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STUDY OF ENDOPHYTIC BACTERIA PRODUCING IAA (INDOLE ACETIC ACID) FOR PLANT GROWTH

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ABSTRACT

This study aimed to obtain endophytic bacteria isolates from peanut plants (Arachis hypogaea) at three locations (Gunungpati, Pakintelan and Klipang) in Semarang, Central Java, Indonesia and to test in vitro the ability of endophytic bacteria isolates for producing IAA. The study was carried out in two stages, The first was isolation of endophytic bacteria from the leaves, stems and roots on common medium for bacteria growth. The second was test in vitro endophytic bacteria isolates on media containing tryptophan to know the ability of IAA production. IAA assay was measured by using a spectrophotometer with a wavelength of 535 nm, the measurements were done once per two days in a week. The results showed that a total of 22 endophytic bacteria isolates were obtained from peanut plans,16 isolates were selected based on IAA-producing ability. IAA amount were produced from isolates indicate different results. The highest and lowest IAA amount after the fourth day of incubation were 69.68 (mg/L) and 8.50 (mg/L) respectively. The kind of isolate, bacteria culture age and plant location affected IAA amount were produced by endophytic bacteria isolates. The Isolates GNP2K21, GNP2K2 and AT produced the highest IAA after the fourth day of incubation.

Key Word: Endophytic bacteria, IAA, Arachis hypogaea

INTRODUCTION

Endophytic bacteria is a kind of bacteria which lives in plant tissue forming colony without harms its host. Every cormophyta, plants that have a stem and root, may contain some endophytic bacteria which is able to produce biological compounds or secondary metabolites which is approximated as the impact of coevolution or genetic transfer from its host into endophytic bacteria (Tan & Zhou, 2001 in Radji, 2004). Types of biological association between endophytic bacteria and its host are varied from neutral, commensialism, to symbiosis. In this condition, a plant is a food source for endophytic bacteria to complete its life cycle (Clay, 1988). Endophytic bacteria can be isolated from the surface of sterile plant tissues or be extracted from inner plant tissues. Particularly, bacteria enter the tissue through the germinated tissue, the root, the stomata, or damaged tissues (Zinniel et al, 2002).

Bacteria that produce IAA can generate phytohormones which can accelerate the growth of plants. IAA hormones is a kind of endogenous auxins which plays a role in cell enlargement, inhibits the growth of side shoots, stimulates abscission, forms xylem and phloem tissue, and also affects the growth and elongation of roots. IAA hormones is a sort of hormones which supports the growth and development of plants so the synthesized by certain bacteria is the reason that led to an increase in plant growth (Aryantha et al., 2004). A number of endophytic bacteria which has been isolated from the inner plant and some crops like ground nut, corn, buckwheat, and sugar cane can increase the production of IAA hormones, James & Olivers (1996) in Susilowati (2003).

Cormophyta can contain some endophytic bacteria capable of producing biological substances or secondary metabolites are expected as a result of genetic transfer or genetic recombination of the host plant to the endophytic bacteria during its evolution (Tan & Zhou, 2001 in Radji, 2005). A number of endophytic bacteria have been isolated from some crops like ground nut, corn, buckwheat, and sugarcane (James and Olivares, 1996). There are some bacteria that are producing IAA hormones which are found in certain plants and producing phytohormones that is helpful for the growth of the plants (Hoflich, 1995 in Aryantha, 2004).

Auxin is a sort of hormones that can spur the growth of plants by increasing the elongation and renewal of stem process as well as cells differentiation (Tarably et al, 2003). In plant tissues, IAA are synthesized in the various parts of plant. Generally, IAA are produced mostly on the parts of the plant which are growing.

Tryptophan is a kind of precursor in auxin biosynthesis which is found in both plants and microorganisms. Tryptophan contains active compound which boosts the growth of the rhizosphere and endophytic microbiome. The availability of suitable precursor is a primary factor of microbial secretion from secondary metabolites. Biosynthesis of IAA microbiome in the soil can be done with the availability of tryptophan which comes from exudates roots or damage cells (Benziri et al, 1998 dalam Husen 2003).

Endophytic bacteria are producing IAA with different parts and conditions so the kind and the ability will be also different. The aim of this study is to isolate endophytic bacteria that produce IAA and examine its capability to produce IAA with in vitro process.

METHODS

The Isolation of Endophytic Bacteria

Parts of ground nut (roots and leaves) were cleaned using running water for about 20 minutes. The sterilization of roots and leaves was done by soaking the pieces of roots and leaves in alcohol solution 70% in 2 minutes, hypochlorite solution 5% in 5 minutes, and alcohol solution 70% in 30 seconds, and then being rinsed with sterile distilled water twice (Radu & Kqueen, 2002). Sterilized roots and leaves were mashed in a mortar aseptically, and then put into test tube containing sterile distilled water with a ratio of 1:10 and made dilution to 103. 1 ml of the roots and leaves was spread on nutrient medium to be sterilized and incubated for 24 hours at room temperature. The growing bacteria colonies were subcultured into the same medium to obtain pure cultures. Characterization of colony morphology, gram stain, and some biochemical tests were conducted to distinguish bacterial isolates from one another.

The Ability of Endophytic Bacteria to Produce IAA with In Vitro Process

The ability of endophytic bacteria to produce IAA with in vitro process was done by generating the bacteria in media containing tryptophan. 3ml bacterial suspension, with the amount of cells of 108CFU/ml/ equal to Mc Farland (Bresson and Borges, 2004) was inoculated into 30 ml of Luria-Bertani Tryptophan solution. Bacterial cultures were incubated at room temperature and shaken at a speed of 150 rpm for 7 days. The level of IAA that was generated during cultivation was measured every two days. The measuring of IAA level was done in colorimetry way with spectrophometer at a wavelength of 535 nm. Culture fluid was centrifuged at 5000 rpm for 25 minutes. The filtrate that had been obtained was mixed with the reagent Salkowski (150 ml concentrated H_2SO_4, 250 ml of distilled water, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$) with a ration 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.

RESULT AND EXPLANATION

The isolation results from 3 location, that is Gunungpati, Pakintelan, and Klipang, earned 22 endophytic bacteria isolates. After examining the bacteria producing IAA, we obtained 16 isolates that have the ability to produce IAA.

Table 1. Characteristics of Bacteria Producing IAA

Isolates	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

The abilities of endophytic bacteria in producing IAA are varied depending on its isolates and age of cultures as presented on Table 2

Table 2. The Productui of IAA from Various Endophytic Bacteria Isolates

Isolates	Level of IAA (mg/L)		
	Day 2	Day 4	Day 6
GNP4K1	22.97	8.501	14.25
DRT	38.92	39.56	27.24
BPK3	36.59	59.42	53.01
K1K1	58.47	65.12	61.21
AT	47.39	69.68	62.26
DM	22.31	38.49	33.74
GNP1K1	16.78	12.54	13.86
GNP1K2	38.88	27.76	41.60
P1K2	36.93	44.99	48.45
K1K2	13.96	7.06	9.68
GNP2K1	55.42	69.68	64.93
GNP3K1	54.76	65.97	61.91
GNP2K21	64.57	69.68	64.93
GNP2K2	60.88	69.68	67.98
GNP1K2	46.43	44.54	44.93
P2K3	30.77	35.58	34.46

Based on Table 2, it can be seen that the production of IAA reach the highest point on day 2, and then on day 4 and 6, it decreased on isolates GNP4K1, GNP1K1, K1K2, and GNP1K2, but on isolates GNP1K2 and P1K2, the production of IAA increased in accordance with the time of the cultures. The highest production of IAA on day 2 occurred on isolates DRT, BPK3, K1K1, AT, DM, GNP2K1, GNP3K1, GNP2K21, GNP2K2, and P2K3. This distinction is approximated due to the variations in the type of bacteria and the planting site. IAA production by bacteria are varied due to environmental factors, the growth rate, the availability of amino acids, and other N sources (Frankenberger & Arshad in Yurnaliza, 2010). The decrease of IAA level on day 4 and day 6 caused by the available nutrients has begun to diminish, in this case is tryptophan, because the use of nutrients in each bacteria is different from one another. Some isolates increased along with the time of incubation because on the second day of incubation time, the enzyme that converts tryptophan to IAA still low. Along with the growth rate of bacteria, enzymes that were used in the conversion of tryptophan to IAA were adequate to generate higher IAA.

Endophytic bacteria are microbes that live in the plant tissue and form a colony without harming its host. Every cormophyta, plants that have a stem and root, may contain some endophytic bacteria which is able to produce biological compounds or secondary metabolites which is approximated as the impact of coevolution or genetical transfer from its host into endophytic bacteria (Tan & Zhou, 2001 in Radji, 2004). Types of biological association between endophytic bacteria and its host are varied from neutral, commensialism, to symbiosis. In this condition, a plant is a food source for endophytic bacteria to complete its life cycle (Clay, 1988). Endophytic bacteria can be isolated from the surface of sterile plant tissues or be extracted from inner plant tissues. Particularly, bacteria enter the tissue through the germinated tissue, the root, the stomata, or damaged tissues (Zinniel et al, 2002).

CONCLUSION

The production of IAA which is generated by endophytic bacteria isolates are affected by the isolates type, the age of cultures, and the location. Isolates GNP2K21, GNP2K2 and AT produced the highest IAA with the age of cultures of 4 days

BIBLIOGRAPHY

Aryantha, I.N., D.P. Lestari., N.P.D. Pangesti. 2004. Potensi Isolat Bakteri Penghasil IAA dalam Peningkatan Pertumbuhan Kecambah Kacang tanah Pada Kondisi Hidroponik. Jurnal Mikrobiologi Indonesia. 9 (2) : 43 -46.

Bresson, W. & M.T. Borges. 2004. Delivery Methods for Introducing Endophitic

E.K. James, F.L. Olivares, J. I. Baldani and J. Dobereiner 1996 . Herbaspirillum, an endophytic diazotroph colonizing vascular tissue in leaves of Sorghum bicolor L. Moench. Journal of Experimental Botany, Vol. 48, No. 308 . 785-789

Gordon, S.A. & R.P. Weber. 1951. Colimetric Estimation of Indol Acetic Acid. Plant Physiol. 26: 192-195.

Hindersah, R, D.H. Arief, & Y. Sumarni. Totowarsa. 2003. Produksi Hormon Sitokinin oleh Azotobacter. Prosiding Kongres dan Seminar Nasional HITI, Padang, Juli 2003 : 549-555

Johan H. J. Leveau and Steven E. Lindow , 2005 . Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by Pseudomonas putida Strain 1290. *Applied And Environmental Microbiology* . 2365–2371

K.A. El-Tarabily, A.H. Nassar , G.E.St.J. Hardy andK. Sivasithamparam Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes . Journal of Applied Microbiology Volume 106, Issue 1: 13–26

Keith Clay 1988. Fungal Endophytes of Grasses: A Defensive Mutualism between Plants and Fungi . Ecology 69:10–16.

Khan, Z. and Doty, S. L. 2009. Characterization of bacterial endophytes of sweet potato plants. *Plant and Soil*322:197-207.

Lestari, P., D. N, Susilowati., E. I, Riyanti. 2007. Pengaruh Hormon Asam Indol Asetat yang Dihasilkan oleh Azospirillum sp. Terhadap Perkembangan Akar Padi. Jurnal Agro Biogen. 3(2): 66 – 71.

Mattos K.A., Padua V.L.M., Romerio A., Hallack L.F., Neves B.C., Ulisses T.M.U., Barros C J. Todeschin A. R., Previat J.O and . Mendoca

Previato L.. 2008. Endophytc Colonization of Rice (Oryza sativa L) By The Diazaotrophic Bacteriu Burkholderia Kururiensis and Its Ability. Ann. Acad. Bras.Cienc. 80 (3):477-493

Prasetyoputri, A dan Ines Atmasukarto. 2006. " Biotrend" Mikroba Endofit Sumber Acuan Baru yang Berpotensi. Volume I No.2 : 13 – 15

Radji, M. 2005. Peranan bioteknologi dan mikroba endofit dalam pengembangan obat

Radu & Kqueen, 2002. Preliminary screening of endophytic fungi from medicinal plants in malaysia for antimicrobial and antitumor activity. Malays J Med Sci. 2002 Jul;9 (2):23-33

Saepen, S., S.Jos. and Roseline.R.2007. Indole-3-Acetic Acid in Microbial and Microorganism and Microorganism Plant Signaling. Departemen of Microbial and Moleculer Systems. centre of Microbial and Plant Genetics ; Belgium

Strobel G., Daisy B., 2003, Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiol. Mol. Biol. Rev., 67 : 491–502

Susilowati, D.N., R. Saraswati., E. Yuniari. 2003 . Isolasi dan Seleksi Mikroba DiazotrofEndofitik an Penghasil Zat Pemacu Tumbuh pada Tanaman Kacang tanah dan Jagung Sumberdaya Genetik Pertanian : 128 – 143

Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC, Khan MR (2004). Characterization and screening of bacteria from the rhizosphere of rice grown in acidic soils of Assam. Curr. Sci. 86: 978-985.

Yarnaliza, Mustika Wildasari Siregar dan Nunuk Priyanti, 2010. Peran Bakteri Endofit Penghasil IAA Terseleksi Terhadap Pertumbuhan Tanaman Padi. Prosiding Seminar Nasional Biologi FMIPA USU: 219 -228

Zinniel DK, P Lambrecht, NB Harris, Z Feng, D Kuczmarski, P Higley, CA Ishimaru, Arunakumari, RG Barletta and AK Vidaver. 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl. Environ. Microbiol. 8:2198-2208.

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