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The endophytic bacteria producing IAA (Indole Acetic Acid) in *Arachis hypogaea*

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Abstract. *Herlina L, Pukan KK, Mustikaningtyas D. 2017. The endophytic bacteria producing IAA* (*Indole Acetic Acid) in* Arachis hypogaea. *Cell Bio Dev 1: 31-35.* Endophytic bacteria are bacteria living in plant tissue and forming colony without harms the host. Every cormophyte, plants that have a stem and root, may contain some endophytic bacteria which can produce biological compounds or secondary metabolites. The objective of the study was to obtain endophytic bacteria isolates from peanut plants (*Arachis hypogaea*) at three locations, and to test in vitro the ability of endophytic bacteria isolates for producing IAA, and also to analyze IAA on the growth and development of mungbean plants. The study was carried out in three stages; the first was the isolation of endophytic bacteria from the leaves, stems, and roots; the second was the test of in vitro endophytic bacteria isolates to know the ability of IAA production. IAA assay was measured by using a spectrophotometer with a wavelength of 535 nm, and the third was the introduction of IAA-producing endophytic bacteria in mungbean. The parameters observed were the length of sprouts and the number of lateral roots. The results showed that 16 isolates were selected based on IAA-producing ability. The isolates could produce different IAA with different morphological characteristics. After the fourth day of incubation, the highest and the lowest of IAA amount were 69.68 (mg L^{-1}) and 8.50 (mg L^{-1}) respectively. Isolates that produce high IAA levels are applied to mungbeans, it affects the number of lateral roots but it does not have effect on the length of the sprouts. DM and K1K1 isolates have the effect of increasing lateral root formation and are expected to be potential sources of bioactive metabolites.

Keywords: Endophytic bacteria, IAA, *Arachis hypogaea*

INTRODUCTION

Endophytic bacteria are microbes that live in tissues which form colonies in plant tissues without harming the host. Each high-level plant may contain several endophytic bacteria which are capable of producing biological compounds or secondary metabolites suspected as a result of coevolution or transfer genetics from host plants to endophytic microbes (Duan et al. 2013). The biological association between endophytic microbes and host plants varies from neutral, commensalism, to symbiosis. Plants are a food source for endophytic microbes in completing its life cycle. Endophytic bacteria can be isolated from the surfaces of sterile plant tissue or extracted from inner plant tissues (Pandey et al. 2012; Ryan et al. 2008). In particular, bacteria enter the tissues through germinated tissue, roots, stomata, and damaged tissue. In recent years, endophytic bacteria are used as biofertilizers to increase crop production as it significantly reduces the chemical input to the environment (Luo et al. 2012; Ahemad et al. 2014).

Endophytic bacteria is one of the microorganisms that are now beginning to develop its role in increasing plant growth through its ability to produce growth hormone and N2 retardation from the air. The ability of endophytic microorganisms to produce plant hormones such as IAA (*Indole Acetic Acid*) or better known as auxin can help plants to grow better as in some food crops such as peanuts, corn, wheat and sugarcane (Mattos et al. 2008). Auxin-producing endophytic bacteria can help plants to grow and develop in addition to endogenous auxin possessed by plants. Auxin in plants is usually present in meristem tissues (Spaepen et al. 2007). Auxin produced by endophytic bacteria *Burkholderia kururiensis* in peanut plants cause plant growth to be better with the number of roots, and it makes lateral roots of the plant increases. Plant growth is rapid, and it gives high yielding products (Mattos et al. 2008).

The mechanism of increasing plant growth by endophytic bacteria can occur in several ways including folic, nitrogen fixation, stimulation lateral root growth and production of growth hormones such as auxin, ethylene, and cytokines (Ahemad et al. 2014). Plants meet the needs of hormones through their ability in synthesizing the auxin hormone from microorganisms in their tissues. IAA-producing bacteria potentially join the physiological process of plants by entering IAA generated crops. The effect on the plant itself is that the plant is more sensitive in altering its IAA concentrations thus helping in the formation of lateral roots, adventitious roots and primary root elongation (Ryan et al. 2008).

Various research results reported that some groups of microbes are capable of producing compounds that can accelerate plant growth. Some soil microorganisms that produce IAA such as *Stenotrophomonas maltophilia* from the banana root can promote plant growth (Ambawade and Pathade, 2015). *Azospirillum* which produces IAA can

accelerate plant growth, lateral root development, stimulate density and root hair length, which in turn leads to increased nutrient uptake in peanut crops to increase peanut plant height and make this bacteria function as bacterial fertilizer (Lestari et al. 2007). The effect of *Azotobacter* in increasing root biomass is due to the income of (*Indol Acetic Acid*) in the root zone. IAA-producing endophytic bacteria successfully isolated from plant roots are Agrobacterium tumafaciens and *Azotobacter vinelandii* (Khan and Doty 2009). Different bacterial groups were reported to produce IAA (indole-3-acetic acid), the most important auxin that regulates plant development such as cell extension, cleavage, differentiation, gene regulation, and other tropical responses (Nath et al. 2013).

Auxin is one type of hormone that can stimulate plant growth by increasing elongation process and stem extension as well as cell differentiation (Tarably et al. 2008). In the IAA plant, the tissue is synthesized in various parts of the plant body. Generally, IAA was mostly produced in the growing parts of plants. Tryptophan is a precursor in auxin biosynthesis both in plants and in microorganisms. Tryptophan contains active compounds that spur the growth of rhizosphere microbes and endophytes. The availability of suitable precursors is a primary factor of microbial secretion of secondary metabolites. IAA microbial biosynthesis in soil may be driven by the presence of tryptophan originating from root exudates or damaged cells (Spaepen et al. 2007). The purpose of this research is to isolate and identify IAA-producing endophytic bacteria, which is expected to also influence the growth and development of green beans.

MATERIALS AND METHODS

The isolation of endophytic bacteria from *Arachis hypogaea*

The roots and leaves of peanut were cleaned for about 20 minutes using running water. Roots and leaves were sterilized by soaking them in alcohol solution 70% for 2 minutes, hypochlorite solution 5% for 5 minutes, and alcohol solution 70% for 30 seconds, and then were rinsed with sterile distilled water twice (RaduandKqueen, 2002). After sterilizing, the roots and leaves were aseptically mashed in a mortar, and then put into a test tube which contained sterile distilled water in a ratio of 1: 10 and made dilution to 103. 1 ml of the roots and leaves were spread on a nutrient medium for sterilizing and incubating at room temperature for 24 hours. To obtain pure cultures, the colonies of growing bacteria were subcultured into the same medium. To distinguish bacterial isolates from one another, the characterization of colony morphology gram stain and some biochemical tests were conducted.

The in vitro process for producing IAA of endophytic bacteria

The in vitro process for producing IAA of endophytic bacteria was done by generating the bacteria in media containing tryptophan 3 ml bacterial suspension, with some cells of 10^8 CFU/ml/ equal to McFarland (Bresson and Borges, 2004) was inoculated into 30 ml of Luria-Bertani Tryptophan solution. At room temperature, bacterial cultures were incubated and shaken at 150 rpm for 7 days. Every two days in a week, the IAA level generated during cultivation was measured. The measuring of IAA level was done in colorimetry way with a spectrophotometer at 535 nm wavelength. Culture fluid was centrifuged for 25 minutes at 5000 rpm. The obtained filtrate was mixed with the Salkowski reagent (150 ml concentrated H_2SO4, 250ml of distilled water, 7.5 ml of 0.5M$FeCl_3$•$6H_2O$) with a ratio of 2:1. The mixture was then incubated at room temperature for an hour before the absorbance was measured at a wavelength of 535 nm. IAA level produced by endophytic bacteria was determined from the linear plot of the absorbance value of a standard IAA.

Introduction of IAA-producing endophytic bacteria in mungbean plants

Positive of bacterial culture which produced IAA were tested in liquid and solid forms to mungbean plant growth. Introduction of endophytic bacteria is done on sterile mungbean sprout. To get sterile sprouts then mungbean seeds were grown in sterile media. The surface of mungbean seeds is washed under running water. The seeds are soaked in a mixture of Agrep (fungicide) solution with two drops of 80% tween solution, and incubated for 30 minutes at 120 rpm. The seeds were washed with sterile distilled water, then soaked with 10% chlorox solution with shaker for 15 minutes and washed again with sterile distilled water for three times. The seeds were soaked with 5% chlorox solution with a shaker for 15 minutes, then they were washed with sterile distilled for three times. The last stage, the seeds were soaked in 70% alcohol for one minute and rinsed with sterile distilled. Seeds of sterile mungbeans were grown in agar medium. The seeds were grown for one week and placed in a room with less light, and then the young sprouts were transferred into a sterile container.

The sprouts are immersed into a production suspension that has equalized the turbidity with a Mc. Farland solution (10^8 cells/ml) for one hour with 50% dilution. Sprouts soaked with aquades were used as controls. Each treatment repeated six times. Any sprouts that have been immersed in a production suspension are grown on sterile soil media in polybags. The growing sprouts are observed after one week. The parameters observed were the length of sprouts, the number of lateral roots and the wet weight of the plant.

RESULTS AND DISCUSSION

The isolation of endophytic bacteria from *Arachis hypogaea*

The isolation results from three location, that is Gunungpati, Pakintelan, and Klipang, earned 22 endophytic bacterial isolates. After examining the bacteria producing IAA, there are 16isolatesthat have the ability to produce IAA (Table. 1). Based on the characterization result at table 1, describe that the isolates have variation of colony morphology. Most of them are Gram negative

bacteria and the cells morphology are coccus and bacillus. It means that the 16 isolates may be different species or different genus. The further research needed to identify the isolate species.

The ability of endophytic bacteria in producing IAA with invitro process

Variation was found in the abilities of endophytic bacteria in producing IAA, depending on its isolates and the age of cultures as presented in Figure 1.

From the Figure 1, it can be seen that the production of IAA by bacteria is mostly (10 isolates) on the fourth day after incubation. While on isolate GNP1K2 and P1K2 IAA, production increased with increasing culture time. 4 isolates i.e. GNP4K1, K1K2, GNP1K1 and GNP2K2 experienced highest IAA production on the second day. This difference is thought to be due to variations in the type of bacteria and location. The production of IAA by bacteria varies due to environmental factors, growth rates and availability of amino acids and other N sources (Yurnaliza. 2010). The decrease of IAA levels on the fourth or sixth day due to the available nutrients (tryptophan) which has begun to decrease. The use of nutrients in every bacterium varies. In some isolates, it increases in line with incubation time because at the time of incubation in the second day, the enzyme that converts tryptophan to IAA is still low. In line with bacterial growth rate, the enzyme used in conversion of tryptophan to IAA is active enough to produce high IAA (Taghavi et al. 2009). It has been reported that endophytic bacteria produce significant IAA, in *B. cereus* (Rana et al. 2011.) and *P. putida* (Jasim et al. 2013). The production rate was found to be maximal in the case of *P. putida* (ECL5) and the minimum in *C. michiganensis* (ECL6) in the presence of tryptophan. IAA is the most common plant hormone, which stimulates plant growth and reproduction (Taghavi et al. 2009). IAA produced by bacteria interacts with plants in a variety of pathogenesis and phytocimulation. IAA is a major auxin in plants involved in cell enlargement and division, tissue differentiation, physiological processes (Spaepen et al. 2007). The amount of IAA produced by bacteria plays an important role in the interaction of microbial plants. Plant growth modulation was performed with optimal IAA concentration range.

Table 1. Characteristics of bacteria producing IAA

Isolate	The colony morphology						Gram	Cells Morphology
	Size	Optical characteristic	Shape	Elevation	Texture	Margins		
K1K1	Moderate	Transluscent	Circular	Raised	Glisten	Serrate	-	Coccus
AT	Pinpoint	Transluscent	Circular	Convex	Smoothly glisten	Entire	-	Bacillus
P1K2	Moderate	Transluscent	Circular	Raised	Smoothly glisten	Undulate	-	Coccus
DM	Moderate	Transluscent	Circular	Raised	Glisten	Undulate	-	Bacillus
GNP2K22	Moderate	Transluscent	Circular	Raised	Glisten	Undulate	-	Coccus
DTR	Small	Transluscent	Circular	Raised	Glisten	Undulate	-	Bacillus
P2K3	Small	Transluscent	Circular	Raised	Glisten	Undulate	+	Bacillus
K1K2	Small	Transluscent	Irregular	Raised	Glisten	Undulate	-	Coccus
GNP2K2	Moderate	Transluscent	Circular	Raised	Glisten	Undulate	-	Bacillus
GNP2K21	Small	Transluscent	Circular	Convex	Smoothly glisten	Entire	-	Bacillus
GNP1K1	Pinpoint	Transluscent	Circular	Raised	Glisten	Undulate	-	Bacillus
BPK3	Moderate	Transluscent	Circular	Rasied	Glisten	Serrate	-	Coccus
GNP1K2	Large	Transluscent	Circular	Raised	Glisten	Entire	-	Bacillus
GNP2K1	Small	Transluscent	Circular	Raised	Glisten	Entire	-	Bacillus
GNP3K1	Small	Transulscent	Circular	Raised	Glisten	Entire	-	Bacillus
GNP4K1	Pinpoint	Opaque	Circular	Raised	Glisten	Undulate	-	Bacillus

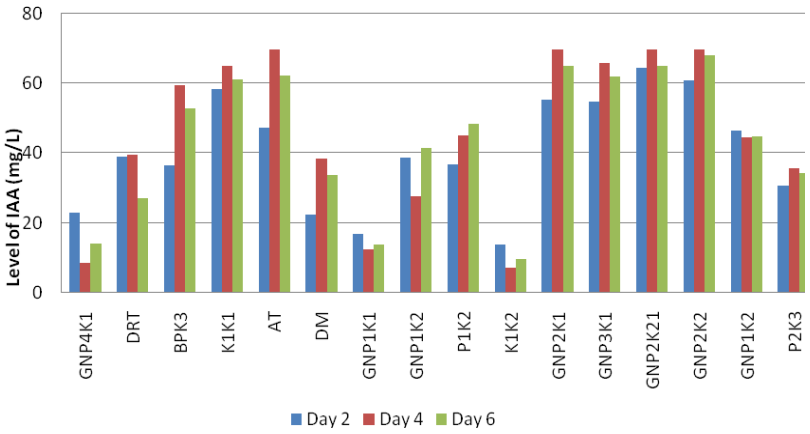

Figure 1. The production of IAA from various endophytic bacteria isolates

The synthesis of IAA by microbes is dependent on the pathway of tryptophan where tryptophan is used as a precursor and different diverse taxonomic and metabolic tissue tissues. Some endophytic microorganisms have the potential to synthesize IAA to increase or stimulate growth when colonization occurs with endophytices (Shi et al. 2009). One of the main contributions of these microorganisms to plant growth is the production of molecules such as auxin (Spaepen et al. 2007). Indole 3 acetic acid (IAA) to auxin can stimulate growth such as cell lengthening and cell division and differentiation (Hasan et al. 2015). IAA-producing bacteria potentially affect the growth process from the amount of IAA into production and tissue sensitivity to IAA concentration changes.

Introduction of IAA-producing endophytic bacteria to mungbean plants

Isolates applied to mungbean plants with high IAA content of 16 isolates obtained were selected 5 isolates, application results to plants can be seen in Table 2.

One way ANOVA analysis results show that IAA-producing bacterial isolates did not affect the length of sprouts but influenced the number of roots. In low concentrations, IAA causes root and shoot elongation, if IAA concentrations are higher, the elongation of shoots and roots becomes inhibited (Moore, 1989). The addition of exogenous IAA has an effect on the increase of IAA concentration in plants causing stunt length inhibition. In contrast to the number of roots, IAA concentrations that exist in plants actually stimulate the formation of lateral roots. IAA bacteria can loosen the cell wall of plants and consequently increase the number of roots that increase exudation that provide additional nutrients to support the growth of bacterial rhizosphere. IAA bacteria stimulate the development of root system of host plants. IAA production of isolates can improve the fitness of microbial plant interactions (Hasan et al. 2015).

Endophytic bacteria do not only generate IAA but also increase the availability of plant nutrients such as nitrogen, phosphate, and other minerals so that plant growth increases. Root is one of the most sensitive organ to IAA fluctuations and is responsible for increasing the number of exogenous IAA useful for primary root elongation process, lateral root formation and adventive root (Ryan et al. 2008) IAA is the major auxin hormone in plants that controls plant growth. Many important physiological processes take place including cell enlargement and cell differentiation. The IAA produced by the bacteria when administered to the plant will have an effect on the sensitivity of the plant tissue.

In conclusion, there were 16 isolates of endophytic bacteria having the ability to produce IAA. The incubation time had an effect on the IAA content produced by bacteria and IAA-producing endophytic bacteria isolate affected the number of lateral roots but it did not affect the length of the sprouts. The largest bacterial isolates stimulating the formation of lateral roots are isolates of DM and K1K1.

Tabel 2. The effect of some isolates of IAA producers on the growth of root length and number of roots

Isolat	Length of sprout	Number of roots
AT	16.025	15 bc
DM	15.025	24.75 a
GNP2K2I	15.425	19.5 ab
K1K1	14.20	23.75 a
P2K3	14.75	19.75 ab
Control	14.65	13.75 c

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