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Indigenous Endophytic Bacteria Potentials to Control Black Rot Disease on Cabbage Towards the Development of Organic Vegetables

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KEYWORDS

Antagonist Agents; Brassicaceae; Endophyte Bacteria.

Abstract Organic farming plays an important role in achieving SDGs 2030, especially on point 2, 3, 13 and 15. Nevertheless, agriculture commodity often meets challenges in organic farming due to plant pests, for instance: on cabbage. Black rot disease on cabbage causes serious losses up to 70%. Most of farmers use chemical pesticides which leads to lots of negative impact towards the environment. Thus, its essential to control the environmentally friendly by applying bio control agents of indigenous endophyte bacteria instead. This article was aimed to examine the indigenous endophyte bacteria ability which excellences on organic cabbage as well as generating black rot caused by the bacterial pathogen *X. campestris*. Narrative reviews along was done through finding out the related material from International Journal articles. The result showed that there were indigenous endophyte bacteria which was able to be bio control agents towards black rot disease on cabbage, namely *Pseudomonas fluorescens, Bacillus subtilis,* and *Streptomyces* sp with effectivity level between 37-63% within resistance zone 1.44 up to 2.4 cm. Indigenous endophyte bacteria on organic cabbage had various kinds antagonist agents especially on *Bacillus* sp. and *Pseudomonas* sp. that able to resist the disease pathogen both in vitro and in vivo.

Introduction

Organic agriculture have an important role in achieving SDGs 2030, especially on point 2 about zero hunger, point 3 about good health and well being, point 13 about climate action, and point 15 about life on land. It happens because the unused synthetic chemicals contributes the continuous agriculture implementation and brings the solution regarding to the food safety as people have already concerned recently. It produces healthier food that is able to increase the immune body system regarding to COVID-19 pandemic that people have already concerned recently (Oetoro *et al*., 2012). Beside that, organic agriculture has role in mitigation and adaptation towards the climate changes because it enables to reduce the greenhouse gas emission. Reducing the use of synthetic fertilizers is believed to result in lower yields per land unitas well as ensuring the biodiversity (Scialabba & MllerLindenlauf, 2010). On the other hand, organic agriculture often meets few challenges due to plant pests and disease. Controlling plant pest and disease on organic agriculture system must be done without involving synthetic and chemical fertilizers. Thus, it is important to have the controlling method development based on organic principal which leads to eco-friendly.

Several agriculture commodities, mainly vegetables such as cabbage, have some challenges relates of plant pest and disease to deal with the organic farming system. Cabbage is a highly nutritious vegetable which contains 1.7 g of protein, 5.3 g of carbohydrate, 64 mg of calcium, 0.9 g of fiber, 75 mg of vitamin A, 62 mg of vitamin C and amount of water 91-93%

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amount per 100 g (Utama & Mulyanto, 2009). Besides that, cabbage belongs to one of essential horticulture commodity and contributes the highest remittances within the third places among the same season-vegetables with the gross is 5.38 thousand tons or equals to USD 1.4 million (Statistics Indonesia, 2018). Yet, the producing result has been decreased recently as in 2017, the percentage reaches 4.67% and decreased in 2018 is getting worse on 2.40% (Statistics Indonesia, 2018). The factor is happen because of the plant pest and disease attack. Further, black rot diseases on cabbage is the most common one that attack on cabbage.

Black rot disease on cabbage is caused by pathogen *X. campestris* bacteria that leads to symptom such as V-shaped lesions at the leaf margins, its affected area may appear black and get rot at the end (Pratama *et al.*, 2016). In addition, the damage that caused by pathogen *X. campestris* bacteria on cabbage is able to reach up to 70% damages. Most of farmers control the black rot disease by using synthetic chemicals with high frequency of chemical compound. In contrast, the extremely misused synthetic chemicals bring negative impacts towards 3 factors namely the environment, food sovereignty of agriculture products and human health. Therefore, it is necessary to developed the eco-friendly controlling method due to the plant pest.

Endophyte is bacteria which thrive inside plants taking role in triggering the host to produce phytoalexin, improve plant growth under stress conditions and as agents that allow directly control. It is assumed having capability to live under challenging conditions and more competitive than non-indigenous bacteria. The potential indigenous bacteria can be taken from healthy plants that also belongs to an organic farming. According to research conducted Xia *et al*. (2015) mentioned that the great number if endophyte bacteria found on some horticulture plants were relatively higher consistent on the organic agriculture (71%) than the conventional (29%). Meanwhile, the various bacteria also found that 32 species bacteria were isolated from organic plants and the other 28 species came from conventional plants. The black rot disease on cabbage was such a threat towards the organic agriculture of cabbage. Thus, it is important to have the controlling method development based on organic principal which leads to eco-friendly. One of the solutions was using antagonist bacteria in which its habit is similar to pathogen, *X. campestris* and able to develop and adapt on Brassicaceae family. Thus, indigenous endophyte bacteria that thrive inside the plants cultivated organically to have the potentials as antagonist bacteria. All this far, there is no indigenous endophyte bacteria which us effective to control the black rot disease on cabbage. Thus, this article was written to obtain the potentials of the excellent endophyte bacteria on organic Brassicaceae and took its benefits as controlling the bacterial pathogen, *X. campestris* that belongs to the main reason of the black rot disease.

Materials and Methods

Narrative reviews along was done through finding out the related material and the Journal International articles on Google Scholar, Science Direct, Springer Link and Research Gate. The journal involved were those that published in 1995 until 2020. There were 30 related literature covering: (1) Controlling methods on black rot disease as well as its effectivity through keywords: black rot disease, *X. campestris*, physical control, mechanic control, chemist control, and bio control, (2) Antagonist bacteria has been used to control the black rot disease on cabbage that was caused by *X. campestris* through keywords: black rot disease, *X. campestris*, and antagonist bacteria, (3) Endophyte bacteria towards

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Brassicaceae family along with its role throughout the keywords: Brassicaceae, and Endophyte bacteria, (4) Endophyte bacteria within antagonistic indigenous towards Brassicaceae family throughout the keywords: Endophyte bacteria, antagonistic bacteria, indigenous cabbage and indigenous Brassicaceae. Those literature reviews were analyzed some factors consisted of how the farmer controlled the black rot disease and its effectiveness, kind and mechanism of antagonistic bacteria towards controlling it as well as the effectiveness both in vitro and in vivo, Endophyte bacteria on Brassicaceae and its role, kinds of indigenous Endophyte bacteria on antagonistic Brassicaceae and its pathogen target, the effectiveness and mechanism of antagonistic indigenous on Brassicaceae family. Once the analysis was done, the consultation and evaluation by plant phytologist were conducted and the next step was constructing the narrative review that contains from analysis result. Mendeley application was used for arranging the references along with Harvard system.

Results and Discussion

The Methods of Controlling the Black Rot Disease

The black rot disease is control-able through these method on Table 1. There were some methods of controlling the black rot disease namely physical, mechanical, chemical, and bio treatment. In line with study conducted by Pratama *et al.* (2016), the most common method used by the farmers was chemical treatment through synthetic fertilizers that contained propineb, chlorpyrifos, chlorantraniliprole, profenofos, mancozeb, and flusulfamide. In contrast, bio controlling was more eco-friendly among the others. According to Suárez-Estrella *et al*. (2014), the involvement of endophyte bacteria namely Bacillus pumilus had high effectivity up to 95% to control the black rot disease on cabbage.

In accordance with the analysis conducted, it was revealed that the most common used was chemical controlling. However, the effectiveness achieved 93.5%. Besides, the effectiveness of mechanical treatment reached 76-85 %, 76% for physical treatment while bio treatment achieved 95%.

Table 1. Kinds of Controlling Method of Black Rot Disease

Kinds of Controlling	How Controlling Process Done	Effectiveness	Reference
Physical treatment	Removing the infected plants and using mulch	76.1 %	Ombuna (2019)
Mechanical treatment	Applying composes and raw milk	85 %	Nuñez *et al.* (2018)
	Using composes made of sewage sludge, pepper waste and almond hulls.	80 %	Suárez-Estrella *et al.* (2014)
	Treating the seeds with hot water and the remnants of the plants	76.1 %	Ombuna (2019)
Chemical treatment	Applying benzoic acid and copper hydroxide The adding up of dioxide chlorine liquid	11.6 % and 32.2 % 93.5 %	Krauthausen *et al.* (2011)
Bio treatment	Applying Bacillus pumilus	95 %	Suárez-Estrella *et al.* (2014)
	Using leaf extract of Agarista salicifolia	33 %	Yemata & Fetene (2016)

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Kinds of Controlling	How Controlling Process Done	Effectiveness	Reference
	Using extract of Agapanthus caulescents on its seeds	84 %	Mandiriza *et al.* (2018)
	Applying Bacillus subtilis on its seeds	83.5 %	

Antagonistic Bacteria Used to Control the Black Rot Disease on Cabbage (X. campestris)

The use of antagonistic bacteria had high potentials as controlling the plants disease. It enables to control the disease both in vitro and in vivo. Some of the antagonistic bacteria used to control black rot caused by pathogen *X. campestris* can be seen in Table 2.

Table 2. Antagonistic Bacteria used to Control Black Rot in Cabbage (*X. campestris*)

Antagonistic Bacteria	Source of Isolates	Obstacle Zone	In Vivo Effectiveness	References
P. fluorescens	Endophyte	3 cm	55%	Singh *et al.* (2010)
B. subtilis	Endophyte	2.4 cm	63%	Singh *et al.* (2010)
B. subtilis	Endophyte	1.75 cm	-	Luna *et al.* (2002)
B. subtilis	Endophyte	-	90%	Wulff *et al.* (2002)
B. cereus	Rhizosphere	1.26 cm	-	Fahmi *et al.* (2014)
P. fluorescens	Rhizosphere	1.44 cm	-	Mishra & Arora (2012)

Antagonistic bacteria can be obtained from the endophytes or rhizospheres of a plant. Antagonistic bacteria can be used to control black rot diseases caused by the pathogen *X. campestris*, namely Pseudomonas fluorescens, Bacillus subtilis, and Bacillus cereus. Endobacterium from the genus Bacillus is a bacterium that can be used as a biocontrol agent because it produces anti-microbial substances in the form of antibiotics and bacteriocins. There are various bacteriocins produced by the genus Bacillus, including cerein produced by B. cereus and subtilisin 6 produced by B. subtillis (Stein *et al.,*2004). The inhibitory activity of anti-microbial compounds, in general, can be done by damaging the cell walls and changing the permeability of cell membranes. Cell membrane damage results in the inhibition of cell growth or cell death. P. fluorescens bacteria can induce plant resistance to disease by inducing phenyl enzyme activity and salicylic acid production (Marwoto *et al.*, 2013).

The disease control using antagonistic bacteria had fairly high effectiveness when compared to chemical disease control. According to Krauthausen *et al.* (2011), disease control using chlorine dioxide solution has a significant value of up to 95%, while Wulff *et al.* (2002) state that the incidence rate of black rot disease has a value of <10% after control using endophytic bacteria antagonist B. subtilis. It can be concluded that the use of endophytic bacteria as antagonistic agents has an effectiveness value of up to 90%.

Endophytic bacteria from the Family Brassicaceae and Their Roles in General

Endophytic bacteria, including bacteria in plant tissue, can provide several benefits for host plants, especially to promote growth and protection from plant pathogens. Several findings of plant endophytic bacteria in the family Brassicaceae can be seen in Table 3.

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Table 3. Endophytic Bacteria in the Brassicaceae Family and their Roles

Vegetable Types	Parts of Plant	Types of Bacteria	Functions	References
Broccoli	Root	*Pseudomonas sp., Pseudomonas flourescents*	PGPR	Gadhave *et al.* (2018)
Cauliflower	Root	*Pseudomonas fluorescens, Bacillus subtilis*	Biocontrol agent	Singh *et al.* (2010)
Mustard Greens	Leaf	*Bacillus* sp., *Bacillus* subtilis, *Bacillus clausii*,	Antibiotic producer, Biocontrol agent	Haque *et al.* (2015)
	Root	*Bacillus pumilus, Bacillus* sp., *Pseudomonas sp.*	Biocontrol agent	Haque *et al.* (2016)
	Root	*Microbispora spp., Streptomyces olivochromogenes*	Biocontrol agent	Lee *et al.* (2008)

Some of the findings of endophytic bacteria in Brassicaceae family plants have fairly high diversity, as shown in Table 3. Endophytic bacteria were found in plants of the Family Brassicaceae, including cabbage, kale, mustard greens, broccoli, and cauliflower. From these results, it can be seen that the most endophytic bacteria found from the genus Bacillus sp. and Pseudomonas sp. (Haque *et al.*, 2015). The endophytic bacteria found can act as PGPR, antibiotics producers, and biocontrol agents. Bacteria Bacillus sp. and Pseudomonas sp. can be used as biocontrol agents to control plant diseases. These bacteria can compete and colonize plant roots, produce toxins, secondary metabolites, siderophores, and act as plant growth-promoting bacteria (PGPR). Therefore, indigenous endophytic bacteria can be used as biological agents to control plant pathogens (Lee *et al*., 2008)

Antagonistic Indigenous Endophytic Bacteria in Family Brassicaceae Plants

Endophytic bacteria are microbes that can inhibit pathogens from a plant. The following are some indigenous endophytic bacteria in the family Brassicaceae that are antagonistic (Table 4).

Table 4. Antagonistic Indigenous Endophytic Bacteria in Family Brassicaceae Plants

The Types of Plant	The Types of Bacteria	Parts of Plant	OPT Target	Obstacle Zone	In vivo Effectiveness	Inhibition Mechanism	References
Cauliflower	*Pseudomonas fluorescens,*	Root	*X. campestris pv campestris*	3 cm	63%	Antibiosis	Singh *et al.* (2010)
	Bacillus subtilis			2.4 cm	55%		
Cabbage	*Pseudomonas fluorescens*	Root	*X. campestris*	1.44 cm	-	Antibiosis	Mishra & Arora (2012)
Cabbage	*Streptomyces sp.*	Leaf and Root	*Alternaria brassicicola*	-	37%	Antibiosis	Hasani *et al.* (2017)

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Indigenous endophytic bacteria which are considered as antagonistic agents against pathogens can be found in several Brassicaceae plants, one of which is cabbage. The group of bacteria found were P. fluorescens, B. subtilis, Bacillus sp. and Streptomyces. The zone of inhibition can be formed because Bacillus sp and Pseudomonas sp. antibiotic properties. Antibiosis is an antagonistic bacteria that produces secondary metabolites in the form of antibiotics and is indicated by the formation of a clear zone in the test antagonist with pathogenic bacteria (Haggag & Mohamed 2007). The ability of Bacillus sp. and Pseudomonas sp. in producing antibiotics causes growth pathogens due to diffusion between antibiotic compounds against the growth media. Antibiotic activity which is bacterio-static can turn into bacteriocidal, the content of antibiotic compounds produced in high amounts. Several strains of bacteria belonging to the genus Bacillus can produce antibiotic compounds, including bacitracin, mycobacilin, zwittermicin, sublilisin, and pumilin. Meanwhile, P. fluorescens bacteria can induce plant resistance to disease by inducing enzyme activity and salicylic acid production (Marwoto *et al.*, 2013).

In the research of Hasani *et al*. (2014) reported that several strains of bacteria belonging to the genus Streptomyces can produce antibiotics such as vancomycin, erythromycin, tetracycline, streptomycin, neomycin, kanamycin, cycloserine, lincomycin, nystatin, sulfonamides, aminoglycosides, aureomycin, chlorineamphenicol, amphomycin, decinomycin, phosphomycin rimfamycin, avermycin, tobramycin, spectinomycin, clindamycin, daptomycin, puromycin, novobiosin, oxytetracycline, chlortetracycline, ribostamycin, platenmycin, viomycin, dimethyl chlortetracycline, spiramycin, and cephalosporins. Antibiotics produced by bacterial strains of the genus Streptomyces have different mechanisms of action, namely by damaging cell walls, disrupting cell membrane function, and disrupting protein and nucleic acid synthesis.

The potential of organically cultivated cabbage indigenous endophytic bacteria has a high chance to be developed as an antagonistic agent because it has a diversity of antagonistic endophytic bacteria that can control pathogens both in vitro and in vivo. (Xia *et al.,* 2015) stated that organic farming is an agriculture that focuses on the preservation and enhancement of microbial abundance and soil biodiversity, the abundance of endophytic bacterial species found is relatively consistently higher in organic farming systems (71%) than in conventional systems (29 %). While various bacteria were found, 32 species of bacteria were isolated from organic plants, and 28 species were isolated from conventional plants. In addition, 61% of isolated bacteria have the potential to increase plant growth. Based on this, it can be seen that the potential for indigenous endophytic bacteria of cabbage can be used as biological agents and can be used for the development of organic vegetables.

Conclusions and Suggestion

The potentiality for bacteria found in the endophyte of a plant from the family Brassicaceae had fairly high diversity. The most common bacterial findings were the genus *Bacillus* sp., *Pseudomonas* sp., *Bacteria Bacillus* sp., and *Pseudomonas* sp. able to inhibit the growth of pathogen *X. campestris*. The potentiality of indigenous endophytic bacteria in organically cultivated cabbage had a high opportunity of being developed as antagonistic agents because bacteria could inhibit the growth of disease pathogens in vitro, and in vivo.

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