--------|----------|
| Bacillus cereus      |                     |                        |                       |        |                   |                    |            |           |          |        |          |
| AGB 1.2 TL           | +                   | OF                     | -                     | +      | +                 | +                  | +          | +         | +        | +      | +        |
| Bacillus toyonensis  |                     |                        |                       |        |                   |                    |            |           |          |        |          |
| AGB E 2.1 TL         | +                   | OF                     | -                     | +      | +                 | -                  | +          | -         | +        | +      | +        |
| Bacillus cereus      |                     |                        |                       |        |                   |                    |            |           |          |        |          |
| SLB E 1.1 SN         | +                   | OF                     | -                     | +      | -                 | +                  | +          | +         | -        | +      | -        |
| Bacillus cereus      |                     |                        |                       |        |                   |                    |            |           |          |        |          |
| SLB E 1.1 AP         | +                   | OF                     | -                     | +      | -                 | +                  | +          | +         | -        | +      | -        |
| Bacillus pseudomycoides|                |                        |                       |        |                   |                    |            |           |          |        |          |
| SLB E 3.1 AP         | +                   | OF                     | -                     | +      | -                 | +                  | +          | +         | -        | +      | -        |
| Bacillus cereus      |                     |                        |                       |        |                   |                    |            |           |          |        |          |
| SLB E 1.1 BB         | -                   | OF                     | -                     | -      | -                 | +                  | -          | +         | -        | +      | -        |
| Bacillus thuringiensis|                   |                        |                       |        |                   |                    |            |           |          |        |          |
| SLB E 2.3 BB         | +                   | OF                     | -                     | -      | -                 | +                  | -          | +         | +        | +      | -        |
| Isolates                      | Salt Tolerance |
|------------------------------|----------------|
|                              | 2%  | 4%  | 6%  | 8%  | 10% |
| Bacillus cereus AGBE 1.2 TL  | +   | +   | +   | -   |    |
| Bacillus toyonensis AGB E 2.1 TL | +   | +   | +   | -   |    |
| Bacillus cereus SLB E 1.1 SN | +   | +   | -   | -   |    |
| Bacillus cereus SLB E 1.1 AP | +   | +   | -   | -   |    |
| Bacillus pseudomycoides SLB E 3.1 AP | +   | +   | -   | -   |    |
| Bacillus cereus SLB E 1.1 BB | +   | +   | +   | -   |    |
| Bacillus cereus SLB E 2.1 BB | +   | +   | -   | -   |    |
| Bacillus thuringiensis SLB E 2.3 BB | +   | +   | -   | -   |    |
| Bacillus cereus SLB E 3.1 BB | +   | +   | +   | -   |    |

Table 3. Carbon source utilization of the endophytic bacteria strains

| Isolates                      | Glucose | Fructose | Sucrose | Lactose | Glycerol | Olive oil |
|------------------------------|---------|----------|---------|---------|----------|-----------|
| Bacillus cereus AGBE 1.2 TL  | +       | +        | +       | +       | +        | +         |
| Bacillus toyonensis AGB E 2.1 TL | +       | +        | +        | -        | +        | +         |
| Bacillus cereus SLB E 1.1 SN | +       | +        | +        | -        | +        | -         |
| Bacillus cereus SLB E 1.1 AP | +       | +        | +        | +        | +        | -         |
| Bacillus thuringiensis SLB E 2.3 BB | +       | +        | -        | +        | -        | +         |
| Bacillus cereus SLB E 3.1 BB | +       | +        | +        | +        | +        | -         |

Table 4. Nitrogen source utilization of the endophytic bacteria strains

| Isolates                      | Peptone | Yeast extract | Urea | NH4Cl | NH4SO4 | NH4NO3 |
|------------------------------|---------|---------------|------|-------|--------|--------|
| Bacillus cereus AGBE 1.2 TL  | +       | +             | -    | +     | +      | +      |
| Bacillus toyonensis AGB E 2.1 TL | +       | +             | -    | -     | -      | -      |
| Bacillus cereus SLB E 1.1 SN | +       | +             | -    | -     | -      | -      |
| Bacillus cereus SLB E 1.1 AP | +       | +             | -    | -     | -      | +      |
| Bacillus pseudomycoides SLB E 3.1 AP | +       | +             | -    | +     | -      | -      |
| Bacillus cereus SLB E 1.1 BB | +       | +             | -    | +     | +      | -      |
| Bacillus cereus SLB E 2.1 BB | +       | +             | -    | +     | +      | -      |
| Bacillus thuringiensis SLB E 2.3 BB | +       | +             | -    | +     | +      | -      |
| Bacillus cereus SLB E 3.1 BB | +       | +             | -    | -     | -      | -      |
3.2. Discussion
In the last decade, most agricultural research has been aimed at studying rhizosphere microorganisms [19]. Among these organisms, bacteria are the most technologically advanced in terms of both production and the use of microbial preparations in agriculture. It opens up the prospects for beneficial prokaryotic microorganisms for plant development under different conditions [20].

Endophytic bacteria are considered one of the options to control vascular wilt disease because of their ability to live and colonize within plants' roots. Our previous research had screened nine selected indigenous endophytic bacteria isolates that can promote growth rate [10]. Further research also confirmed that those strains suppressed R. syzigii subsp. indonesiensis, without developing any symptoms, five isolates could suppress symptoms of Foc [21]. The current study was conducted to characterize the biochemical characteristics of the selected indigenous endophytic bacteria. This present study can be used to develop the strains' ability for its new bio formulations development.

PGPR produces hydrolytic enzymes (chitinase, glucanase, protease, and cellulase). These enzymes have responsible for phytopathogens' lysis process through hyper-parasitism. The antagonistic characteristic of hydrolytic enzymes against various phytopathogens plays an essential role in biocontrol [22, 23]. Our current study found that all the strains showed various hydrolytic activity (Table 1). The hydrolytic action may directly impact the endophytic bacteria strain's ability to control pathogens [21].

Chitinase [EC 3.2.1.14] plays an essential role in the biocontrol of many plant diseases by lysing phytopathogenic fungal cell walls through the degradation of chitin polymers in cell walls [24]. Protease [E.C. 3.4.24] plays a vital role in phytopathogenic fungal cell wall lysis because chitin and/or β-glucan fibrils are embedded in the protein matrix, and proteolytic activity is a prerequisite for all fungal cells lysis [25]. Protease enzymes break down major proteins into chains of peptides and/or amino acids, making up phytopathogens and causing phytopathogenic ability to act on plant cells to be lost [24]. Bacillus sp. produce extracellular proteases. Several species of Bacillus such as Bacillus cereus, Bacillus stearothermophilius, Bacillus mojavensis, Bacillus megaterium, and Bacillus subtilis are known to produce proteases [26–29]. Cellulase [EC 3.2.1.4] catalyzes the hydrolysis of 1,4-β-D-glycosidic linkages in cellulose and plays an essential role in nature by recycling polysaccharides. Cellulase is a linear polymer unit of β-D-glucose linked through a 1,4-β relationship with polymerization rates ranging from 2,000 to 25,000 [30]. Cellulase causes cell wall degradation and further penetration into the host mycelium [31].

Several approaches have explained the optimization of biocontrol agents' formulations, including applying appropriate carriers and formulation additives. Ideal formulation additives must enhance biocontrol agents' ability without supporting pathogen growth or damaging host plants [32]. The formulation is a crucial step to develop microbes into biocontrol agents for plant pathogens. Burges and Jones [33] define biocontrol agents' formulation as a technique to preserve and send antagonists to their targets and increase their activity. Wiyono et al. [32] investigated the role of defined nutrients (C- and N-source) as formulation additives in PfB5 ad planta's efficacy. In this study, it expanded in current research through the use of nitrogen compounds and trace elements. The use of nitrogen compounds in formulations has been reported for B. thuringiensis Berliner with D-L-tryptophan and L-tyrosine [34]. Tailor and Joshi [35] further state that urea as a nitrogen and tyrosine source as a carbon source are the most suitable for Siderophore production by S-11. Schmidt et al. [36] also suggested that peptone (1%) can increase the efficacy of B. subtilis (Ehrenberg) Cohn and Pantoea agglomerans against Eutypa lata in grapes (Vitis vinifera L.). Nitrogen fertilizer containing a mixture of NO3 and NH4 can also increase the capacity of P. fluorescens to enhance plant growth and inhibit Fusarium growth in wheat (S. cereale) [37].

4. Conclusion
This study showed that all the endophytic bacteria strains characterized had various biochemical characteristics. All strains showed the different ability to utilize nitrogen and carbon source. Some strains survived to grow at 4°C except Bacillus cereus AGBE 1.2 TL. All strains tolerate growth in 4% NaCl concentrations, while some strains can tolerate up to 6%. This study suggests promising
endophytic bacterial strains for growth promoters and biocontrol agents to develop their commercial formulations further. New research concerning nutrition should be given more attention in developing biological control methods in the future.

Acknowledgments
This research was funded by Hibah Riset Percepatan Guru Besar Universitas Andalas, Contract No. T/1/UN-1617/PP.Pangan_PTU-KRP2GB/LPPM/2020 on Juni 21, 2020. The authors would like to sincerely thanks the Rector of Andalas University.

References
[1] Partridge DE, Sutton TB, Jordan DL, Curtis VL, Bailey JE. Management of sclerotinia blight of peanut with the biological control agent Coniothyrium minitans. Plant Dis. 2006;90(7):957–63.
[2] Compton S, Clément C, Sessitsch A. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem. 2010;42(5):669–78.
[3] Paul NC, Ji SH, Deng JX, Yu SH. Assemblages of endophytic bacteria in chili pepper (Capsicum annuum L.) and their antifungal activity against phytopathogens in vitro. Plant Omi J. 2013;6(6):441–8.
[4] Reinhold-Hurek B, Hurek T. Living inside plants: Bacterial endophytes. Curr Opin Plant Biol [Internet]. 2011;14(4):435–43. Available from: http://dx.doi.org/10.1016/j.pbi.2011.04.004
[5] Mercado-Blanco J, Lugtenberg B. Biotechnological Applications of Bacterial Endophytes. Curr Biotechnol. 2014;3(1):60–75.
[6] Ren JH, Ye JR, Liu H, Xu XL, Wu XQ. Isolation and characterization of a new Burkholderia pyrrocinia strain JK-SH007 as a potential biocontrol agent. World J Microbiol Biotechnol. 2011;27(9):2203–15.
[7] Wang X, Liang G. Control efficacy of an endophytic bacillus amyloliquefaciens strain BZ6-1 against peanut bacterial wilt, ralstonia solanacearum. Biomed Res Int. 2014;2014.
[8] Habazar T, Yanti Y, Ritonga C. Formulation of Indigenous Rhizobacterial Isolates from Healthy Soybean’s Root, which Ability to Promote Growth and Yield of Soybean. Int J Adv Sci Eng Inf Technol [Internet]. 2014 [cited 2015 Dec 18];4(5):377. Available from: http://insightsociety.org/ojaseit/index.php/ijaseit/article/view/438
[9] Nakkeeran S, Kawitha K, Chandrasekar G, Renukadevi P, Fernando WGD. Induction of plant defence compounds by Pseudomonas chlororaphis PA23 and Bacillus subtilis BSCBE4 in controlling damping-off of hot pepper caused by Pythium aphanidermatum SO - Biocontrol Science & Technology. 16(4):403-416, 2006 Feb. MH - Growth-promot. Biocontrol Sci Technol. 2007;16(4):403–16.
[10] Yanti Y, Astuti FF, Habazar T, Nasution CR. Screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili. Biodiversitas, J Biol Divers [Internet]. 2017;18(1):1–9. Available from: http://biodiversitas.mipa.uns.ac.id/D/D1801/D180101.pdf
[11] Cappuccino J, Sherman N. Microbiology: A Laboratory Manual. New York: Benjamin/Cummings Pub; 1992.
[12] Mackie T, Mc Cartney J. Handbook of Practical Bacteriology. 9th ed. Wallingford: E. & S. Livingstone; 1956.
[13] Chernin L, Ismailov Z, Haran S, Chet I. Chitinolytic Enterobacter agglomerans Antagonistic to Fungal Plant Pathogens Downloaded from http://aem.asm.org/ on September 12 , 2015 by University of Pretoria : Academic Information Service. Appl Environ Microbiol. 1995;61(5):1720–6.
[14] Miller J. Experiments in Molecular Genetics. New York: Cold Spring Harbor; 1972.
[15] Cattelan AJ, Hartel PG, Fuhrmann JJ. Screening for Plant Growth-Promoting Rhizobacteria to Promote Early Soybean Growth. Soil Sci Soc Am J. 1999;63:1670–80.
[16] Walsh GA, Murphy RA, Killen GF, Headon DR, Power RF. Technical Note: Detection and Quantification of Supplemental Fungal B-Glucanase Activity in animal feed. J Anim Sci. 1995;73:1074–6.

[17] Lányi B. Current Methods for Classification and Identification of Microorganisms. In: Colwell R, Grigorova R, editors. Methods in Microbiology [Internet]. London: Academic Press; 1988. p. 1–67. Available from: http://www.sciencedirect.com/science/article/pii/S0580951708704070

[18] Nakbanpote W, Panitlurtumpai N, Sangdee A, Sakulpone N, Sirisom P, Pimthong A. Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd contaminated soil: Identification and effect on rice under saline conditions. J Plant Interact [Internet]. 2014;9(1):379–87. Available from: http://dx.doi.org/10.1080/17429145.2013.842000

[19] Chebotar VK, Malfanova N V., Shcherbakov A V., Ahtemova GA, Borisov AY, Lugtenberg B, et al. Endophytic bacteria in microbial preparations that improve plant development (review). Appl Biochem Microbiol. 2015;51(3):271–7.

[20] Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: Recent developments and applications. FEMS Microbiol Lett. 2008;278(1):1–9.

[21] Yanti Y, Warnita, Reflin, Busniah M. Indigenous endophyte bacteria ability to control Ralstonia and Fusarium wilt disease on chili pepper. Biodiversitas. 2018;19(4):1532–8.

[22] Kim KJ, Yang YJ, Kim JG. Purification and characterization of chitinase from Streptomyces sp. M-20. J Biochem Mol Biol. 2003;36(2):185–9.

[23] Shaikh S, Sayyed R. Role of Plant Growth-Promoting Rhizobacteria and Their Formulation in Biocontrol of Plant Diseases. In: Arora N, editor. Plant Microbes Symbiosis: Applied Facets. New Delhi: Springer; 2015. p. 337–351.

[24] Jadhav H, Shaikh S, Sayyed R. Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: An overview. In: Mehnaz S, editor. Rhizotrophs: Plant growth promotion to bioremediation. Singapore: Springer; 2017. p. 183–203.

[25] Elad Y, Kapat A. The role of Trichoderma harzianum protease in the biocontrol of Botrytis cinerea. Eur J Plant Pathol. 1999;105(2):177–89.

[26] Banik RM, Prakash M. Laundry detergent compatibility of the alkaline protease from Bacillus cereus. Microbiol Res. 2004;159(2):135–40.

[27] Beg QK, Gupta R. Purification and characterization of an oxidation-stable, thiol-dependent serine alkaline protease from Bacillus mojavensis. Enzyme Microb Technol. 2003;32:294–304.

[28] Sookkheo B, Sinchaikul S, Phutrakul S, Chen ST. Purification and characterization of the highly thermostable proteases from Bacillus stearothermophilus TLS33. Protein Expr Purif. 2000;20(2):142–51.

[29] Gerze A, Omay D, Guvenilir Y. Partial purification and characterization of protease enzyme from Bacillus subtilis megatherium. In: Davison B, Evans B, Finkelstein M, Mcmillan J, editors. Twenty-sixth Symposium on Biotechnology for Fuels and Chemicals. New York: Humana Press; 2005. p. 335–345.

[30] Kuhad RC, Singh A, Eriksson KE. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol. 1997;57:45–125.

[31] Fridlender M, Inbar J, Chet I. Biological control of soilborne plant pathogens by a β-1,3-glucanase-producing Pseudomonas cepacia. Soil Biol Biochem. 1993;25(9):1211–21.

[32] Wiyono S, Schulz DF, Wolf GA. Improvement of the formulation and antagonistic activity of Pseudomonas fluorescens B5 through selective additives in the pelleting process. Biol Control. 2008;46(3):348–57.

[33] Burges H, Jones K. Trends in formulation of microorganisms and future research requirements. In: Burges H, editor. Formulation of Microbial Biopesticides, Beneficial Microorganisms, Nematodes, and Seed Treatment. Dordrecht: Kluwer Academic Publication; 1998. p. 311–332.

[34] Bernhard K, Holloway P, Burges H. A catalogue of formulation additives: function, nomenclature, properties, and suppliers. In: Burges H, editor. Formulation of Microbial Biopesticides—Beneficial Microorganisms, Nematodes, and Seed Treatment. Dordrecht: Kluwer
[35] Tailor AJ, Joshi BH. Characterization and Optimization of Siderophore Production From Pseudomonas Fluorescens Strain Isolated From Sugarcane Rhizosphere. J Environ Res Dev. 2012;6(3A):688–94.

[36] Schmidt CS, Lorenz D, Wolf GA, Jäger J. Biological control of the grapevine dieback fungus Eutypa lata II: Influence of formulation additives and transposon mutagenesis on the antagonistic activity of Bacillus subtilis and Erwinia herbicola. J Phytopathol. 2001;149(7–8):437–45.

[37] Kurek E, Jaroszuk-Ściseł J. Rye (Secale cereale) growth promotion by Pseudomonas fluorescens strains and their interactions with Fusarium culmorum under various soil conditions. Biol Control. 2003;26(1):48–56.