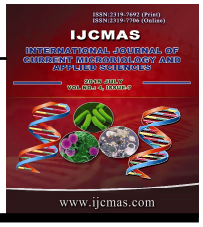
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 593-598

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 593-598**
http://www.ijcmas.com



Original Research Article

Morphological Characteristics and Ability Isolate of *Azotobacter sp.* to Produce IAA Origin from Cocoa Rhizosphere

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Keywords

Azotobakter, Cocoaclones, IAA, Rhizosphere, Sulawesi

A B S T R A C T

Azotobacter sp. is a non-symbiotic bacteria which have the capability of N fixation in the atmosphere and produce hormones IAA, there by potentially as bio-fertilizer and bio-stimulant. The purpose of this study was to characterize the morphology and analyze the ability of *Azotobakter* origin isolates of Sulawesi cocoa clones producing IAA. From the results, 38 isolates *Azotobakter* which has a characteristic morphological with different characters from colony colors and has avaried production capability of IAA concentrations in the range of 0.48 ppm-45.87 ppm. Rhizosphere isolates Az 12 origin cocoa clones Palopo (South Sulawesi) produce the highest IAA concentration of 45.87 ppm or 101.93 times higher than isolates Az 33 origin cocoa clones Sigi (Central Sulawesi) and is selected isolates suspected to function as bio-stimulant.

Introduction

Soil microbial ecosystem includes the total number of microbes, soil composition, and physical properties of soil, includes the biotic and abiotic components. In the ecosystem is an interaction between microbes, where such interactions maybe neutral, mutualism and commensalism, antagonism, competition, parasitism and predation. Microbesin the soil as biochemical agent in the conversion of complex organic compounds in to compounds those are beneficial top lants.

The complexity of the compound formed can be a hormone produced by microbes. *Azotobakter* is a non-symbiotic bacterium that has the ability to fixation of nitrogen and active colonizes to increasing growth and crop production because of the ability to produce growth hormones such as auxin, cytokinin, and gibberellin (Leveau and Lindow, 2005). Interactions between plant crops such as cocoa and soil microbes Azotobacter mutually beneficial effects as Azotobacter from the rhizosphere of plants, known as PGPR (Nasaruddin, 2014).

Indole acetic acid (IAA) is the main auxin hormone in the plant that control various of important physiological processes include the division and growth of cells, differentiation of tissue, and response to light and gravity (Salisbury and Ross,1992). The role of rhizo-bacteria produce the IAA supports the growth of cocoa plants in soils with the poor nutrient and very interesting to be researched.

The purpose of this research was to isolate, characterize and analyze the capability of rhizosphere azotobakter isolate origin from Sulawesi cocoa clones to produce IAA were grown in various environments. Results of this study are expected to be found of isolates as a bio-stimulant function to increase the availability of nutrients and boost the physiological activity and productivity of the cocoa plant.

Materials and Methods

Source of isolates

Azotobakter bacterial isolates originating from Sulawesi cocoa production centers are: Palopo district and Pinrang (South Sulawesi), Polman district and Mamuju (West Sulawesi), and Donggala and Sigi Biromaru (Central Sulawesi). Each location was taken at 4 points and rhizosphere soil samples taken at a distance of 8 cm from the base of the roots at a depth of 5-10 cm.

Isolation and characterization of bacteria Azotobacter

Isolation of bacteria was done using serial dilution method. Soil samples were weighed as much as 2 g and resuspended into10 ml of sterile water, then dilution was carried out from10^{-1} to 10^{-6}. 0.1 ml of the suspension of each dilution was cultured on selective media for 24 hours. Colonies that grew on selective media indicated as a colony isolate of *Azotobacter sp*. Visually after 24 hours of incubation white colonies will reveal characteristic wet and turned into a dark brown after 3–5 days. Morphological characterization was observed on the growth of colonies, form colonies of cells (microscopic), form colonies, colony color, catalase test and Gram reaction by reference to standard procedures.

Production of IAA

Azotobacter is capable of producing IAA in test use Triptic Soy Broth (TSB) media 50% (half-strength) were added with 200 ppm of L-tryptophan as a precursor. IAA production quantitatively based methods spectophotometric according Gravel *et al.* (2007). One milliliter of sample results centrifuged supernatant for 10 minutes at 8,000 rpm, then added 2 ml of solution of reagent Salkowski (Gordon and Weber, 1951), incubated in the dark for 30 minutes and then the absorbance was measured with a spectrophotometer at a wavelength of 530 nm. Pink color change indicates that Azotobacter isolates tested were able to produce IAA. Concentration levels produced by Azotobacter isolates calculated based on the standard curve (Kesaulya *et al.,* 2015). The standard used was pure IAA solution is measured in the same circumstances and conditions.

Results and Discussion

Isolation and characterization of bacteria Azotobacter

Azotobacter bacteria isolation results obtained 38 isolates with varying morphological characters (Table 1). Early stage, all isolates were observed with macroscopic characterization of bacteria. The main characteristics of the genus

Azotobacter is shaped cells cocoid, oxidase negative, catalase positive and form cysts (Figure 1), which serves to protect against extreme environmental conditions, such as drought, ultraviolet light and ionizing radiation (Madigan *et al.,* 1997).

Azotobacter is a cocci bacterium with sized 1.5–2.0μm, not forming endospores but cyst. Move with flagella, are aerobic and chemo organotroph, using glucose, alcohol, salt of organic material to grow, catalase positive. The optimum pH at 7-7.5 and commonly found in soil and water. Certain species can be associated with the roots of plants (Holt, *et al.,* 1999). Shape colony isolates obtained from generally spherical and irregular, varied colonies such as wavy edges, parted, intact and curly. The most dominant elevation colonies on bacterial isolates are flat and the other curved and flat arise. Colors of bacteria are white and yellow, while the rounded shape and stem cells. The results of gram reaction test showed that all isolates are Gram negative.

Hormone production IAA

All Azotobacter isolates tested were able to produce the IAA with different concentration levels. Azotobacter isolates ability to produce IAA marked with a color change to pink. This color change occurs when reacted with Salkowsky solution that caused the interaction between IAA produced by Azotobacter with Fe which forms a complex compound [Fe_2 $(OH)_2$ $(IA)_4$]. The interaction occurs in acidic conditions (Kovacs *et al.,* 2009). According to Kovacs *et al.* (2009) reaction formed there are two kinds of complex reactions and redox reactions. The color pink is increasingly concentrated showed that IAA content generated by higher bacteria.

Results of testing against 38 isolates of Azotobacter were found the concentration range of 0.48 to 45.87 ppm (Table 2). Az 12 isolates origin cocoa clones Palopo (South Sulawesi) has a production capability IAA highest with 45.87 ppm concentration or 101.93 times higher than the lowest concentration of 0.45 ppm achieved by Az 33 Isolates origin cocoa clones Sigi (Central Sulawesi). Found also isolates Az 29 origin cocoa clones Sigi (Central Sulawesi) which is capable of producing IAA with 31.59 ppm concentration or 65.81 times higher than isolates Az 33.

This means the isolates Az 12 is selected isolates and have potential compared to other isolates. Differences in the levels of production of IAA presumably because differences in sources of isolates, species and strains, culturing conditions, stage of growth and the availability of substrate (Mirza *et al.,* 2001), and was also influenced by genetic structure, growth rate and enzyme activity (Khalid, 2004) and is influenced by its ability to convert L-tryptophan as a precursor contained in the medium into IAA.

Spaepen *et al.* (2007) says that the IAA stripe synthesis in bacteria through the indole-3-acetamide (IAM). First tryptophan is converted to IAM by the enzyme tryptophan-2-monooxygenase (IaaM), encoded by the gene IaaM. The second step is converted to IAA by IAM hydrolase enzyme (IaaH), encoded by the gene IaaH.

The diversity of production capabilities IAA by Azotobacter isolates characterized by the degree of concentration produced and influenced also by the ability of the metabolism of each isolate bacteria Azotobacter, where each different bacteria have the ability to convert tryptophan to IAA. Novarro and Barea (1976) found

concentrations of auxin, gibberellins and cytokinins in the culture of *Azotobacter vinelandii* dan *Azotobacter beijerinckia, Azotobacter chroococcum* dan *Azotobacter paspali*.

Obtained 38 isolates of *Azotobacter* sp. isolated from cocoa crop plantations in Sulawesi has characters morphology of colony cell is shaped stem, catalase positive and the same of Gram-negative reaction with different colors of each colonies. Az 12 Isolates has the capability of producing higher IAA as big as 45.87 ppm or by 101.93 times then the lowest concentration of 0.45 ppm is achieved by Isolates Az 33. Isolates Az 12 selected isolates suspected to function as bio-stimulant.

Table.1 Characters morphology and biochemical Azotobacter isolate from cocoa rhizosphere

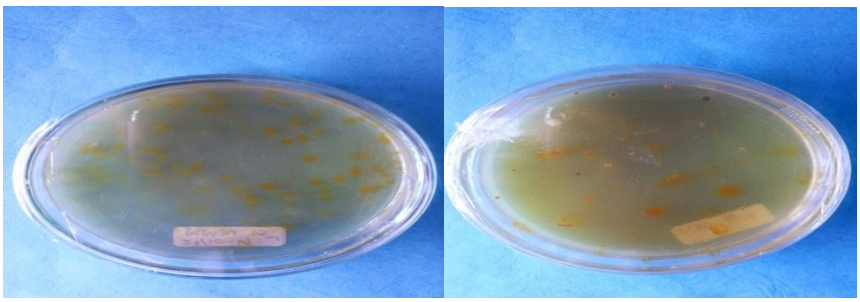
Code of Isolatess	Growth of Colonies	Number of Colonies (24hours)	Form of Cells Colonies	Color of Colonies (24 hours)	Color of Colonies (3-5 days)	Diameter Of colony (mm)	Catalase Test	Gram Test
Az1	+	120	Stem	Yellow	Brown	1,90	(+)	(-)
Az2	+	224	Stem	Yellow	Brown	1,70	(+)	(-)
Az3	+	127	Stem	White	Brown	1,93	(+)	(-)
Az4	+	167	Stem	White	Brown	2.00	(+)	(-)
Az5	+	158	Stem	Yellow	Brown	1,90	(+)	(-)
Az6	++	206	Stem	Yellow	Brown	2.45	(+)	(-)
Az7	+	143	Stem	White	Brown	0,90	(+)	(-)
Az8	+	128	Stem	Yellow	Brown	1,80	(+)	(-)
Az9	+	173	Stem	Cream	Brown	1,40	(+)	(-)
Az10	+	122	Stem	White	Brown	1,80	(+)	(-)
Az11	++	224	Stem	Clear White	Brown	2,50	(+)	(-)
Az12	++++	380	Stem	Yellow	Brown	3,00	(+)	(-)
Az13	+	110	Stem	Yellow	Brown	1,90	(+)	(-)
Az14	+	125	Stem	Cream	Brown	1,90	(+)	(-)
Az15	+	123	Stem	White	Brown	1,70	(+)	(-)
Az16	+	147	Stem	Translucent White	Brown	1,50	(+)	(-)
Az17	+	156	Stem	White	Brown	1,87	(+)	(-)
Az18	+	173	Stem	Yellow	Brown	1,90	(+)	(-)
Az19	+	163	Stem	Yellow	Brown	1,89	(+)	(-)
Az20	+	154	Stem	White	Brown	1,20	(+)	(-)
Az21	+	143	Stem	White	Brown	1,10	(+)	(-)
Az22	++	267	Stem	Yellow	Brown	2,25	(+)	(-)
Az23	+	143	Stem	Translucent White	Brown	1,90	(+)	(-)
Az24	+	161	Stem	Translucent White	Brown	1,87	(+)	(-)
Az25	++	252	Stem	Translucent White	Brown	2.35	(+)	(-)
Az26	+	128	Stem	Yellow	Brown	1,90	(+)	(-)
Az27	+	146	Stem	Translucent White	Brown	1,87	(+)	(-)
Az28	+	139	Stem	Yellow	Brown	1,70	(+)	(-)
Az29	+++	310	Stem	Yellow	Brown	2,67	(+)	(-)
Az30	+	149	Stem	Translucent White	Brown	1,60	(+)	(-)
Az31	+	137	Stem	Clear White	Brown	1,75	(+)	(-)
Az32	+	126	Stem	White	Brown	1,50	(+)	(-)
Az33	+	119	Stem	Yellow	Brown	1,70	(+)	(-)
Az34	+	173	Stem	White	Brown	0,20	(+)	(-)
Az35	+	146	Stem	Yellow	Brown	0,90	(+)	(-)
Az36	+	134	Stem	White	Brown	1,50	(+)	(-)
Az37	+	128	Stem	Yellow	Brown	0,90	(+)	(-)
Az38	+	123	Stem	White	Brown	1,80	(+)	(-)

Description: (-) Gram Negative reaction, (+) positive catalase, +weakly growth, ++ weak growth, +++ strong growth, ++++ very strong

Table.2 Production of hormones IAA (IndoleAcetic Acid) *Azotobacter* isolate

Code of Isolates	Source Location Isolate	Clon	Production IAA (ppm)
Az1	Polman, West Sulawesi	S2	3.65
Az2	Polman, West Sulawesi	S1	9.68
Az3	Polman, West Sulawesi	S2	1.90
Az4	Polman, West Sulawesi	S2	7.78
Az5	Polman, West Sulawesi	S2	1.59
Az6	Mamuju,West Sulawesi	S2	15.71
Az7	Mamuju,West Sulawesi	S2	6.03
Az8	Mamuju,West Sulawesi	S1	2.70
Az9	Mamuju,West Sulawesi	S1	4.29
Az10	Palopo, South Sulawesi	S1	1.54
Az11	Palopo, South Sulawesi	S2	14.44
Az12	Palopo, South Sulawesi	S2	45.87
Az13	Palopo, South Sulawesi	S2	1.59
Az14	Palopo, South Sulawesi	S1	8.73
Az15	Palopo, South Sulawesi	S1	6.67
Az16	Palopo, South Sulawesi	S2	4.29
Az17	Palopo, South Sulawesi	S2	7.94
Az18	Palopo, South Sulawesi	S1	2.38
Az19	Palopo, South Sulawesi	S1	3.33
Az20	Palopo, South Sulawesi	S1	5.56
Az21	Pinrang, South Sulawesi	S2	5.24
Az22	Pinrang, South Sulawesi	S2	15.08
Az23	Pinrang, South Sulawesi	S2	1.90
Az24	Pinrang, South Sulawesi	S2	1.75
Az25	Pinrang, South Sulawesi	S2	14.29
Az26	Pinrang, South Sulawesi	S2	4.29
Az27	Pinrang, South Sulawesi	S2	4.29
Az28	Sigi, Central Sulawesi	S1	11.27
Az29	Sigi,Central Sulawesi	S1	31.59
Az30	Sigi, Central Sulawesi	S1	2.38
Az31	Sigi, Central Sulawesi	S2	6.67
Az32	Sigi, Central Sulawesi	S1	3.33
Az33	Sigi, Central Sulawesi	S2	0.48
Az34	Donggala, Central Sulawesi	S1	3.17
Az35	Donggala, Central Sulawesi	S2	5.40
Az36	Donggala, Central Sulawesi	S2	3.17
Az37	Donggala, Central Sulawesi	S1	3.02
Az38	Donggala, Central Sulawesi	S1	4.92

Figure.1 Characteristic isolates of genus *Azotobacter sp.*



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