

Isolation and Characterization of Endophytic Bacteria in Ciplukan Plant (*Physalis angulata*)

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Abstract

Biodiversity of endophytic bacteria is a potential biological resource to be applied in various fields, especially in the medical field. Endophytic bacteria are bacteria that live in the host plant tissues which belongs to a symbiosis of mutualism. Ciplukan plant (*Physalis angulata*) is an herbal plant that is useful as antihyperglycemic, antimicrobe, immunostimulant, anti-inflammatory, antioxidant, and cytotoxic. Active compounds obtained from plants need a more complicated time and process compared to extracting compounds from bacteria. The study aims to obtain isolates and characterization of endophytic bacteria in Ciplukan plant (*Physalis angulata*). The method that is used is the isolation of microbe by Nutrient Agar (NA), and morphological characterization with a gram staining technique. The result of isolation of endophytic bacteria in Ciplukan plant (*Physalis angulata*) obtains the data which the highest mean is in the rhizosphere part that is 13.13×10^6 . Isolation of a single colony of endophytic bacteria obtains 20 bacterial isolates which are 12 bacterial isolates of Gram-positive and 8 bacterial isolates of Gram-negative. The result of isolation of a single colony obtains 1 bacterial isolate of endophytic root (AC2) that has characteristic as Actinobacteria which has a form of spherical spore of colony and Gram-positive. Actinobacteria colonies grow by attached to the surface of the media and can produce spores such as powder. Metabolite compounds produced by Actinobacteria have the role of inhibiting the growth of bacteria and fungi. Furthermore, the metabolites produced by Actinomycetes are widely developed as medicinal materials that can cope with various diseases.

1. INTRODUCTION

Endophytic bacteria are bacteria that live in host plant tissues without causing symptoms of a particular disease (Shore & Satisha, 2010). These microorganisms can live in cell vessels or in the intercellular space, roots, stems, leaves, and fruit (Simamarta et al., 2007). The number of endophytic bacteria in plants cannot be certainly determined. However, these bacteria can be detected by isolating the agar medium (Bacon et al., 2007). Endophytic bacterial diversity is a rich and reliable resource to be applied in various fields, especially in medicine and agriculture. Endophytic bacteria have been popularly known as microbes that live in the tissues of living plants without causing any significant direct negative effects (Hallmann, 2001).

Endophytic bacteria can be obligate or facultative in colonizing their host plants. Moreover, a host plant generally consists of several genus and species. Although these bacteria have a broad range of the hosts, there are a number of endophytic bacteria that can only be associated with specific host plants. The symbiosis happened between plants and endophytic bacteria are neutral, mutualism, or commensalism. The symbiosis of mutualism happens between endophytic bacteria and plants. In this case, endophytic bacteria obtain nutrients from the result of plant metabolism and protect plants against disease. Then, the plants get nutrients and active compounds needed during their lives (Tanaka et al., 1999). Some endophytic bacteria are capable of producing potential products including: endophytic bacteria *Bacillus polymixin* isolated from

Anuma plants (*Artemisia annua*) can produce a chemical compound of antimalarial artemisinin in synthetic liquid media (Simanjuntak et al., 2004). *Streptomyces griseus* from the Kandelia candle plant produces paminacetophenonic acid as an antimicrobe, *Streptomyces* NRRL 30562 from the *Kennedia nigricans* plant produces munumbicin and munumbicin D (antimalaria), *Serratia marcescens* from the *Rhyncholacis penicillata* plant produces oocydin A as an antifungal. In addition, *Paenibacillus polymyxin* from wheat plants produce fusaricidin A-D as an antifungal (Guan et al., 2005; Beck et al., 2003).

Ciplukan plant (*Physalis angulata*) is an herbal plant that is useful as an antihyperglycemic, antimicrobial, immunostimulant, and immunosuppressant (immunomodulatory), anti-inflammatory, antioxidant, and cytotoxic. *Physalis angulata* has rich polyphenols and flavonoids which flavonoids is one of the antioxidants found in plants that are needed by the body. The antioxidant effect of Flavonoids found in *Physalis angulata* can enhance the regeneration process caused by free radicals by synthesizing competitive substrates for unsaturated lipids and accelerating the natural repair mechanism of the damaged cells. *Physalis angulata* also contains an active component of physalins, withanolides, phytosterols and polyunsaturated fatty acids such as linoleic acid and oleic acid which are antioxidant and hypocholesterolemic (Jyothibasu & Venkata, 2014). Then, this study aims to isolate and characterize endophytic bacteria in ciplukan plant (*Physalis angulata*).

2. RESEARCH METHODS

a. Place and time

This research is conducted in August 2018 until June 2019 in the Microbiology Laboratory of FMIPA Universitas Negeri Jakarta (UNJ) and the Bacteriology Laboratory of the Faculty of Agriculture Institut Pertanian Bogor (IPB). The sample of Ciplukan plant (*Physalis angulata*) is obtained from the Jasinga area of Bogor Regency, West Java, Indonesia.

b. Tools and Materials

The tools used in this study are Petri dishes, autoclaves, ose needles, bunsen, airflow laminar, test tubes, chemical beakers, knives, Duran bottles, hotplates, stirrers, rulers, shovels, Erlenmeyer tubes, glass objects, glass covers, and light microscopes.

The materials used in this study are roots and soil around the roots of the Ciplukan plant (*Physalis angulata*), Nutrient Agar (NA) media, Nutrient Broth (NB) media, cotton, aquades, methylated spirits, 5.25% Chlorox solution, alcohol, nystatin, violet crystal solution, safranin solution, iodine.

c. Sample Preparation of Ciplukan plant (*Physalis angulata*)

The sample of Ciplukan plant (*Physalis angulata*) is obtained from Bogor region, West Java, Indonesia. The parts of Ciplukan plant (*Physalis angulata*) used are leaves, stems, roots, and soil around the roots (Rhizosfer) of Ciplukan plants (*Physalis angulata*).

d. Isolation of endophytic bacterial in Ciplukan plant (*Physalis angulata*)

The plant samples in fresh conditions cleaned with fresh water and cut into 2 cm lengths. Then, the surface of the sample pieces is sterilized by immersing them in alcohol for 1 minute, 5.25% Chlorox solution for 5 minutes, and alcohol for 2 minutes. The sterilized sample pieces are cut, chopped, and planted in NA media containing nystatin. Media containing the sample is incubated in room temperature in the dark and observed every day until there is a growth of the colonies. Then, Endophytic bacteria that grow are purified one by one and preserved in tilt NA.

e. Isolation of a Single Colony on Endophytic Bacteria

The isolation of a single colony is done by taking a bacterial colony. Then, it is transferred to new media. Moreover, a single colony is transferred to the new Agar media by using the quadrant stroke method and incubated. A single colony in the cup is incubated for approximately 48 hours.

f. Characterization of Endophytic Bacterial Cells

Characterization of endophytic bacterial cells is done through gram staining techniques. Firstly, aquades dripped on a slide is added 1 sample ose. Then, it is fixed on fire and is dropped the violet crystal staining and leave it for 1 minute. Then, wash it with water, drop the alcohol and let it for one minute, and wash it again with water. Next, drop 96% alcohol, leave it for 30 seconds, wash it with water, add safranin, leave it again for 30 seconds, and wash again with water. The next step is to dry it by using absorbent paper, add emersion oil, and observe it with a microscope. If the staining result obtains red bacteria, the bacteria must be gram-negative bacteria. Surely, if the staining result obtains purple bacteria, the bacteria must be gram-positive.

3. RESULTS AND DISCUSSION

Ciplukan plant (*Physalis angulata*) is a herbal plant that is useful as an antihyperglycemic, antimicrobial, immunostimulant and immunosuppressant (immunomodulatory), anti-inflammatory, antioxidant, and cytotoxic. Endophytic bacteria are bacteria that live in host plant tissues without causing any symptoms of the

disease (Shore & Sathisha, 2010). The result of isolation of endophytic bacteria in Ciplukan plant (*Physalis angulata*) obtains the data which the

highest mean is in the rhizosphere part that is 13.13×10^6 (Table 1).

Table 1. The result of isolation of endophytic bacteria in Ciplukan plant (*Physalis angulata*)

No.	Part of the plant	Bacteria amount			Mean
		Deuteronomy 1	Deuteronomy 2	Deuteronomy 3	
1	Leaves	9.70×10^4	1.98×10^5	8.60×10^4	12.70×10^4
2	Stems	4.30×10^6	2.10×10^6	1.89×10^6	2.76×10^6
3	Roots	4.40×10^4	5.40×10^4	1.10×10^5	6.90×10^4
4	Rhizosphere	9.20×10^6	2.96×10^7	1.27×10^6	13.13×10^6

Table 2. Characterization of endophytic bacteria in Ciplukan plant (*Physalis angulata*)

No.	Part of the plant	Isolate Code	Morphologycolony				Gram Type
			Colour	Shapes	Elevation	Edge	
1	Leaves	DC1	Orange	Round	Convex	Full	+
		DC2	Yellow	Round	Flat	Wavy	+
		DC4	Grayish White	Round	Convex	Full	+
		DC5	Yellow	Round	Convex	Wavy	+
		DC6	Yellow	Round	Flat	Full	+
2	Batang	BC1	Yellow	Round	Convex	Full	+
		BC2	Clear Yellow	Round	Convex	Full	-
		BC3	Clear Yellow	Round	Flat	Full	-
		BC4	Grayish White	Round	Convex	Full	+
		BC5	Milky White	Round	Flat	Wavy	-
3	Akar	AC1	Yellow	Round	Convex	Full	-
		AC2	Milky White	Spored Round	Flat	Wavy	+
		AC3	Milky White	Round	Convex	Jagged	+
		AC4	Clear White	Round	Convex	Full	-
4	Rhizosfer	RC1	Milky White	Round	Flat	Wavy	+
		RC2	Yellow	Round	Convex	Full	+
		RC3	Grayish Yellow	Round	Convex	Full	-
		RC4	Brown	Round	Flat	Wavy	+
		RC5	Grey	Round	Flat	Wavy	-
		RC6	Grayish White	Round	Convex	Full	-

The result of the isolation of a single bacterial colony obtains 20 bacterial colonies with different characteristics of morphological colonies (Table 2). There are twelve isolates of endophytic bacteria which are Gram-positive. The twelve isolates are 5 isolates on leaves (DC1, DC2, DC4, DC5, DC6), 2 isolates on stems (BC1, BC4), 2 isolates on roots (AC2, AC3), and 3 isolates from rhizosphere (RC1, RC2, RC4). Moreover, there are eight isolates of endophytic bacteria which are Gram-negative. The eight isolates are 3 isolates from the stem (BC2, BC3, BC5), 2 isolates from the roots (AC1, AC4), and 3 isolates from the rhizosphere (RC3, RC5, RC6) (Table 2). The result of Gram Bacteria staining is purple Gram-positive and red Gram-negative. Gram-positive bacteria look purple because ribonucleic acids in the cytoplasm of gram-positive cells form stronger bonds with the purple crystal violet complex, so that the chemical bonds are not easily broken by colour pales (Hadioetomo, 1993). That reaction is based on the differences in the chemical composition of the cell wall. Gram-positive cells have a thick layer of

peptidoglycan wall (Sunatmo, 2007). While Gram-negative bacteria look pink because they contain lipids and fat in a higher percentage than gram-positive bacteria (Sudarsono, 2008). Besides, Gram-negative bacteria also have thinner peptidoglycan than gram-positive bacteria (Sunatmo, 2007). In addition, several types of endophytic bacteria are known to be able to produce active compounds that are antibiotic (Castillo et al., 2003), antimalarials (Simanjuntak et al., 2004), and antifungal (Beck et al., 2003).

Actinobacteria are gram-positive microbes that produce the most antibiotic compounds. There are about 80% of antibiotics which are produced by actinomycetes especially the genus *Streptomyces* (Khamna et al., 2009). The result of this study obtains that bacterial isolates AC2 have characteristics like Actinobacteria which are spherical and have gram-positive round colony form (Table 2). Actinomycetes colonies grow attached to the surface of the media and can produce spores such as powder. Moreover, one of the characteristics of Actinobacteria is having a colony that is covered

by air mycelium. It also has a hypha surrounded by a hydrophobic sheath from the surface of the colony to the air (Holt et al., 1994).

Actinobacteria have different colony colours due to the differences in the pigment of each constituent cell. Actinomycetes become very important in the pharmaceutical industry because of their benefit to produce various metabolite compounds, both in the structure and the function. Metabolite compounds produced by actinobacteria have the activity of inhibiting the growth of bacteria and fungi. Furthermore, the metabolites produced by Actinobacteria are widely developed as the ingredients of medicine that can cope with many diseases (Nurkanto et al., 2010).

The ability of endophytic bacteria to produce active compounds is potential. Moreover, it can be well developed since the active compounds are generally obtained by extracting plants, especially medicinal plants. The active compounds obtained from plants require a more complicated time and process compared to extracting compounds from bacteria. However, *Physalis angulata* is rich in polyphenols and flavonoids in which flavonoids are one of the antioxidants found in plants that are needed by the body. The effect of antioxidant from Flavonoids found in *Physalis angulata* can enhance the regeneration process caused by free radicals by synthesizing competitive substrates for unsaturated lipids in the membrane and accelerating the repair mechanism of damaged cell membranes. *Physalis angulata* also contains active components such as physalins, withanolides, phytosterols, and polyunsaturated fatty acids. Moreover, it also has linoleic acid and oleic acid which are antioxidant and hypocholesterolemic (Jyothibasu & Venkata, 2014).

4. CONCLUSION

The result of isolation of endophytic bacteria in Ciplukan plant (*Physalis angulata*) obtains the data which the highest mean is in the rhizosphere part that is 13.13×10^6 . Next, the isolation of a single colony of endophytic bacteria obtains 20 bacterial isolates which are 12 bacterial isolates of Gram-positive and 8 bacterial isolates of Gram-negative. Moreover, one of the isolates that is potential as antibiotics is from Gram-positive namely actinomycetes. Practically speaking, the root of bacterial isolates "AC2" have characteristics like Actinobacteria which are spherical and have gram-positive round colony form.

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