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Endophytic bacterial consortia as biocontrol of purple blotch and plant growth promoters of shallots

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Endophytic bacterial consortia as biocontrol of purple blotch and plant growth promoters of shallots

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Abstract. Endophytic bacteria have been reported to have roles as biocontrol agents and plant growth promoters. The application of endophytic bacteria in the form of mixed culture [consortia] will provide better effectiveness than when applied alone. This study aimed to obtain a consortium of endophytic bacteria that is effective as a biological control for purple blotch [*Alternariaporri]*, promote the growth and yield of shallots. The research was arranged in a completely randomized design [CRD] consisting of 7 treatments with three replications. The consortium used was a mixture of two strains of *Bacillus* sp., two strains of *B. cereus*, two strains of *Serratia marcescens*, *Bacillus subtilis*, *Azotobacter*, *Azospirillum*, and *P. fluorescens*. The variables observed in this study included disease progression [incubation period, disease incidence, and disease severity], plant growth [number of leaves and plant height], and yield [fresh weight and dry weight of bulbs]. The results showed that the C consortium [*Bacillus* sp. strain SJI, *Bacillus* sp. strain HI, *Serratia marcescens* strain ULG1E4 and *Serratia marcescens* strain JBIE3] and G consortium [*Bacillus* sp. strain SJI, *Bacillus* sp. strain HI, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Serratia marcescens* strain ULG1E4 and *Serratiamarcescens* strain JBIE3] were the best consortia in suppressing purple blotch disease, as well as in improving the growth and yield of shallots.

Keywords: *Alternaria porri,* endophytic bacteria, consortia, purple blotch, shallots

1. Introduction

Shallots are a vegetable crop of global importance and are known as protective food because of their special nutritive value in Indonesia. One of the important diseases in shallots is purple blotch diseases [*Alternaria porri*], which causes considerable yield loss in Indonesia up to 50–70% and under severe infection even total crop loss [2]. This disease causes symptoms of purplish-colored spots surrounded by circular yellow patches[9]. This symptom can be found in shallots during tuber formation and can cause failure in forming tubers [3]. Control of pathogens in shallots is commonly carried out using synthetic pesticides. Meanwhile, the intensive and unwise use of synthetic pesticides harms humans and the environment. Accordingly, it is necessary to use other control alternatives that are safer and more environmentally friendly. In this study, biocontrol was used the endophytic bacteria. Biocontrol of plant diseases using endophytic bacteria is an alternative control that is environmentally friendly and sustainable, which can be integrated into an integrated pest control [IPM] program

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Endophytic bacteria can be suppressing the diseases, several types of nematodes, and insects through direct or indirect mechanisms. The direct mechanism is by producing siderophores, lytic enzymes[7], and antimicrobial compounds[20], by causing competition to obtain iron, nutrients, and space, as well as through parasitism. The indirect mechanism is through the resistance induction mechanism in host plants. Induced systemic resistance [ISR] is the interaction of certain bacteria with roots that allows the plant to develop resistance to potential pathogens[19]. Endophytic bacteria also have the benefit of plant growth promoters, such as *Burkholderiacepacia*, *P. fluorescens*, and *Bacillus* sp. [6]. *Burkholderia* sp. PsJN line can stimulate the growth of grapes [*Vitisvinifera* L.] [1]. *Bacillus* sp. can induce resistance of cotton plants to damp-off disease caused by *Rhizoctonia solani* by increasing plant defense enzymes [10].

The endophytic bacteria is not applied only singly but also with a consortium of biological agents. The bacterial consortium has several advantages because combining several endophytic bacteria with various disease control mechanisms will be more effective than a single application. The consortium of bacteria was able to suppress the growth of the *Xanthomonasoryzae* pv. *oryzae in vitro*. The consortium of *Bacillus firmus*, *Bacillus cereus*, and *Pseudomonas aeruginosa* has the potential to suppress the growth of the fungus *Pyriculariaoryzae* that causes blast disease by 69.92% [17]. The study aimed to determine endophytic bacterial that are effective in controlling purple blotch disease, as well as stimulating growth and yield of shallot.

2. Materials and Method

The treatment of the endophytic bacteria consortium is shown in Table 1

Table 1. The treatment of the endophytic bacteria consortium

Consortium	Endophytiv consortium strain
A	Control
B	*Bacillus cereus* strain Se07; *Bacillus cereus* strain P14
C	*Bacillus* sp strain SJI; *Bacillus* sp strain HI; *Serratia marcescens* strain ULG1E4; *Serratia marcescens* strain JBIE3
D	*Bacillus* sp strain SJI; *Bacillus* sp strain HI; *Bacillus subtillis*; *Pseudomonas fluorescens*
E	*Serratia marcescens* strain ULG1E4; *Serratia marcescens strain* JBIE3; *Azetobacte*r; *Azosprillium*; *Pseudomonas fluorescens*
F	*Bacillus* sp strain SJI; *Bacillus* sp strain HI; *Serratia marcescens* strain ULG1E4; *Serratia marcescens* strain JBIE3; *Bacillus subtillis; Pseudomonas fluorescens*
G	*Bacillus* sp strain SJI; *Bacillus* sp strain HI; *Bacillus subtillis*; *Pseudomonas fluorescens*; *Serratia marcescens* strain ULG1E4; *Serratia marcescens* strain JBIE3; *Azetobacter*; *Azosprillium*

The endophytic bacteria used were obtained from the collection o Zurai Resti [11]. The bacteria were cultured and purified on NA [*Nutrient Agar*] and the population density was 10^8 cells/ml. The bacteria were confirmed by Gram test [18] and hypersensitivity reaction [5]. The consortia were made by combining several endophytic bacteria that had been propagated. Each endophytic bacterial liquid culture was prepared according to the treatment was put into a culture bottle containing 50 ml of NB media and 1 ml of liquid culture transferred to 200 ml of sterile coconut water and incubated for 72 hours on a rotary shaker at a speed of 150 rpm at room temperature.

Pathogenic fungi [*A. porrii*] were isolated by direct planting method and cultured on PDA [*Potato Dextrose Agar*] media. Pure cultures of the pathogenic fungi were macroscopically and microscopically identified. Identification included the growth rate, colony shape, color, and texture of the colony. The identification performed referred to [9]. The pathogenicity tested using the spray method [3].

The growing media [soil and manure] in a ratio of 2:1, sterilized at 100°C for 1 hour, and transferred to a 5 kg polybag, and placed in a wirehouse. The shallot used is Bauji cultivar that is susceptible to purple blotch disease. The shallot bulbs were sterilized with 5% NaOH for 1 minute and dried. The top 1/3 of the bulb was cut and soaked in 200 ml of the consortia suspension for 15 minutes. The control was immersed in sterile distilled water, then planted.

Inoculation of *A. porri* pathogenic fungi was carried out on the leaves of the shallot plants using the spray method. Then the plants were maintained until harvest. Measurement of disease progression is the incubation period, disease incidence, and disease severity. The growth variables observed were the number of leaves and plant height. Observations were made once a week, starting from a week after planting until before harvest. The observation of yield was by calculating the fresh weight and dry weight of the shallot bulbs.

The experiment was arranged in a completely randomized design [CRD] consisting of seven endophytic bacterial consortia with three replications. Data was analyzed using the ANOVA test [analysis of variance] if the significant difference was continued with the Least Significant Different [LSD] at 5%

3. Results and Discussion

3.1. The development of purple blotch disease [A. porrii]

The endophytic bacteria consortium was able to extend the incubation period and reduce the incidence and severity of disease compared to control [Table 2]. The consortia significantly suppressed the purple blotch disease compared to controls. The incubation period of the disease was ranging from 3.33 - 10.67 days after inoculation. The introduction of the endophytic bacterial consortia could reduce the incidence of purple blotch disease in the range between 50% - 100%. The disease severity can also be reduced in the range of 24.78% - 47.28% [Table 2]. The best endophytic bacterial consortia in suppressing purple blotch disease were the C and G consortia.

The ability to suppress the development of this disease is because the endophytic bacterial consortium can act as a biocontrol agent. The endophytic bacteria composing the consortium are bacteria that had been tested before to control several plant pathogens, including wilt caused by *Ralstonia solanacearumin* chili plants [15]. Furthermore, according to [16], the best endophytic bacterial consortia to control bacterial leaf blight in rice were the C [*Bacillus* sp. strain SJI and *Bacillus* sp. strain HI] and D [*Bacillus* sp. strain SJI and *S. marcescens* strain JB1E3].

The mechanism involved in the consortium's ability to suppress disease is suspected due to the induction of resistance because the endophytic bacteria composing the consortium have the potential to induce resistance. According to[14], endophytic bacteria from the *Bacillus* group [*Bacillus* sp. strain HI, *Bacillus* sp. strain SJI, *Bacillus cereus* strain Se07, and *Bacillus cereus* strain P14] have physiological characters in producing salicylic acid between 13.96 ppm - 14.72 ppm. [12] also reported that the introduction of endophytic bacteria could increase the activity of the peroxidase enzyme in the leaves and roots of shallots that are resistant to bacterial leaf blight. According to [4], the consortium application is better than the single application because various mechanisms and synergistic effects of the consortium source microbes can suppress disease compared to control.

Table 2. Incubation period, incidences, and severity of purple blotch disease on shallots introduced with a consortium of endophytic bacteria.

Consortium	Incubation Period [DAI]	Diseases Incidence [%]	Diseases Severity [%]
A [Control]	3.33 a	100 c	47.28 b
B	3.33 a	50 a	31.35 a
C	7.00 b	50 a	28.66 a
D	8.33 bc	83 bc	37,67 b
E	10.67 c	67 ab	32.4 0 a
F	5.00 ab	83 b	35.15 ab
G	6.00 ab	50 a	24.78 a

The numbers followed by the same lowercase letter in the same row are not significantly different according to the LSD test at the 5% level

3.2. Growth of shallots

The growth of shallot plants significantly increased after being treated with the endophytic bacterial consortia compared to control [Table 3]. The number of leaves ranged from 38 to 49.67, showing an increase of 30.63% compared to the control. The plant height ranged from 38.67 - 46.67 cm, showing an increase of 20.68% compared to the control. The introduction of the endophytic bacterial consortia could increase plant growth because the endophytic bacteria used as a source of the consortia are a group of bacteria that can produce IAA hormones and dissolve phosphate [13]. The best consortia showing benefits in improving the growth of shallots were the C and G consortia.

Table 3. Effect of endophytic bacteria consortium in increasing the number of leaves height of shallot plants fresh weight and dry weight of shallots tuber.

Consortium	Number of leaves	Plant height (cm)	Tuber fress weight (g)	Tuber dry weight (g)
A (Control)	38.00 a	38.67 a	17.60 a	9.90 a
B	43.67 a	46.00 b	20.50 a	10.87 a
C	44.00 a	46.67 b	28.87 a	13.97 a
D	38.33 a	44.67 b	17.30 a	8.01 a
E	42.33 a	44.00 b	23.30 a	13.10 a
F	49.67 a	45.67 b	21.79 a	10.90 a
G	43.33 a	46.67 b	19.99 a	10.30 a

The numbers followed by the same lowercase letter in the same row are not significantly different according to the LSD test at

The increase in bulb fresh weight reached 64.03%, while the increase in bulb dry weight reached 41.08% compared to the control [Table 3]. The best consortia to increase the yield of shallot were the C and E consortia. According to [4], endophytic bacterial consortia also increased plant growth and yield. Furthermore, according to [11], the endophytic bacteria of *Bacillus cereus* strain P14 could increase the yield of shallots with an increase in dry weight of 214.85% compared to control.

The results of this study indicate that the endophytic bacteria consortium can suppress purple spot disease indirectly through the induction of plant resistance. Besides, the bacterial encoding consortium is also able to increase the growth and yield of shallot plants. According to [21], the direct mechanism is by producing antimicrobial compounds, then according to [7], siderophores and lytic enzymes compete in obtaining iron, nutrients and space, and parasitism. Indirectly through the systemic resistance induction mechanism in host plants. Induced systemic resistance [ISR] is the interaction of certain bacteria with roots that allows the plant to develop resistance to potential pathogens [25]. Then, according to [19], the endophytic microbial consortium significantly increases plant growth and yield and increases tolerance to stress due to lack of water in wheat.

4. Conclusion

The C consortium [*Bacillus* sp. strain SJI, *Bacillus* sp. strain HI, *Serratia marcescens* strain ULG1E4, and *Serratia marcescens* strain JBIE3] and G consortium [*Bacillus* sp. strain SJI, *Bacillus* sp. strain HI, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Serratia marcescens* strain ULG1E4, *Serratia marcescens strain* JBIE3, *Azetobacter*, and *Azospirillum*] were the best consortia in suppressing the purple blotch disease and improving the growth and yield of shallots.

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References

[1] Compant, S., Duffy, B., Nowak, J., Clément, C., and Barka.E A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microb.71:4951–4959.

[2] Farid N. 2012. Assembling of high yield and purple blotch resistant shallot clones [dissertation]. Indonesia: Bogor Agricultural University.

[3] Hadisutrisno, B, Sudarmadji, S, Sitidan P, Achmad 2005, Peranan Faktor Cuaca Terhadap Infeksi dan Perkembangan Penyakit Bercak Ungu pada Bawang Merah. Indon. J. Plant Prot, 1, [1] : 56-64

[4] James. D, and Methew KS. 2015. Evaluation of Endophytic microbial consortium for the management of bacterial wilt of tomato caused by *Ralstoniasolanacearum*.J.Biological Control 29[3]: 148-156.

[5] Klement, Z.K., Rudolph, and Sand, D.C. 1990. Methode in phytobacteriology. Academic Kiado. Budapest

[6] Kloepper, J.W., Leong, J., Teintze, M, and Scrhorth, M. N. 1999.Enhanced plant growth by sideophores produced by platrit growth promtingrhizobacteria. Nature.1980,286:885-886..

[7] Lugtenberg, B. and Kamilova, F . 2009. Plant-growth-promoting Rhizobacteria.Annu Rev Microbiol. 63:541–56.

[8] Manihuruk, G, 2007,Uji efektivittas pestisida nabati untuk mengendalikan penyakit bercak ungu [Alternaria porri Ell.Cif] pada bawang merah[*Allium ascalonicum L*.] di lapangan, Departemen ilmu hama dan penyakit, Fakultas pertanian, USU, Medan

[9] Marlitasari, E, Liliek, S, Restu, K2016. *Hubungan Ketebalan Lapisan Epidermis Daun TerhadapI nfeksi Jamur Alternaria porri penyebab Penyakit Bercak Ungu Pada Empat Varietas Bawang Merah.*Jurnal HPT, [4] 1:

[10] Rajendran, L. and Samiyappan, R. 2008. Endophytic *Bacillus* species confer increased resistance in cotton against damping off disease caused by Rhizoctoniasolani, Plant Pathology Journal 7: 1–12.

[11] Resti, Z., Habazar, T., Putra, D.P. and Nasrun. 2013. Skrining dan identifikasi isolat bakteri endofit untuk mengendalikan penyakit hawar daun bakteri pada bawang merah. J.HPT Tropika. 13[2] : 167 –178.

[12] Resti, Z., Habazar, T., Putra, D.P. &Nasrun. 2016. Aktivitas enzim peroksidase bawang merah yang di introduksi dengan bakteri endofit dan tahan terhadap penyakit hawar daun bakteri [*Xanthomonas axonopodisp vallii*].*J.HPTTropika*, 16[2], 131–137.

[13] Resti, Z., Reflin,&Gani, S. 2017. Antagonistic and plant growth promoting potentials of indigenous endophytic bacteria of shallots.*International Journal of Science and Applied Technology*,2 [2], 42-49.
[14] Resti, Z, Habazar, T., Putra, D. P. &Nasrun. 2018a. Characterization of endophytic*Bacillus* isolated from shallot root as biocontrol of bacterial leaf blight disease. *Jurnal Hamadan PenyakitTtropika*, 18[1], 31-38.
[15] Resti, Z., Sulyanti, E. &Reflin.2018b. Endophytic bacterial consortium as biological control to Ralstoniasolanacearum and growth promoter for chili plant.*Prosidin Seminar NasionalMasyarakatBiodiversitas Indonesia*, 4[2], 208-214.
[16] Resti, Z. Liswarni, Y. and Martinius. 2020. Endophytic bacteria consortia as biological control of bacterial leaf blight and plant grwoth promoter of rice [*Oryza sativa*. L]. Journal of Applied Agricultural Science and Technology E-ISSN: 2621-25284 [2]: 134-145 [2020]
[17] Riana, E, 2011, SeleksidanFormulasiKonsorsiumBakteriuntukMengendalikanPenyakit Blas [Pyricularia oryzae] padaTanamanPadi.Skripsi, InstitutPertanian Bogor, Bogor.
[18] Schaad, N.W., Jones, J.B., Chun, W. 2001. Laboratory Guide for Identification of Plant.Pathogenic Bacteria. St Paul: The American Phytopatology Society.
[19] Ud-Din, GM, M A, Ali, M, Naveed, K.Naveed, A. Abbas, J. Anwar, M.H, Tanveer. 2018. Consortium Application of Endophytic Bacteria and Fungi Improves Grain Yield and Physiological Attributes in Advanced Lines of Bread Wheat,Turkish Journal of Agriculture - Food Science and Technology, 6[2]: 136-144,.
[20] Van Loon LC. 2007. Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243-254.
[21] Wang, Y., Zeng, Q., Zhang, Z. 2010. Antagonistic bioactivity of an endophytic bacterium H-6.*African Journal of Biotechnology*, 9[37]:6140-6145.