

Endophytic Bacteria Research in Indonesia: A Review

*Dwi Hartanti, Risda Yunita

Fakultas Farmasi Universitas Muhammadiyah Purwokerto

*Email: dwihartantihamad@gmail.com

ABSTRACT

In this review, a considerable amount of works that have been published on the endophytic bacteria isolated from host plants collected in Indonesia is reported. The objective of this review is to give an overview of the reported studies from the selection of host plants, the isolated bacteria, and the screened bioactivity of endophytic bacteria derived from Indonesian host plants.

Keywords: bioactivity, endophytic bacteria, host plant, Indonesia.

ABSTRAK

Review ini membahas tentang data-data terkait bakteri endofit yang diisolasi dari tumbuhan inang yang tumbuh di Indonesia. Tujuan dari review ini adalah untuk memberikan gambaran secara detail tentang seleksi tumbuhan inang, bakteri yang berhasil diisolasi dan juga bioaktivitas yang diuji dari bakteri endofit yang berhasil diisolasi tersebut.

Kata kunci: bakteri endofit, bioaktivitas, Indonesia, tumbuhan inang.

I. INTRODUCTION

Endophytes are those grow intra- or intercellularly in the tissues of higher plants without causing overt symptoms of disease (Schulz and Boyle 2006). The most studied endophytic organisms are fungi, but bacteria is also common

microbes existing as endophytes (Strobel and Daisy 2003).

Endophytic bacteria are those colonize inner host tissues, sometimes in high numbers, without damaging the host or eliciting strong defense responses (Reinhold-Hurek and Hurek 2011). They can be found inside the endorhiza, in

stems, leaves as well as inside plant reproductive organs of different host plants, from both monocotyledonous and dicotyledonous ones. Endophytic bacteria have been isolated from woody tree to herbaceous plants (Brader *et al.*, 2014; Ryan *et al.*, 2008). It is believed that endophytic bacteria produce metabolites in order to adapt the environment, that are used by both endophytes and their respective host plants in defense and competition, bacterial communication, specific signaling and nutrient acquisition processes (Brader *et al.*, 2014). Endophytic bacteria and their host plants communicate in those processes through certain pathways that one metabolism will trigger changes in that of its symbion (Hardoim *et al.*, 2008).

This review aims to take a comprehensive look at the progress of endophytic bacteria research in Indonesia as December 2015

II. HOST PLANTS

There are 33 host plants studied for isolation of endophytic bacteria in Indonesia. The majority of those plants are from crops (43%) and medicinal plants (39%) category (figure 1). The most studied host plants are dahlia (*Dahlia viriabilis*), banana (*Musa paradisiaca*), black pepper (*Piper nigrum*), binahong

(*Anredera cordifolia*), and potato (*Solanum tuberosum*).

Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents. The potency of endophytic bacteria isolated from crops as biocontrol agent is widely studied, as the increasing demand for chemical free grown crops from the society (Berg *et al.*, 2005). The crops that have been studied for their endophytic bacteria symbion are onion (*Allium cepa*) (Resti *et al.*, 2013), sweet potato (*Ipomoea batatas*) (Vionita *et al.*, 2015), bitter gourd (*Momordica charantia*) (Pujjiyanto & Ferniah 2010), water melon (*Citrulus lunatus*) (Rangkuti *et al.*, 2014), peanut (*Arachis hypogea*) (Arios *et al.*, 2014), soybean (*Glycine max*) (Habazar *et al.*, 2012), banana (Balosi *et al.*, 2014; Hastuti *et al.*, 2014; Marwan *et al.*, 2011; Nawangsih 2007), black pepper (Munif & Kristiana 2012; Wulandari *et al.*, 2012), rice (*Oryza sativa*) (Munif *et al.*, 2012b), coffee (*Coffea arabica*) (Harni & Khaerati 2013), potato (Kartini *et al.*, 2014; Utami *et al.*, 2012), tomato (*Lycopersicon esculentum*) (Munif *et al.*, 2012a), chilli (*Capsicum annum*) (Gofar 2007), and tea (*Camilla sinensis*) (Pranoto *et al.*, 2014).

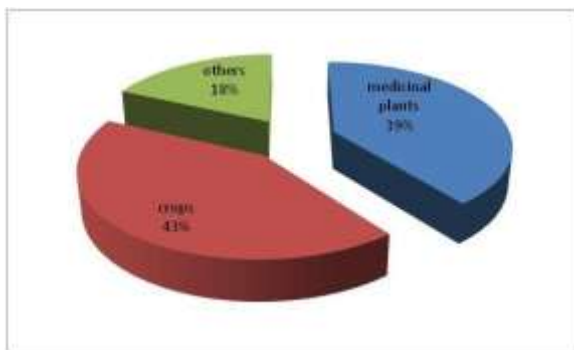


Figure 1. The nature of host plants

Most of medicinal plants studied for endophytic bacteria isolation were based on ethnomedicinal use by one or more of hundreds of Indonesian tribes. It has been reported that endophytic fungi undergo a sophisticated communication with their host plants and resulted their capability to produce the same or similar metabolites produced by their respective host plants (Kusari et al. 2012). Hence, the medicinal plants are the promising resources to obtain bacteria capable of producing medicinally important metabolites. The medicinal plants reported as host plant for endophytic bacteria in Indonesia are king of bitter (*Andrographis paniculata*) (Gusmaini et al. 2013), *purwoceng* (*Pimpinella alpine*) (Widayat and Soetarto 2012), *sambung nyawa* (*Gynura procumbens*) (Simarmata et al. 2007), *Athyrium bantamense* (Melliawati and Sulistiyowati 2012), *binahong* (Desriani et al. 2014; Nursulistyarini and Ainy 2012), *trengguli* (*Cassia fistula*) (Kumala et al. 2006), *johor* (*C. siamea*) (Nursanty and

Suhartono 2012), ketepeng china (*C. alata*) (Desriani et al. 2014), *miana* (*Coleus scutellarioides*) (Kusumawati et al. 2014), *Helixanthera* sp., *Scurulla* sp. (Walpajri et al. 2014), betle (*Piper betle*) (Purwanto et al. 2014), and *Leucosyke capitellata* (Melliawati and Sulistiyowati 2012).

Aside from crops and medicinal plants, endophytic bacteria also have been isolated from dahlia (*Dahlia viriabilis*) (Elita et al. 2013; Prima et al. 2015; Purba et al. 2012; Robi'a et al. 2012), marine organisms such as *lamun* (*Thalassia hemprichii*) and *Jaspis* sp. (Abubakar et al. 2011; Permata et al. 2014), patchouli (*Pogostemon cablin*) (Harni et al. 2007), *suren* (*Toona sureni*) (Djamaan et al. 2012), and *Freycinetia angustifolia* (Melliawati and Sulistiyowati 2012).

III. THE ISOLATED ENDOPHYTIC BACTERIA

The majority of studies of endophytic bacteria in Indonesia report the bacteria isolation process and their bioactivity. Hence, the identity of the isolated endophytic bacteria are remain unknown (figure 2). When the identifications of endophytic bacteria are performed, the methods used are usually based on the characterization of the morphology and biochemistry of those bacteria. Some studies combine them with statistical approach, while other uses fatty

acid constitution as the base of identification (table 1).

Table 1. The isolation of identified endophytic bacteria

No	Plants	Isolated bacteria	Method of identification	Refs
1	Onion	<i>Bacillus cereus</i> P14, <i>B. cereus</i> Se07, <i>Bacillus</i> sp. H1, <i>Bacillus</i> sp. SJ1, <i>Serratia marcescens</i> PPM4	Morphology, gram staining and hypersensitivity reaction	(Resti et al. 2013)
2	Dahlia	<i>Pseudomonas stutzeri</i> , <i>P. cepacia</i> , <i>B. coagulans</i> , <i>Acenitobacter antratus</i> , <i>B. pantothenicus</i> , <i>Actinobacillus actinomycetemcomitans</i> , <i>Alcaligenes faecalis</i> , <i>A. odorans</i>	Gram staining and biochemistry identification	(Elita et al. 2013; Prima et al. 2015; Purba et al. 2012)
3	Binahong	<i>Bacillus</i> sp., <i>Pseudomonas</i> sp., <i>Staphylococcus</i> sp.	Phenotypic characterizations analyzed with dendogram by MVSP (Multivariate Statistical Package)	(Nursulistyarini and Ainy 2012)
7	Tomato	<i>Acidovorax avinae</i> , <i>Agrobacterium radiobacter</i> , <i>A. xylosoxydans</i> , <i>Alteromonas haloplanktis</i> , <i>Arthrobacter citreus</i> , <i>B. cereus</i> , <i>B. circulans</i> , <i>B. mycoides</i> , <i>B. megaterium</i> , <i>B. pumilus</i> , <i>B. sphaericus</i> , <i>B. thuringiensis</i> , <i>Burkholderia cepacia</i> , <i>B. gladioli</i> , <i>B. pickettii</i> , <i>Cellulomonas cellulans</i> , <i>Chryseobacterium balustinum</i> , <i>C. meningosepticum</i> , <i>C. indologens</i> , <i>Curtobacterium flaccumvaciens</i> , <i>Comamonas acidovorans</i> , <i>Enterobacter tylosae</i> , <i>Erwinia aminoflora</i> , <i>Gluconobacter oxydans</i> , <i>Kluyvera cryocrescens</i> , <i>Lactobacillus fermentum</i> , <i>Macrococcus lutens</i> , <i>Methylobacterium mesophilicum</i> , <i>Ochrobactrum anthropi</i> , <i>Paenibacillus pabuli</i> , <i>Paracoccus denitrificans</i> , <i>Peinococcus erythromixa</i> , <i>Phyllobacterium myrcenacearum</i> , <i>P. rubiacearum</i> , <i>P. aeruginosa</i> , <i>P. chlororaphis</i> , <i>P. corugata</i> , <i>P. fluoroscens</i> , <i>P. flekteus</i> , <i>P. mendocina</i> , <i>P. putida</i> , <i>P. savastanoi</i> , <i>Rathayibacter rathayi</i> , <i>Spingobacterium multirorum</i> , <i>Staphylococcus sciari</i> , <i>Stenotrophomonas maltophilia</i> , <i>Serratia marcescens</i> , <i>Weeksella verosa</i> , <i>Xanthobacter agilis</i> , <i>Xanthomonas campestris</i>	FAME-GC and MIDI system	(Munif et al. 2012a)

The most often isolated bacteria are from *Bacillus* and *Pseudomonas* genus. Bacteria from *Alcaligenes*, *Burkholderia*, *Chryseobacterium*, *Phyllobacterium*, *Serratia*, and *Staphylococcus* genus are isolated for multiple times (Munif et al. 2012a; Nursulistyarini and Ainy 2012; Purba et al. 2012; Resti et al. 2013; Robi'a et al. 2012).

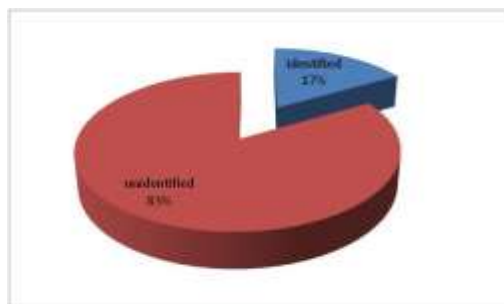


Figure 2. The identity of the isolated bacteria

IV. SCREENED BIOACTIVITY OF ENDOPHYTIC BACTERIA

Bioactivity guided isolation is commonly performed in order to obtain bioactive compounds from endophytes (Strobel and Daisy 2003). The selection of the screened bioactivity of endophytic bacteria is based on the bioactivity of their respective host plant. The most often studied bioactivity of endophytic bacteria in Indonesia is antimicrobial, and followed by antinematodic, promoting growth, inducing host metabolism, and antidiabetic. Another screening parameter used is producing certain active metabolites and nitrogen fixation. A minor number of the

studies do not test the bioactivity of the isolated bacteria (Figure 3).

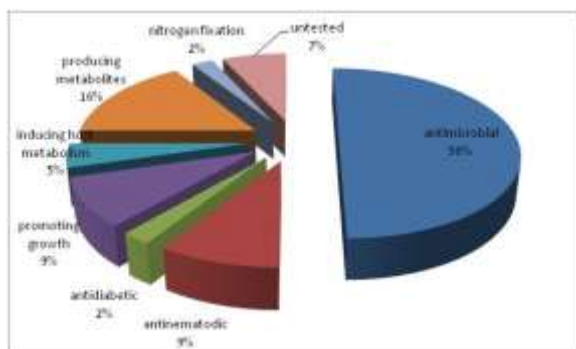


Figure 3. The screened bioactivity of endophytic bacteria

There are two main purposes of studying antimicrobial activity of endophytic bacteria, they are producing antibiotic for human disease and developing bioagent to combat plant pathogenic fungi and bacteria. Endophytic bacteria as the source of new antibiotics, such as Ecomycins, Pseudomycins, Munumbicins, and Kakadumycins, has been extensively reviewed before (Christina et al. 2013) The endophytic bacteria screened for producing antibiotics were mainly isolated from antimicrobial medicinal plant such as *sambung nyawa* (Simarmata et al. 2007), *binahong* (Desriani et al. 2014; Nursulistyarini and Ainy 2012), *dahlia* (Elita et al. 2013), *trengguli* (Kumala et al. 2006), *johor* (Nursanty and Suhartono 2012), *ketepeng cina* (Desriani et al. 2014), *miana* (Kusumawati et al. 2014), *Helixanthera* sp., *Scurulla* sp. (Walpajri et al. 2014), *suren* (Djamaan et al. 2012), betle

(Purwanto et al. 2014), and *Jaspis* sp. (Abubakar et al. 2011). For this purpose, the extract of selected endophytic fungi were tested against common human pathogenic microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Salmonella typhi*, *S. enteritidis*, *Vibrio harveyii*, *Candida albicans*, and *C. tropicalis*.

In order to obtain biocontrol agents against plant pathogenic microorganisms, endophytic bacteria were isolated from crops such as onion (Resti et al. 2013), water melon (Rangkuti et al. 2014), peanut (Arios et al. 2014), soybean (Habazar et al. 2012), banana (Balosi et al. 2014; Hastuti et al. 2014; Marwan et al. 2011; Nawangsih 2007), black pepper (Wulandari et al. 2012), potato (Kartini et al. 2014), tomato (Munif et al. 2012a), and rice (Munif et al. 2012b). The tested organisms for finding new biocontrol agents against plant pathogenic bacteria are *Xanthomonas axonopodis*, *Erwinia* sp., *Ralstonia solanacearum*, blood disease bacteria, and blight disease bacteria, while the plant pathogenic fungi used as tested organisms are *Colletotrichum* sp., *Sclerotium* sp., *Septobasidium* sp., *Rhizoctonia solani*, *Pyricularia grisea*, and *Fusarium oxysporum*.

Endophytic bacteria have been studied for antinematodic and biopesticide

activity. Bacteria isolated from black pepper were active against *Meloidogyne incognita* and *Radopholus similis* (Munif and Kristiana 2012). Similar activity was exhibited by bacteria isolated from patchouli, coffee, and potato, which were tested against *Pratylenchus brachyurus*, *Pratylenchus coffeae*, and *Globodera rostochiensis*, respectively (Harni et al. 2007; Munif et al. 2012a; Utami et al. 2012).

It has been reported that endophytes undergo a sophisticated communication with their host plants (Kusari et al. 2012). It resulted, among others, in production of metabolites capable of promoting the growth of the host plant. The detailed roles of endophytic bacteria in plant growth has been explained (Hardoim et al. 2008). This activity has been studied in Indonesia, using endophytic bacteria isolated from king of bitter (Gusmaini et al. 2013), water melon (Rangkuti et al. 2014), patchouli (Harni et al. 2007), and banana (Marwan et al. 2011).

There are some study reported productions of metabolites by endophytic bacteria. Those isolated from *A. bantamense*, *F. angustifolia*, and *L. capitellata* collected from Taman Nasional Gunung Halimun were capable of producing amylase (Melliawati and Sulistiyowati 2012). Another study

reported that endophytic bacteria derived from patchouli produced chitinase, cellulase, protease, HCN, and fluorescence agents (Harni et al. 2007). Auxin, a plant growth promoting hormone, was detected in culture of endophytic bacteria from chili (Gofar 2007). Beside enzymes and hormones, endophytic bacteria were also known for capability of producing primary and secondary metabolites. For example, endophytes from dahlia were reported producing cellulose, while those from *T. hemprichii* were able to produce carotenoid (Permata et al. 2014; Prima et al. 2015).

Besides producing metabolites on their own, endophytic bacteria were also known for inducing certain metabolites of their respective host plants. The recent studies showed that endophytic bacteria isolated from purwoceng increasing the capacity of its host to produce coumarine, while those from king of bitter inducing andrographolide production (Gusmaini et al. 2013; Gusmaini et al. 2013; Widayat and Soetarto 2012).

V. CONCLUSION

It is believed that searching for natural products produced by endophytes could be a promising way to discover new bioactive. In Indonesia, the rationale for host plant selection has been followed, resulted in the variety of host plants and

their respective isolated endophytic bacteria. Bioactivities of endophytic bacteria have been reported, somehow the isolation of metabolites responsible for those activities was still limited to date.

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