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Research Article

Isolation, Characterization, and Selection of *Bacillus* sp. from Shallot Rhizosphere that Inhibits *Fusarium oxysporum* Growth

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ABSTRACT

Bacillus sp. is a Plant Growth Promoting Rhizobacteria (PGPR) species that lives in the rhizosphere. This bacterium can produce antifungal compounds that suppress pathogenic fungi, such as *Fusarium oxysporum*. The aim of this research was isolate, characterize, and obtain *Bacillus* sp. that can inhibit *F. oxysporum* which causes twisted disease in shallots. Isolation was done by sampling 10 g of soil from shallot roots, placing it in 9 mL of sterile water, and carrying out a series of dilutions. The first dilution was incubated at 80 °C for 20 minutes to obtain *Bacillus* sp. on the selective medium *HiChrome*. Colony morphology, shape, cell color, Gram staining, catalase and endospore staining were observed from obtained isolates. An antagonist test was done to determine the inhibitory effects of isolates against the pathogenic fungus *F. oxysporum*. Four isolates of *Bacillus* sp. were obtained with irregular colony shape, dull white color without muccus, edges varying in shape between lobate and undulate, and raised elevation. The results of the antagonist test showed that the four isolates had the ability to inhibit the growth of the pathogenic fungus *F. oxysporum* with the greatest inhibition shown by isolate BM1 at 30.12%, with an antibiosis mechanism and hyphae swelling.

Keywords: *Bacillus*; dual culture; *Fusarium oxysporum*; shallot

INTRODUCTION

Base rot or known as twisted disease is caused by *F. oxysporum* f.sp. *cepae* and is a major challenge for shallot production that can cause up to 50% of yield loss (Dinata *et al.*, 2021). Potential yield loss due to twisted disease infection during early and late plant development can cause 20–100% of yield loss depending on shallot cultivation practices (Triwidodo & Tanjung, 2020). Management using synthetic pesticides is commonly done, but may pollute soil that later result in reduce yield in shallot productions (Supriatna *et al.*, 2021).

Alternative management strategies for twisted disease that have been done include the application of *Trichoderma* sp. that have to be effective to 43.68% (Deden & Umiyati, 2017). *Bacillus* sp. is a species of plant growth promoting rhizobacteria (PGPR) that can be used to manage twisted disease and produce various plant hormones, such as gibberellins, cytokinins, indole acetic acid, and ethylene, fix nitrogen, produce siderophore, dilute phosphate, and produce antibacterial or antifungal compounds (Ismy *et al.*, 2019). Several species of *Bacillus* genera can induce plant defense (Prihatiningsih *et al.*, 2017).

Bacillus sp. is an antagonic bacteria with the ability to produce endospore that can withstand extreme condition and broaden its possibility to be used as a natural enemy and growth promoting symbiont. Rhizosphere are areas surrounding plant roots that are suitable sampling zones to identify beneficial microorganisms for isolation. Wulandari *et al.* (2019) stated that rhizospheres contains more bacteria, fungi, and *Actinomycetes* compared to soil without root systems. Several abundant bacteria in the rhizosphere include *Pseudomonas*, *Azotobacter*, *Agrobacterium*, *Flavobacter*, *Cellulomonas*, *Micrococcus*, *Mycobacter*, and *Bacillus* (Mukamto *et al*., 2015). Shallots that are known as host of *F. oxysporum* is a suitable isolation site for beneficial bacteria, such as *Bacillus*, which can be later used to effectively manage twisted disease (Tuhuteru *et al*., 2019). Previous research showed that *Bacillus* sp. isolated from potato rhizosphere was able to suppress *Ralstonia solanacearum* using an antibiosis mechanism (Prihatiningsih *et al*., 2020) while the rice root endophyte, *Bacillus subtilis*, was able to suppress rice leaf blight by producing protease and chitinase (Prihatiningsih *et al*., 2022). This study aims to isolate, characterize, and obtain *Bacillus* sp. from shallot rhizosphere with ability to suppress *F. oxysporum* that later can be transfered to agriculture settings.

MATERIALS AND METHODS

Research was done at the Plant Protection Laboratory of the Faculty of Agriculture, Universitas Jenderal Soedirman from May to August 2023. This research was divided into three stages, namely isolation, characterization, and selection. Rhizosphere soil samples were collected from healthy shallot plants.

Bacillus specific mediums were used in this study, namely HiCrome Bacillus agar base, Nutrient Agar (NA), and Potato Dextrose Agar (PDA). Approximately 10 g of pepton was placed into HiCrome medium with 1 g of HM extracts, 10 g of D-manitol, 10 g of Sodium chloride, 3.2 g of Chromogenic mixture, 0.025 g of phenol red, and 15 g of agar. Gram staining was done using crystal violet, iodine, alcohol, and safranin. Endospore coloring used malachit green and safranin; while catalase test used hydrogen peroxide (H_2O_2). Other materials used in this research was 96% alcohol. Tools used in this study were petri dishes, bunsen burner, reaction tubes, micropipettes, brush, object glass, cover glass, microscope, oven, autoclave, LAF, and incubator.

Soil from healthy plant rhizosphere was collected using brushes and placed on sterile Petri dishes. As much as 1 g of soil samples were dilluted into 9 mL of sterile water in reaction tubes. Samples were oven for 20 minutes at 80 °C to select *Bacillus* sp. due to their ability to survive at this temperatures compared to other bacterium genera. Serial dillution were done by taking 1 mL of isolate samples and dilluted into 9 mL of sterile water, homogenized for 20 s and repeated until a dilution of 10^{-3} was obtained. As much as 50 µL of suspension from the 2nd and 3rd dilutions were taken and grown on *Bacillus* selective medium and incubation was done for 24 h and colony was then purified on NA medium.

Isolate characterization was done using Gram staining, catalase test, endospore staining. Endospores are *Bacillus* defence mechanisms against extreme conditions where other bacteria species cannot withstand. Pandyala *et al.* (2019) stated that endospore is not produced by all bacteria, except *Bacillus* and *Clostridium*. Differences between endospores produced by *Bacillus* and *Clostridium* is where endospores are formed. Bacillus endospores were round or oval and formed within cells while *Clostridium* endospores were elongated and formed outside of the cell. Antagonistics test of *Bacillus* sp. against *F. oxysporum* growth was done on PDA by placing both organisms on the petri dishes at room temperature, observed daily, and inhibition was calculated using the formula as of the following (Ainy *et al*., 2015):

$$P = \frac{R1 - R2}{R1} \times 100\%$$

Notes: P = Growth inhibition (%); R1= radius of *F. oxysporum* colony growing against *Bacillus* sp. colony; R2= radius of *F. oxysporum* colony growing towards *Bacillus* sp. colony.

RESULTS AND DISCUSSION

Thirteen isolates were obtained and later selected based on colony morphology and catalase test. *Bacillus* is a genus of bacteria that can produce catalase (Bais *et al*., 2004). From the catalase test, four *Bacillus* isolates were obtained and later named BM1, BM2, BM3, and BM4.

All four colonies were dull white and had irregular with lobate edges for BM1 and BM4, while BM2 and BM3 had undulate edges (Figure 1 and Table 1). Isolated bacteria were rod shaped with various sizes (Figure 2) which was consistent with findings from Mukamto *et al.* (2015) that state the same characteristics for the *Bacillus* bacteria found in their work. Gram staining and catalase test showed positive results for all isolates (Table 2).

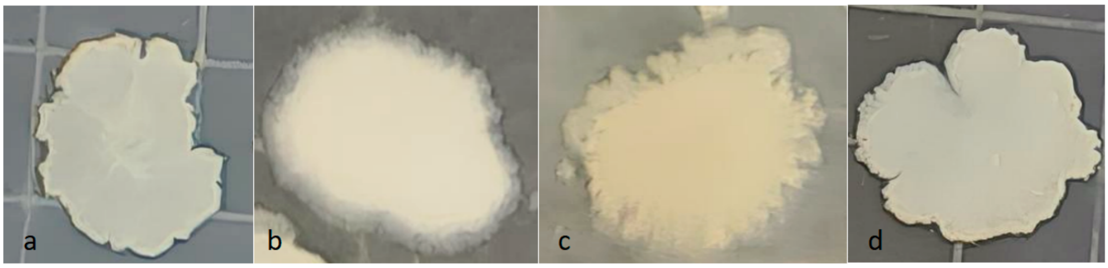

Figure 1. Isolate colonies: BM1 (a), BM2 (b), BM3 (c), and BM4 (d)

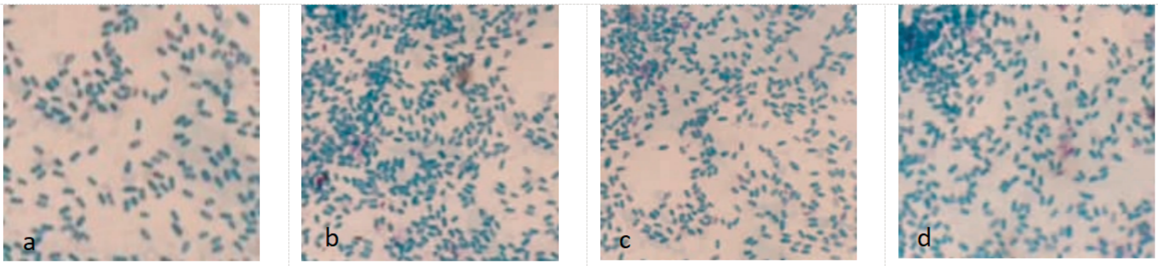

Figure 2. Cell shape of collected isolates. BM1 (a), BM2 (b), BM3 (c), and BM4 (d)

Bacillus is a genus of bacteria that is commonly found in soil and plant tissue (Saxena *et al.*, 2020). *Bacillus* can be isolated from soil by heating samples at 80 °C for 10−30 minutes. This temperature will kill other bacteria besides *Bacillus* due to their ability to form endospores (Kavitha *et al.*, 2022). Prihatinigsih *et al.* (2023) patent No. IDS000005984 demonstrated that heating at 80 °C for 20 minutes was able to select for *Bacillus* sp. isolates. Endospore staining showed that all isolates had endospore and shows *Bacillus* ability to survive in extreme conditons (Table 2). Endospore are structures with thick walls, low amounts of water, and formed by bacteria as a respond to physical and chemical conditions. *Bacillus* endospores are oval, round, of cylindrical and formed in the middle or edge of vegetative cells (Yuliana & Hidayati, 2022). *Bacillus* sp. is bacteria species known to inhibit fungi growth by producing antifungal compounds, such as bacillomicin D, surfactin, and fengcyn (Jo *et al.*, 2021). Antagonistic test showed that all four Bacillus isolates could suppress *F. oxysporum* growth with BM1 showing the highest inhibition of 30.12% (Figure 3).

Table 1. *Bacillus* sp. isolate morphology

Isolate	Shape	Margin	Color	Elevated
BM1	Irregular	Lobate	White	Raised
BM2	Irregular	Undulate	White	Raised
BM3	Irregular	Undulate	White	Raised
BM4	Irregular	Lobate	White	Raised

Table 2. Chemical test *of Bacillus* sp. isolates

Isolate	Gram staining	Endospore coloring	Catalase test
BM1	+	+	+
BM2	+	+	+
BM3	+	+	+
BM4	+	+	+

Suppresing ability of each isolate were different and may be due to the different antibiotic that each isolate can produce. Besides previously mentioned compounds, *Bacillus* sp. is able to produce chitinase that can suppress and degrade chitin of fungi wall cell (Figure 4) (Lestari *et al.*, 2017; Rahayuniati & Mugiastuti, 2012). Inhibition mechanisms showed swelling of hyphae with swelling appearing more on fungi closer to bacteria colony compared to fungi in the middle.

Mycelium growth was suppressed due to the ability of *Bacillus* sp. isolates to produce various secondary metabolites, such as pyrrolnitrin, phenazine and cepabactin that known to affect mycelium morphology and growth (Prihatiningsih *et al.*, 2019; Wulansari *et al.*, 2017). *Bacillus* sp. can produce antibiotics that can penetrate pathogen cells and disrupt their

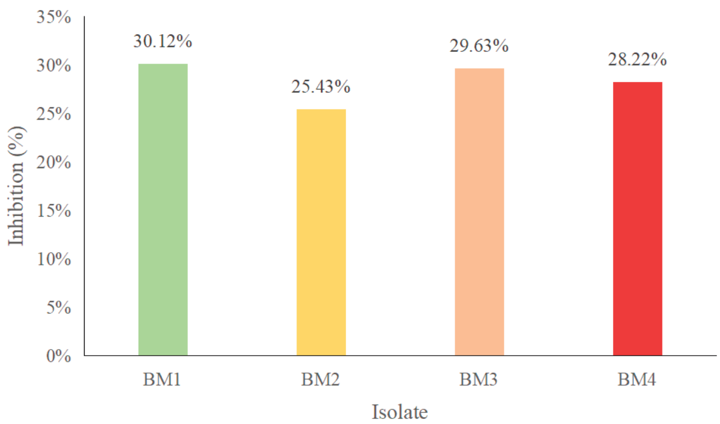

Figure 3. *Fusarium oxysporum* growth inhibition by *Bacillus* sp. isolates from shallot rhizospheres

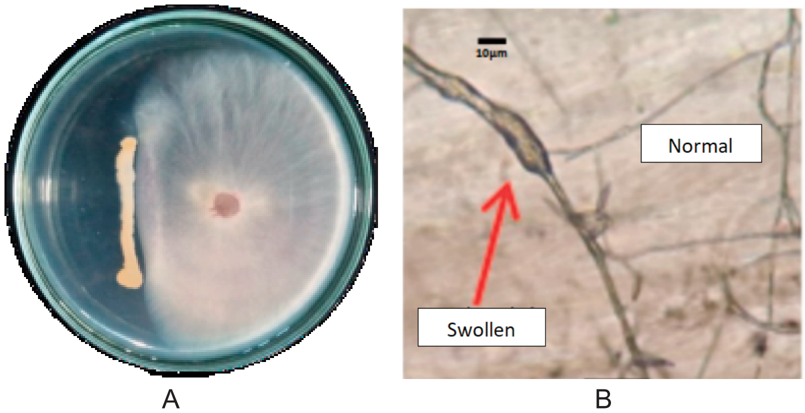

Figure 4. *Fusarium oxysporum* f.sp. *cepae* growth inhibition by *Bacillus* sp. isolate from shallot rhizosphere (A) and hyphae swelling as a inhibition mechanism (B)

activity by damaging sel and hyphae (Moreira *et al.*, 2014). *Bacillus* sp. also produce and secrete siderophore and hydrogen cyanide that are toxic against pathogens (Nadarajah & Ali, 2015; Prihatiningsih *et al.*, 2017; Wang *et al.*, 2009). Hyphae malformation can result in swelling, lysis, shrinking, cutting, thinning, and reduce of size (Widiantini *et al.*, 2020).

CONCLUSION

Four *Bacillus* sp. isolates were isolated from healthy shallot rhizosphere. All isolates had irregular shaped colonies, with lobat and undulate shaped edges, white in color, and raised elevation. Chemical test indicated that isolates were Gram positive, catalase positive, and were able to form endospores that were characteristics of *Bacillus* sp. Antagonis tests demonstrated that all four isolates could suppres *F. oxysporum* with the highest inhibition shown by BM1 with inhibition of 30.12%.

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LITERATURE CITED

Ainy, E.Q., Ratnayani, R., & Susilawati, L. (2015). Uji Aktivitas Antagonis *Trichoderma harzianum* 11035 terhadap *Colletotrichum capsici* TCKR2 dan *Colletotrichum acutatum* TCK1 Penyebab Antraknosa pada Tanaman Cabai. *Prosiding Seminar Nasional XII Pendidikan Biologi FKIP UNS*, *12*(1), 892–897. Retrieved from https://jurnal.uns.ac.id/prosbi/article/view/7122/6350

Bais, H.P., Fall, R., & Vivanco, J.M. (2004). Biocontrol of *Bacillus subtilis* against Infection of Arabidopsis Roots by *Pseudomonas syringae* Is Facilitated by Biofilm Formation and Surfactin Production. *Plant Physiology*, *134*(1), 307–319. https://doi.org/10.1104/pp.103.028712

Dinata, G.F., Ariani, N., Purnomo, A., & Aini, L.Q. (2021). Pemanfaatan Biodiversitas Bakteri Serasah Kopi Sebagai Solusi Pengendali Penyakit Moler pada Bawang Merah. *Jurnal HPT (Hama Penyakit Tumbuhan)*, *9*(1), 28–34. https://doi.org/10.21776/ub.jurnalhpt.2021.009.1.5

Deden, & Umiyati, U. (2017). Pengaruh Inokulasi *Trichoderma* sp. dan Varietas Bawang Merah terhadap Penyakit Moler dan Hasil Tanaman Bawang Merah (*Allium ascalonicum* L.) [The Effect of Trichoderma sp. Inoculation and Shallot "Bawang merah" Variety on Moler Diseases and Yield of Shallot]. *Jurnal Kultivasi*, *16(*2), 340–348. Retrieved from https://jurnal.unpad.ac.id/kultivasi/article/view/12213/0

Ismy, A., Syauqi, A., & Zayadi, H. (2019). Keanekaragaman Koloni Mikroorganisme Rizosfer Lahan Tebu (*Saccharum officinarum*) pada Penggunaan Pupuk Bio-Slurry dan Pupuk Kimia [Colony Diversity of Rizosphere Microorganisms of Sugarcane (*Saccharrum officinarum*) Field on Bio-Slurry and Chemical Fertilizers Utilization]. *Jurnal Ilmiah Biosaintropis (Bioscience-Tropic)*, *5*(1), 25–30. Retrieved from https://biosaintropis.unisma.ac.id/index.php/biosaintropis/article/view/223

Jo, J., Subroto, S.T., Victor, H., & Sanjaya, A. (2021). Pengujian Aktivitas Antijamur *Bacillus amyloliquefaciens* strain n1 [Assessment on Antifungal Activity of *Bacillus amyloliquefaciens* strain n1]. *FaST-Jurnal Sains dan Teknologi (Journal of Science and Technology)*, *5*(2), 126–133. Retrieved from https://ojs.uph.edu/index.php/FaSTJST/article/view/4681/0

Kavitha, T.R., Suneetha, C., & Sunitha, T.R. (2022). Heat-Treatment Method for Isolation of *Bacillus* spp. from Plant Tissues. In A. Sankaranarayanan, N. Amaresan, M.K. Dwivedi (Eds.), *Endophytic Microbes: Isolation, Identification, and Bioactive Potentials* (pp. 7–13). New York, NY: Springer US. https://link.springer.com/protocol/10.1007/978-1-0716-2827-0_2

Lestari, P., Prihatiningsih, N., & Djatmiko, H.A. (2017). Partial Biochemical Characterization of Crude Extract Extracellular Chitinase Enzyme from *Bacillus subtilis* B 298. *IOP Conference Series: Materials Science and Engineering*, *172*, 012041. https://doi.org/10.1088/1757-899X/172/1/012041

Moreira, R.R., Nesi C.N., & De Mio, L.L.M. (2014). *Bacillus* spp. and *Pseudomonas putida* as Inhibitors of the *Colletotrichum acutatum* Group and Potential to Control Glomerella Leaf Spot. *Biological Control*, *72*, 30–37. https://doi.org/10.1016/j.biocontrol.2014.02.001

Mukamto, Ulfa, S., Mahalina, W., Syauqi, A., Istiqfaroh, L., & Trimulyono, G. (2015). Isolasi dan Karakterisasi *Bacillus* sp. Pelarut Fosfat dari Rhizosfer Tanaman Leguminosae [Isolation and Characterization of Phosphate Solubizing Bacteria *Bacillus* sp. from the Rhizosphere of Leguminosae Plants]. *Sains dan Matematika*, *3*(2), 62–68. Retrieved from https://journal.unesa.ac.id/index.php/sainsmatematika/article/view/221

Nadarajah, K. & Ali, H.Z. (2015). *Trichoderma* and *Bacillus* as Biocontrol Agents against Fusarium in Rice. *Journal of Pure and Applied Microbiology*, *9*(4), 3245–3255. Retrieved from https://microbiologyjournal.org/trichoderma-and-bacillus-as-biocontrol-agents-against-fusarium-in-rice/

Pendyala, B., Patras, A., Gopisetty, V.V.S., Sasges, M., & Balamurugan, S. (2019). Inactivation of *Bacillus* and *Clostridium* Spores in Coconut Water by Ultraviolet Light. *Foodborne Pathogens and Disease*, *16*(10), 704–711. https://doi.org/10.1089/fpd.2019.2623

Prihatiningsih, N., Djatmiko, H.A., & Lestari, P. (2017). Aktivitas Siderofor *Bacillus subtilis* sebagai Pemacu Pertumbuhan dan Pengendali Patogen Tanaman Terung [Siderophore Activity of *Bacillus subtilis* as Plant Growth Promoters and Biological Control Agent of Eggplants Pathogens]. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *17*(2), 170–178. Retrieved from https://jhpttropika.fp.unila.ac.id/index.php/jhpttropika/article/view/420

Prihatiningsih, N., Djatmiko, H.A., Erminawati, & Lestari, P. (2019). *Bacillus subtilis* from Potato Rhizosphere as Biological Control Agent and Chili Growth Promoter. *Jurnal Perlindungan Tanaman Indonesia*, *23*(2), 179–184. https://doi.org/10.22146/jpti.40606

Prihatiningsih, N., Arwiyanto, T., Hadisutrisno, B., & Widada, J. (2020). Characterization of *Bacillus* spp. from the Rhizosphere of Potato Granola Variety as an Antibacterial against *Ralstonia solanacearum*. *BIODIVERSITAS*, *21*(9), 4199–4204. https://doi.org/10.13057/biodiv/d210934

Prihatiningsih, N., Djatmiko, H.A., & Lestari, P. (2022). Antagonistic Feature Displayed by Endophytic Bacteria Consortium for Control Rice Pathogens. *Journal of Tropical Plant Pests and Diseases*, *22*(2), 154–161. Retrieved from https://jhpttropika.fp.unila.ac.id/index.php/jhpttropika/article/view/657

Prihatiningsih, N., Djatmiko, H.A., & Lestari, P. (2023). Metode Isolasi Bakteri Endofit Akar Padi Khusus *Bacillus* sp. Paten No. IDS000005984

Rahayuniati, R.F., & Mugiastuti, E. (2012). Keefektifan *Bacillus* sp. dan *Pseudomonas fluorescens* Mengendalikan *Fusarium oxysporum* f. sp. *lycopersici* dan *Meloidogyne* sp. Penyebab Penyakit Layu pada Tomat secara *In Vitro*. *Jurnal Pembangunan Pedesaan*, *12*(1), 65–70.

Saxena, A.K., Kumar, M., Chakdar, H., Anuroopa, N., & Bagyaraj, D.J. (2020). *Bacillus* species in Soil as a Natural Resource for Plant Health and Nutrition. *Journal of Applied Microbiology*, *128*(6), 1583–1594. https://doi.org/10.1111/jam.14506

Supriatna, S., Siahaan, S., & Restiaty, I. (2021). Pencemaran Tanah oleh Pestisida di Perkebunan Sayur Kelurahan Eka Jaya Kecamatan Jambi Selatan Kota Jambi (Studi Keberadaan Jamur Makroza dan Cacing Tanah). *Jurnal Ilmiah Universitas Batanghari Jambi*, *21*(1), 460–466. https://doi.org/10.33087/jiubj.v21i1.1348

Triwidodo, H., & Tanjung, M.H. (2020). Hama Penyakit Utama Tanaman Bawang Merah (*Allium ascalonicum*) dan Tindakan Pengendalian di Brebes, Jawa Tengah [Shallot (*Allium ascalonicum*) Pests and its Control Measures in Brebes, Central Java]. *Agrovigor: Jurnal Agroekoteknologi*, *13*(2), 149–154. https://doi.org/10.21107/agrovigor.v13i2.7131

Wang, H., Wen, K., Zhao, X., Wang, X., Li, A., & Hong, H. (2009). The Inhibitory Activity of Endophytic *Bacillus* sp. strain CHM1 against Plant Pathogenic Fungi and its Plant Growth-Promoting Effect. *Crop Protection*, *28*(8), 634–639. https://doi.org/10.1016/j.cropro.2009.03.017

Widiantini, F., Yulia, E., & Kurniawan, A. (2020). Penghambatan Pertumbuhan *Rhizoctonia oryzae* dan *Cercospora oryzae* oleh Senyawa Volatil yang Dihasilkan Bakteri Endofit Padi [Inhibiton Growth of *Rhizoctonia oryzae* and *Cercospora oryzae* by Volatile Compound Produced by Rice Endophytic Bacteria]. *Jurnal Agrikultura*, *31*(1), 61–67. https://doi.org/10.24198/agrikultura.v31i1.27323

Wulandari, N., Irfan, M., & Saragih, R. (2019). Isolasi dan Karakterisasi Plant Growth Promoting Rhizobacteria dari Rizosfer Kebun Karet Rakyat [Isolation and Characterization of Plant Growth Promoting Rhizobacteria from the Rizosphere of Folk Rubber Plantations]. *Dinamika Pertanian*, *35*(3), 57–64. https://doi.org/10.25299/dp.2019.vol35(3).4565

Wulansari, N.K., Prihatiningsih, N., & Djatmiko, H.A. (2017). Mekanisme Antagonis Lima Isolat *Bacillus subtilis* terhadap *Colletotrichum capsici* dan *C. gloeospoiroides In Vitro* [Antagonistic of Five Isolates of *Bacillus subtilis* to *Colletotrichum capsici* and *C. gloeospoiroides In Vitro*]. *Agrin*, *21*(2), 127–139. http://doi.org/10.20884/1.agrin.2017.21.2.371

Yuliana, N., Sarkono, Hidayati, E., & Faturrahman. (2022). Isolasi, Karakterisasi, dan Identifikasi *Bacillus* spp. Berasosiasi Abalon (*Haliotis asinina*) [Isolation, Characterization and Identification of *Bacillus* spp. Associated with Abalone (*Haliotis asinina*)]. *Samota Journal of Biological Science*, *1*(1), 1–10. Retrieved from https://journal.unram.ac.id/index.php/samota/article/view/1322