Screening Of Free-Living Indole Acetic Acid Producing Rhizobacteria From Shallot Rhizospheres In The Island Of Sulawesi

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Abstract: Auxin producing bacteria can be isolated from plant rhizospheres and used as inoculants for improving the growth and yield of agricultural crops. The present study focuses on the screening of indole-3-acetic acid producing rhizobacterial isolated from the rhizosphere soil of shallot plants grown at five provinces in Sulawesi. A total of 125 bacterial isolates were screened and characterized for the production of bioauxin, Indole-3- Acetic Acid (IAA) by colorimeter method. The IAA activity was induced when the isolates were grown in the presence of L-Tryptophan, a physiological precursor of auxins. 51 out of 125 rhizobacterial isolates were found to produce IAA in the culture filtrates with amount ranged from 0.76 to 2.33 ppm, of these, five isolates (MG9, LB3, MK6-1-1, MK11, and GR25) were able to produce 2.05, 2.14, 2.20, 2.33, and 2.33 ppm of IAA respectively. Therefore, these five isolates are needed to be further studied due to their potential to be developed as plant growth promoting rhizobacteria in the field application.

Keywords: Rhizobacteria, Indole Acetic Acid, Shallot, Sulawesi

1. INTRODUCTION

Shallot is an important tropical and subtropical crop on the basis of its high consumption, nutritional, and cash value to farmers and consumers worldwide. The economically important organ of shallot is bulb, therefore, induction of the bulb growth is important factor in producing a high quality of shallot bulbs. IAA is a type of phytohormone that plays a role in induction of bulbs (Brewster, 2002)[1] and promotes plant growth. Khalid *et al.,* (2004)[2] were successful screened IAA producing rhizobacteria isolated from rhizosphere of wheat and demonstrated increases in root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 37.7%) and shoot dry weight (up to 36.3%) of inoculated wheat seedlings. Mohamed-Yassen *et al*., (1994)[3] reported the in vitro highest multiplication of cv. Red California shallot grown on medium containing 0.15 µM Thidiazuron and 0.1 µM NAA. found that combination of 2 mg L^{-1} IAA and 4 mg L^{-1} Kinetin gave the best plantlet production of in vitro shallot plants. It has been well known that normal plant growth and development is controlled by hormone compounds produced by the plant itself.

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However, plants may not have the capacity to synthesize sufficient endogenous plant hormones for optimal growth and development under suboptimal environmental conditions. Additional input of the IAA by bacteria could modify endogenous auxin synthesized by the plant to optimal level, resulting in the induction of growth and development in desired direction (Pattern and Glick, 1996)[4]. However, supply of exogenous plant hormones such as auxin on shallot crop by a method of spraying is not effective due to the presence of a wax layer on the leaves surface. Another potential and economical source of these phytohormones is the soil microbiota. A majority of soil microorganisms, such as *Pseudomonas* sp., *Azospirillium* sp., *Azotobacter* sp., *Bacillus* sp., *Lactobacillus* sp., *Paenibacillus polymyxa*, *Enterobacter* sp., *Serratia marcescens*, *Klebsiella* sp., *Alcaligenes faecalis* and cyaonobacteria release these secondary metabolite compounds (Torres-Rubio *et al.,* 2000[5]; Leveau and Lindow, 2005)[6]. The activities of rhizosphere microbiota, may provide a continuous source of active substances for plant uptake and therefore for improving the growth and developing of shallot bulbs, which is better than one time application of synthetic compounds. Khalid *et al.,* (2004)[2] suggested that auxins produced by microbial origin in the area of plant roots may raise a physiological response in the host plant, therefore, screening of the auxin production rhizobacteria in vitro could provide a reliable method for selection of effective Plant Growth Promotion Rhizobacteria (Khalid *et al.,* 2004)[2]. The present work aims to screen the collection of rhizobacteria originating from different sites of shallot rhizospheres soil in Sulawesi island for their ability to produce IAA. The availability of a suitable precursor is one of the primary factors affecting microbial secretion of these secondary metabolite products. Studies have shown that microbial production of phytohormones can be increased several fold by providing their suitable precursors. L-Tryptophan (L-TRP) is considered an efficient physiological precursor for the biosynthesis of auxin as the addition of L-TRP into the bacterial culture can increase the production of IAA (Khalid *et al.,* 2004[2]; Chaiharn and Lumyong, 2011)[7]. Therefore, in this study the in vitro production of IAA by the rhizobacteria was tested in the presence of L-TRP.

2. MATERIALS AND METHODS

Microbial Isolation

Bacterial used in this work were independently isolated from the rhizosphere of different shallot fields grown in the Sulawesi region. Soil samples were taken from the vicinity of the roots (rhizosphere) of healthy shallot plants grown in Malakaji (South Sulawesi), Limboro (West Sulawesi), Sidera (Central Sulawesi), Maligano (Southeast Sulawesi) and Ilomangga (Gorontalo). The soil samples were serially diluted and 5 ml of samples solution were spread on Nutrient Agar (NA) medium and incubated at 28 ± 2℃ for 24 h. Colonies showing prolific growth were selected and purified by streaking five times on fresh plate of NA. 125 bacterial isolates having different morphological appearance were stored in slants NA medium before used for measurement auxin production as IAA equivalents.

1. IAA Production

A collection of 125 bacterial isolates were previously screened for production of IAA production using the modified method as described by Zakry *et al.,* (2010)[8]. The bacterial isolates were grown on nutrient agar medium supplemented with 0.1 g/l L-Tryptopane and incubated at 28 ± 2℃ for 3 days.

2. Colorimetric Analysis

The bacterial isolates were assayed for production of IAA using the modified method developed by Loper and Scroth (1986)[9]. Bacterial colonies growing on nutrient agar as described above were harvested by pouring 5 ml of distilled water and centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of ortophosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of sulphuric acid, 1 ml 0.5 M $FeCl_3$). The Development of a pink-colored in the culture filtrate after 24 hours of incubation indicates the occurrence of IAA. Intensity of the color was measured on spectrophotometer at 530 nm. Concentration of IAA produced by cultures was measured using standard graph of IAA (Sigma-Aldrich) obtained in range of 0.5 – 10 ppm.

3. RESULTS AND DISCUSSION

Rhizobacterial Collection

This study has successfully collected 125 pure bacterial isolates with prolific growth and assayed the ability of the isolates in producing IAA in vitro. Of these isolates, 29% were isolated from shallot rhizosphere in South Sulawesi (36 isolates), 22% (28 isolates) were originating from shallot rhizosphere soils in Gorontalo, 21 isolates (17%) were recovered from Southeast Sulawesi, 16% of rhizobacterial (20 isolates) were isolated from Center and West Sulawesi shallot rhizospheres respectively (Figure 1). Data revealed that some bacteria isolated from rhizosphere of shallot plants had the ability to produce IAA in the presence of auxin precursor L-TRP.

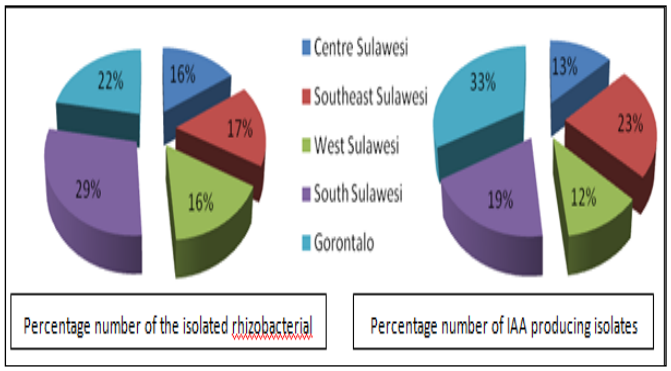

Fig 1. Percentage Number Of The Rhizobacterial Isolates Produced Indole Acetic Acid

Production of indole Acetic acid

Indole acetic acid is a type of auxin that essential for plant growth and development. IAA involved in various plant physiological processes such as root initiation, cell elongation, vascular tissue differentiation and flowering (Husen and Saraswati, 2003)[10].

Fig 2. Samples Of The Pink Colour Solution Containing Indole Acetic Acid

Salkowski reagent used in production of IAA assay serves as an indicator of the IAA biosynthesis. This reagent reacts with indole pyruvic acid that accumulates in the filtrate resulting in the formation of pink color. Indole pyruvic acid catalysis is a product of tryptophan by tryptophan transaminase in the biosynthesis of IAA (Husen and Saraswati, 2003)[10]. About 41% of the bacterial isolates had the ability to produce IAA, which is known to stimulate both rapid and long term responses in plants. The highest number of IAA producing isolates (61%) were detected from shallot rhizosphere soils in Gorontalo (17 out of 20 isolates), followed by bacteria isolated from Southeast Sulawesi (57%), Centre Sulawesi (35%), West Sulawesi (30%), and South Sulawesi (28%) soils respectively (**Table 1**).

Table 1. Indole Acetic Acid Activity By Isolates Rhizobacterial From 5 Province In Sulawesi

Origin	Isolate Code	Number of Isolates Screening	Number of IAA producing Isolates	Percentage (%)
Centre Sulawesi	PL	20	7	35
Southeast Sulawesi	MG	21	12	57
West Sulawesi	LB	20	6	30
South Sulawesi	MK	36	10	28
Gorontalo	GR	28	17	61
Total		125	51	

Indole acetic acid, a type of auxin, is one of important phytohormone which is known to affect plant growth throughout ontogeny. This hormone plays a role in many aspects of plant growth, including cell division and elongation, differentiation, tropisms, apical dominance, senescence, abscission and flowering (Zhao *et al.,* 2001)[11]. The percentage of IAA producing bacteria isolated from the shallot rhizospheres soil in Sulawesi region was lower than the 80% reported for rhizosphere bacteria by Patten and Glick (1996)[4]. A previous study by Barea *et al.,* (1976)[12], reported that about 86% of the 50 bacteria isolated from the rhizospheres soil of some plants were able to produce auxin. However, the ability of the isolates used in this study in producing IAA was higher than bacteria isolated from the rhizosphere soil of rice grown in northern Thailand (Chaiharn and Lumyong, 2011)[7] and from the rhizosphere soils of wheat grown at different sites (Khalid *et al.,*2004)[2].

Table 2. Indole acetic acid concentration produced by rhizobacteria of shallot rhizospheres soil

Origin	Isolate Code	IAA Quantity (ppm)	Origin	Isolate Code	IAA Quantity (ppm)
Centre	PL 1	1.08	South	MK2	1.53
Sulawesi	PL 2	1.02	Sulawesi	MK 5-1	1.14
	PL 6	1.81		MK 6-1-1	2.20
	PL 8	1.74		MK 8	1.03
	PL 9	1.38		MK 11	2.33
	PL 12	0.92		MK 12-1	0.76
	PL 18	1.65		MK 13	1.21
Southeast	MG 1	1.01		MK 13-1	1.38
Sulawesi	MG 2	1.02		MK 15-2	1.46
	MG 3	1.90		MK 22	1.46
	MG 5	1.10	Gorontalo	GR 1	1.64
	MG 7	1.16		GR 3	1.12
	MG 9	2.05		GR 5	1.71
	MG 10-1	1.72		GR 7	1.18
	MG 10-2	1.17		GR 8	0.92
	MG 11	1.17		GR 11	1.83
	MG 12	1.46		GR 13	1.63
	MG 13	1.51		GR 14	0.95
	MG 14	1.60		GR 15	0.92
West	LB 3	2.14		GR 16	1.67
Sulawesi	LB 4	1.20		GR 18	1.15
	LB 5-2-1	1.71		GR 19	1.38
	LB 8	1.97		GR 20	1.08
	LB 11	1.66		GR 21	1.11
				GR 22	1.55
				GR 24	1.99
				GR 25	2.33

Colorimetric analysis showed variable amount of auxins (ranging from 0.76 to 2.33 ppm) produced by the rhizobacteria in vitro (Table 2). 17 rhizobacteria isolated from Gorontalo's shallot rhizosphere soils (GR) were able to produce IAA in culture filtrates with quantity ranged from 0.2 to 2.33 ppm. Isolate GR 25 produced the highest amount of IAA (2.33 ppm) while isolates GR 8 and GR 15 produced the lowest amount of IAA (0.92 ppm). 12 bacteria isolated from the rhizosphere of Southeast Sulawesi shallot (MG) produced IAA in vitro (amount ranging from 1.01 to 2.05 ppm). Maximum IAA (2.05 ppm) was produced by isolate

MG 10-1 while minimum IAA (1.01 ppm) was produced by isolate MG 5. Altogether 20 isolates originating from the rhizosphere shallot in Centre Sulawesi (PL) produced IAA with amount ranged from 0.92 to 1.81 ppm. Isolate PL 6 produced the highest quantity of IAA (1.81 ppm) while isolate PL 12 produced the minimum amount of IAA (0.92 ppm). Only ten isolates from total of 36 bacteria isolated from South Sulawesi (MK) shallot rhizosphere can produced IAA with amount ranged from 0.76 to 2.33 ppm. Isolate MK 11 produced the highest quantity of IAA (2.33 ppm) and minimum IAA was produced by isolate MK 12-1 (0.76 ppm). Two isolates recovered from West Sulawesi shallot rhizosphere (LB 3 and LB 4) produced maximum (2.14 ppm) and minimum IAA (1.20 ppm) respectively. The rhizobacteria tested in this study using Salkowski reagent produced similar levels of IAA to those recorded by other researchers (Ahmad *et al.,* 2008;[13] Zakry *et al.,* 2010 [8]; Ahmed, 2010[14]; Ponmurugan *et al.,* 2011[15]; Kieu and Le, 2011)[16].

4. CONCLUSIONS

The Indole acetic acid activity was induced by the presence L-Tryptophan, a physiological precursor of auxins. Of the 125 rhizobacterial isolates, 51 isolates were able to produce IAA in culture filtrates after added ortophosphoric acid and reagent Salkowski and 5 isolates (MG9, LB3, MK6-1-1, MK11, and GR25) were more prolific IAA producers than other isolates.

REFERENCES

[1]. Brewster, JL., 2002. Crop Production Science in Horticulture 3 : Onions and Other Vegetable Alliums 2nd ed. CAB International. Amsterdam. 227p.

[2]. Khalid A, Arshad M and Zahir ZA. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol. 96 (3): 473-80.

[3]. Mohamed-Yassen, Y., Splittstoesser, W. E., & Litz, R. E. (1994). *In vitro* Shoot Proliferation and Production Sets from Garlic and Shallot. *Plant Cell Tiss. Org. Cult.*, Vol. 36, pp. 243-247, ISSN 0167-6857.

[4]. Patten CL, Glick BR. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Can J Microbiol* 42:207-220.

[5]. Torres-Rubio MG, Valencia-Plata SA, Bernal-Castillo J, Martínez-Nieto P (2000). Isolation of enterobacteria, Azotobacter sp. And Pseudomonas sp., producers of Indole-3-Acetic Acid and siderophores, from Colombian rice rhizosphere. Rev. Lat Am. Microbiol., 42: 171-176.

[6]. Leveau JH., Lindow SE., 2005. Utilization of the plant hormone indole-3-acetic acid for growth by Pseudomonas putida strain 1290. Appl Environ Microbiol 71: 2365–2371.

[7]. Chaiharn M and Lumyong S. 2011. Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. Current Microbiology. 62 (1): 173-181.

[8]. Zakry, F.A.A., Halimi, M.S., Abdul Rahim, K.B., Osumanu, H.A., Wong, S.K., Franklin, R.K., Stephen, L.C.T., and Make J., 2010. Isolation and Plant Growth Promoting Properties of Rhizobacterial Diazotrophs from Pepper Vine. Malaysia Application Biology. 39(2):41-45.

[9]. Loper, J.E., Scroth, M.N., 1986. Influence of bacterial sources on indole-3 acetic acid on root elongation of sugarbeet. Phytopathology 76, 386-389.

[10]. Husen E, Saraswati R. 2003. Effect of IAA-producing bacteria on the growth of hot pepper. *J Mikrobiol Indones* 8: 22-26.

[11]. Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J. A role for flavin monooxygenase–like enzymes in auxin biosynthesis. *Science* 291 : 306 - 309.

[12]. Barea, J. M., E. Navarro, and E. Montoya. 1976. Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. J. Appl. Bacteriol. 40: 129-134.

[13]. Ahmad, Farah., Igbal Ahmad and M.S. Khan. 2008. Sreening of free-living rhizobacteria for their multiple plant growth promoting activities. Microbiological Research 163: 173 -181.

[14]. Ahmed, Ambreen and Shahida Hasnain. 2010. Auxin-producing *Bacillus* sp.: Auxin quantification and effect on the growth of *Solanum tuberosum*. Purre Appl. Chem. 82 (1): 313 – 319.

[15]. Ponmurugan, P., K. Manjukarunambika and M.S. Reddy. 2011. *In vitro* studies on efficacy of *Pseudomonas* spp. For plant growth promoting traits and biocontrol of diseases in tea plants. Proceeding of the 2an Asian PGPR Conference, August 21-24, 2011, Beijing, P.R. China. Plant Growth-Promoting Rhizobacteria (PGPR) for Sustainable Agriculture. 166 - 178.

[16]. Kieu, Le Nhu and Le Thu Thanh Thuy. 2011. Isolation and Selection plant growth promoting microorganism from the soil of rubber in Son La, Dien Bien and Lai Chau Provinces – Vietnam. Proceeding of the 2an Asian PGPR Conference, August 21-24, 2011, Beijing, P.R. China. Plant Growth-Promoting Rhizobacteria (PGPR) for Sustainable Agriculture. 166 - 178.