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Isolation and Molecular Identification of Indole Acetic Acid-Producing Endophytic Bacteria from Daun Dewa Plant (Gynura divaricata (L.) DC)

Ratna Dewi Setyowati*, Artini Pangastuti, Ari Susilowati
Bioscience Study Program, Graduate Program, Universitas Sebelas Maret,
Jl. Ir. Sutami 36 A, Kentingan, Surakarta, 57126, Indonesia
*Corresponding author: ratnadewisetyowati7@gmail.com

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Abstract. *Indole acetic acid (IAA) is an auxin hormone that can regulate plant growth and development. Bacteria produce IAA through L-tryptophan metabolism. The purpose of this study was to determine the ability of endophytic bacteria isolated from Daun Dewa plants to produce IAA to increase the germination and growth of tomato seedlings. Isolation of endophytic bacteria was carried out by sterilizing plant surfaces. The production of IAA is done by adding L-tryptophan precursors. The results showed that there were six endophytic bacteria, and three isolates of them have the potential to produce IAA, namely: AD1.1, UD1.1, and UD2.1. The highest IAA concentration was produced by isolate AD1.1. Growth in sprouts is significantly influenced by the concentration of IAA; the higher the concentration of IAA, the longer the shoots and the roots. Based on the 16S rRNA gene sequence, these isolates belong to the genus* Klebsiella.

Keywords: *Daun Dewa Plant, Endophytic bacteria, Indole acetic acid (IAA), 16S rRNA*

INTRODUCTION

Endophytic bacteria are symbiotic bacteria that spend part of their life cycle in plant tissue without harming the host cell. Each plant has several endophytic microbes that also produce metabolite compounds due to coevolution or gene transfer from host plants into endophytic microbes. Endophytic bacteria have an association between bacteria and plants that has an impact on plant productivity, both directly and indirectly. One direct impact is the ability of bacteria to act as growth stimulants by producing growth regulators, also known as phytohormones, as described by Gamalero and Glick in 2011.

Numerous research have been conducted to determine the potential of the metabolite chemicals found in endophytic bacteria. IAA hormone can be produced by endophytic bacteria, and this hormone is good for plant growth. It is known that some endophytic bacteria, including *Enterobacter* sp., *Azotobacter, Klebsiella, Bacillus, Serratia Pseudomonas* sp., and *Azospirillium* sp., can generate and manufacture indole acetic acid (Tangapo, 2020).

To maximize plant growth, plants can't produce enough IAA (indole acetic acid). In order to encourage plant growth from the outside, symbiotic microorganisms, including the assistance of endophytic bacteria, are required. Plants will take up hormones released by bacteria, causing them to grow more quickly or larger. The IAA hormone has the biological ability to create compounds that can boost plant growth, height, and germination (Grobelak et al., 2015).

Indole Acetic Acid (IAA) is one of the auxin hormones that regulate plant growth and development processes such as vascular tissue differentiation, apical dominance, root initiation (lateral roots and root fibers), cell division, elongation of roots and stems, and seed germination. Natural IAA hormones, in addition to being synthesized by plants that are actively developing, can also be synthesized by bacteria, such as endophytic bacteria.

Auxin, a hormone that encourages plant growth, is typically present in meristem tissues (Gavrel et al., 2007). To maximize plant growth, plants can't produce enough IAA (indole acetic acid). In order to encourage plant growth from the outside, symbiotic microorganisms, including the assistance of endophytic bacteria, are required. Plants will take up hormones released by bacteria, causing them to grow more quickly or larger. According to Grobelak et al. (2015), the IAA hormone can physiologically produce chemicals that promote seed germination, plant height, and growth.

Tomatoes have a wide range of uses, both in the form of vegetables and fruit and in the form of processed foods. This very common use of tomatoes causes the demand for tomato production to be high. Tomatoes have a high nutritional content, such as proteins, carbohydrates, fats, minerals, and vitamins (Bernadus and Wahyu, 2002). To increase production, approaches can be taken. One approach is the utilization of microbes as biological agents, with the ability of endophytic bacteria to produce the phytohormone IAA.

MATERIALS AND METHODS

1. Isolation of Endophytic Bacteria

Gynura divaricata (L.) DC obtained from Flora Herba Nusantara. Daun Dewa plants were washed with running water to remove dirt. in the form of dust and soil. The plant sample is cut in 1-2 cm. The samples were treated by soaking Tween 20 for 5 minutes by shaking. The sample was washed with sterile aquades several times, soaked with 0.5% sodium hypochlorite for 5 minutes, rinsed with 70% alcohol for 2 minutes while shaken and then washed with sterile distilled water three times. For the isolation of endophytic bacteria, the surfaces of sterilized and cut plants are then macerated in pH 7.4 and serially diluted to 10^3 dilution, from 100 µL gilded into the media to be incubated for 24 hours at 37°C then periodically observed for bacterial growth (Jasim *et al.*, 2014).

2. In vitro IAA Production Test

IAA production quantitatively uses 100 µL of each endophytic bacterial inoculum (108 CFU/ml) inoculated to the nutrient broth (NB) medium without and with the addition of L-tryptophan (100, 200, 300 µg ml/L and incubated for 3 days at 120 rpm at 37°C. The bacterial inoculum was centrifuged at a speed of 3000 rpm for 20 minutes. One ml of the centrifuged supernatant was transferred to a sterile tube and added 2 mL of Salkowski reagent were then incubated for 30 minutes in the dark. The absorbance value measured using a UV-Vis spectrophotometer at 530 nm. The quantity was determined by IAA standard solution (Khan *et al.*, 2010).

3. In vitro tomato seed germination test

Tomato seeds are surface sterilized using sodium hypochlorite (NaOCl) 1% for 5 minutes and then washed with sterile distilled water 3 times. Inoculum preparation, Nutrient broth media were added with L-tryptophan 100, 200, 300 µg/ml respectively. Seed coatings were done with 100 µL of IAA positive bacterial culture. Each bacterial culture was given to tomato seeds then incubated according to treatment at 37°C with 120 rpm for 24 hours in a shaker incubator. Each seed was transferred into a sterile Petri dish and given with sterile cotton soaked with sterile distilled water. The seed was then stored at room temperature for one week (Rui *et al.*, 2016). The shoot length was measured from the base of the stem until the end of the leaves with a ruler. While for the root length measured based on the number of and position the root (lateral root). All data were analyzed with analyses of

variance (ANOVA) using SPSS software. Futher test data analyzed using Duncan's Multiple Range Test (DMRT) test 5% (Taiz *et al.*, 2002).

4. Amplification of 16S rRNA gene using PCR method

Endophytic bacteria genomic DNA were extracted using Presto™ Mini gDNA Bacteria Kit (Geneids). The 16S rRNA gene of the endophytic bacteria was amplified using forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387r (5'-GGG CGG TGT ACA AGG C-3'). Amplification of Polymerase Chain Reaction (PCR) mixture consisted of 12,5 µl 2X myTaq Hs Red Mix DNA polymerase, 1,25 µl of each primer (10 pmol), 1 µl DNA template (100 ng) and 9 µl of ddH2O. Thermocycling conditions included pre-denaturation at 95°C for 1 minute, and 35 cycles of denaturation at 95°C for 15 seconds, annealing of 58°C for 15 seconds, elongation 72°C for 15 minutes. The finalizing process was performed at 72°C for 3 minutes (Marchesi *et al.*, 2002), and then the checked by with electrophoresis. PCR products of the 16S rRNA gene were then sequenced by sending it to 1st Base Singapore. The 16S rRNA gene sequences were analyzed using BLAST Nucleotide software on the NCBI website to identify the species of the endophytic bacteria base on the sequence similarity with the GenBank database.

RESULT AND DISCUSSION

1. *In vitro* IAA production

Six endophytic bacterial isolates were obtained from the Daun Dewa plant. The six isolates were Ud1.1, Ud2.1, Ad1.1, Bd2.1, Bd2.2, and Ad1.2. Among the six isolates, three isolates produced the highest IAA production, namely Ud1.1, Ud2.1, and Ad1.1 in the nutrient broth media, which was marked by a change in color from reddish yellow to pink, which was caused by the Salkowski reagent, where FeCl3 is incorporated in the Salkowski reagent, so it is bound to IAA. The color change is first seen at the highest IAA concentration within minutes and continues to increase in intensity after 30 minutes of incubation. The higher the color density, the more it indicates that the amount of IAA produced by the bacteria is also increasing. This opinion is consistent with the research. According to Yuniarti & Purwani (2007), qualitative analysis on agar plate media enriched with tryptophan is characterized by the formation of a pink color around the colonies after being given Salkowski reagent, and according to the opinion of (Shaharoona et al., 2006; Joshi & Bath, 2011) showed that, the potential for color change will be greater if the concentration of Salkowski used is higher.

Endophytic bacterial isolates are known to have the ability to produce IAA in the nutrient broth medium without tryptophan or with the addition of tryptophan (Table 1).

Table 1. IAA production by endophytic bacterial isolates of Daun Dewa plant

L-tryptophan Concentration	IAA concentration the result of isolat					
	Ud1.1	Bd2.1	Bd2.2	Ud2.1	Ad1.1	Ad1.2
NB + L-trp 100	359.8 [a]	118.2[ab]	253.2[b]	556.5[b]	621.5[b]	181.5[b]
NB + L-trp 200	386.5[b]	134.8[b]	233.2[b]	589.9[c]	651.5[c]	188.8[b]
NB + L-trp 300	511.5[c]	111.5[ab]	259.8[b]	786.5[d]	671.5[d]	206.5[c]
Control	354.5[a]	71.5[a]	66.5[a]	354.8[a]	433.2[a]	93.2[a]

Note: letters on the same column show no significant difference based on Duncan's test of 5% level.

In the medium added with L-tryptophan, the three isolates, such as Ud1.1, Ud2.1, and Ad1.1, were able to produce higher IAA compared to other isolates. This is because the amino acid L-tryptophan functions as a precursor in IAA synthesis by bacteria through tryptophan dependent pathways (Marag *et al.*, 2018) and also indicates that the microbe can utilize L-tryptophan as a precursor compound for IAA production (Matsuda *et al.*, 2005). However, for treatments without the addition of tryptophan, bacteria can still produce IAA, even in small amounts. The three isolates showed significant differences, which means that there was an influence from the addition of L-tryptophan to the endophytic bacterial isolates.

Except for isolates Bd2.1, Bd2.2 and Ad1.2, these three isolates were able to produce IAA but with low yields. The addition of L-tryptophan with different concentrations was carried out to find out the effect of L-tryptophan on producing IAA.

The difference in IAA production from various isolates can be influenced by the type of bacteria and its ability to convert L-tryptophan contained in the media into IAA. When viewed in terms of the media used and the process of IAA production, the concentration of IAA produced by these bacteria cannot be used as a basis for assessing the superior quality of a bacterium in producing the phytohormone IAA. This can be influenced by several variations in composition and conditions. This is consistent with Bambang's (2013) variation of media composition and production process conditions (such as temperature, pH, stirring speed, and incubation time), which remained the same for each bacterial isolate in this study. In addition to variations in bacterial isolates, media composition and production process conditions also have an impact on the concentration of IAA produced. Each bacterial isolate requires optimal conditions in media composition and production process in order to produce IAA phytohormone maximally.

2. Germination of tomato seeds *in vitro*

Tomato seeds were incubated with the bacterial culture that produced the highest IAA (Ad1.1, Ud1.1, Ud2.1). Bacterial cultures were given L-tryptophan with different concentrations. The bacterial isolate Ad1.1 showed a very good increased in seed growth (Table 2).

Table 2. Effect of seed coating with IAA-producing endophytic bacteria on tomato seed germination (*in vitro*)

L-Tryptofan concentration (g/ml)	Name of isolates					
	Ud1.1		Ud2.1		Ad1.1	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
100	1.876^{b} ± 0.15	0.341 ^{a} ± 0.05	1.238^{b} ± 0.07	0.626 ^{a} ± 0.13	3.029^{b} ± 0.22	1.351 ^{b} ± 0.10
200	2.269 ^{c} ± 0.07	0.446^{b} ± 0.05	1.371^{c} ± 0.05	0.640^{a} ± 0.01	4.184^{c} ± 0.08	2.433^{c} ± 0.09
300	2.547 ^{d} ± 0.08	0.628 ^{c} ± 0.004	1.862^{d} ± 0.09	0.774^{b} ± 0.06	5.282^{d}± 0.02	2.756^{d} ± 0.12
Control	0.813^{a}± 0.04	0.377^{a} ± 0.04	0.093^{a} ± 0.03	0.601^{a} ± 0.10	0.942^{a} ± 0.04	0.773^{a}± 0.010

Note: mean ± standard deviation of 3 replications p <0.05, Letters in the same line show no significant difference based on Duncan's test of 5% level.

Seed coating with endophytic bacteria has the ability of different to promote growth and increase seed germination yield. The germination rate in the treatment of endophytic bacterial isolates was higher than in the control this shows there is a significant change. The germination rate will also affect the shoot and root length of the sprouts. Therefore, the use of endophytic bacterial isolates or biological agents can increase shoot and root length.

In the Ad1.1 with a concentration L-tryptophan different able to encourage seed germination, so in the concentration L-tryptophan 100, 200 dan 300 µg/ml influential significantly against shoot and the root length germination tomatoes compared to control. in the Ad1.1 produces higher seed germination compared with the treatment endophytic bacterial another. This proves that at low concentrations of IAA can cause elongation of roots and stem shoots as well as at high concentrations. While for Ud1.1 treatment showed results that were not different from Ad1.1 treatment although lower than Ad1.1 treatment. Then for Ud2.1 with the addition of IAA concentrations from 100 to 300 µg/ml was able to cause elongation of the stem, while the roots had no effect compared to isolates Ad1.1 and Ud2.1. According to (Patten and Glick2002)., High concentrations of exogenous IAA can induce lateral and adventitious roots, while low concentrations of exogenous IAA can stimulate the growth of primary roots and shoots.

The use of bacterial isolates and L-tryptophan concentration in tomato seed soaking has the potential to accelerate the germination process and increase the number of germinated seeds in Ud1.1 and Ad1.1 varieties. As a result, the faster the tomato seeds germinate, the better their growth as shown in (Table 3).

Table 3. Percentage of effect seed coating with IAA-producing endophytic bacteria on tomato seed germination (*in vitro*) in one-week observation.

Concentration L-tryptophan µg/ml	Number of seeds germinated (%)		
	Ud1.1	Ud2.1	Ad1.1
100	17^{b} ± 1.0	15 ^{b} ± 1.0	20^{b} ± 1.0
200	19^{b} ± 1.0	10 ^{a} ± 1.0	16 ^{a} ± 1.0
300	23^{c} ± 2,1	10 ^{a} ± 1.5	25^{c} ± 1.0
Control	14^{a} ± 1,0	13^{b} ± 1.0	20^{b} ± 2.0

Seed Coating stored for one week with the addition of L-tryptophan to every isolate shown percentage different (Tabel 3). The treatment Ud1.1 with L-tryptophan 100, 200 dan 300 have increased significantly compared with the control. This is indicated that the Ud1.1 with L-tryptophan potentially improves seed germination. While to Ud2.1 in the L-tryptophan 100 did not significant different against the control and concentration 200 and 300 showed the same. It is likely caused by several things that was a layer of coating hard and dry resulted in time water inhibition become longer so hamper radicular to come out. On the show result, the isolate Ad1.1 higher compared to the constitution of is Ud1.1 and Ud2.1. this is indicated that the isolate Ad1.1 with L-tryptophan potentially improves the germination of the seeds of tomatoes with the influence of the hormone indole acetic acid (IAA).

Based on the results obtained indicate that the increase in seed germination results, which is due to the direct influence of endophytic bacteria. The direct effect comes from endophytic bacteria that can produce IAA growth regulating-hormones, giving different responses to shoot and root lengths. In the same IAA, a concentration gives a different growth response to each part of the plant organs (Silva *et al.*, 2004). This is similar to research (Kunkel *et al.*, 2006) that, the ability of biological agents can increase crop yields, and is closely related to the ability of biological agents to synthesize plant hormones such as IAA, IBA, and GA

3. Endophytic bacterial identity based on 16S rRNA gene sequences

Endophytic bacterial isolates were identified molecularly based on sequences of genes of encoding 16S rRNA. Bacterial genomic DNA was amplified by the PCR method with primers 63f and 1387r discovered that those primers could amplify 16S rRNA gene sequences with a size of about ± 1.400 bp. PCR products from each of the three endophytic bacterial strains showed almost the same size, which was about ± 1.400 bp. Description: M= Marker DNA ladder, (1) = Ud1.1, (2) = Ud2.1, (3)= Ad1.1. Visualization of PCR products could be seen in Figure 1 and the closed species of the endophyte producing IAA isolates based on the 16S rRNA sequence presented in (Table 4 and Figure 1).

Table 4. Identity of IAA producing endophytic bacteria from Daun Dewa plant based on sequences of 16S rRNA.

No	Isolation Name	Closest Species Identity	Identity %	Accession Number
1	Ud1.1	*Klebsiella quasipneumoniae* subsp st P64	98.04	MK336743
2	Ud2.1	*Klebsiella pneumoniae* strain KP1804	98.38	MK386785
3	Ad1.1	*Klebsiella pneumoniae* strain GBA758	97.06	HM209778

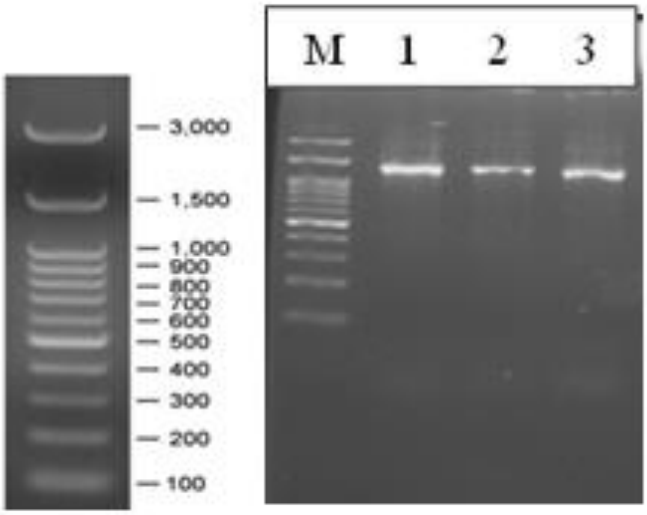

Figure 1. Agarose gel electropherogram of 16S rRNA gene amplification of endophytic bacteria.

Genuse of the *Klebsiella* are widely distributed in nature and they are often found in soil and plants. Although these strains were confirmed as endophytes, under some conditions they might be opportunistic pathogens (Kunkel *et al.*, 2006). From the studies of (Celloto *et al* ., 2012), it is evident that a large number of bacterial diazotrophs including Klebsiella have the ability to synthesis IAA. According to Bhattacharyya and Jha (2012), various types of bacteria have been identified as IAA producers, such as Aeromonas veronii, Agrobacterium sp., Alcaligenes piechaudii, Azospirillum brasiliense, Bradyrhizobium sp., Enterobacter cloacae, Rhizobium leguminosarum, and Klebsiella pneumoniae (Sachdev et al. 2009). In addition, several bacterial species from the genus Pseudomonas, Aerobacter, Bacillus, and Klebsiella are also known to have the potential to fix nitrogen and produce the hormone IAA. (Chen et al. 2013) and (Rosenblueth et al, 2008).

The high-level similarity of those isolates compared to GenBank data confirmed that these isolates belonged to the same genus as mention in the database. According to (Drancourt *et al* ., 2009) based on comparison sequences of genes encoding 16S rRNA in GenBank, two bacteria that had the maximum degree of similarity (Maximum identity) ≥ 99% indicates that the species being compared are the same, while the maximum rate ≥ 97% similarity can be stated that the isolates are in the same genus and similarities between 89-93% showing different family.

CONCLUSION

Endophytic bacterial isolated from *Gynura divaricata* (L.) DC, medicinal plant, obtained six endophytic bacteria and three isolates of endophytic bacteria that exhibit activity in producing hormones indole acetic acid (IAA) and increase in seed germination, shoot and root growth *in vitro* by seed coating. These isolates belong to the genus *Klebsiella*.

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REFERENCES

A,G. Moat, J.W. Foster, and .P. Spector. 2007. *Micrbial Physiology.* Fourth Edition. New York: Wiely-liss. Inc

Berkum,V.P. and B.B.BOHLOOL.1980. Evaluation of nitrogen fixation by bacteria in association with roots of tropcal grasses. *Microbiol Rev.* 44 (3): 491-517

Bernadus, T dan W. Wahyu. 2002. Bertanam Tomat. Agromedia Pustaka. Jakarta.

Bhattacharyya PN, Jha DK(2012)Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture.World Journal of Microbiology & Biotechnology.28:1327 –1350.

Bianco C, Imperlini E, Calogero R, Senatore B, Pucci P, Defez R. 2006. Indole-3-acetic acid regulates the central metabolic pathways in Escherichia coli. *Microbiology* 152: 2421-2431

Chen M, Lin L, Zhang Y, Sun L, An Q. 2013. Genome sequence of Klebsiella oxytoca SA2, an endophytic nitrogen-fixing bacterium isolated from the pioneer grass Psammochloa villosa. Genome Announc. 1(4):e00601–13.

Davies PJ. 2004. The plant hormonrs: Their nature, occurrence and function, In : Davies pj (ED) Plant Hormones: Biosyntetis, Signal Transduction, Action. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp 1-15

Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA). Soil *Biol Biochem* 39: 1968-1977

Grobelak A, Napora A, Kacprzak M (2015) Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecol Eng* 84: 22-28

Jasim B, Joseph Agnes Aswathy, Jimtha John C, Mathew J, Radhakrishnan EK (2014). 2014 Isolation and Characterization of Plant Growth Promoting Endophytic Bacteria from The Rhizome of *Zingiber officinale. Biotech* 4: 197-204

Khan, M. S., Zaidi,A., Ahemad, M., Oves, M.,& Wani, P.A. (2010). Plant Growth Promoting by Phosphate Solubilizing Fungi-Current perspective. *Archives of Agronomy and Soil Science,* 56(1): 73-98

Rosenblueth, M and E. Martínez-Romero. 2006. The American Phytopathological Society. MPMI Vol. 19, No. 8 :827–837

Rui S. Oliveira, Ines Rocha, Ying Ma; Miroslav Vosatka & Helena Freitas. 2016. Seed coating with arbuscular mycorrhizal fungi as an ecotechnologicalapproach for sustainable agricultural production of common wheat (*Triticum aestivum* L.). *Journal of Taxicology and Environmental Health, Part A.*

Taiz I, Zeiger E. 2002. Plant Physiology Third Edition Sunderland: *Sinaeur Associates* Inc.

Marchesi JR, Sato T, Weighman AJ, Martin TA, Fry JC, Himpm SJ, Wade WG. 1998. Design and evaluation of useful bacterium specific PCR primer that amplify genes coding for bacterial 16S rRNA. Appl. Environ Microbiol 63: 795-764.

Marag, Shivsing., Premsing & Suman, Archana. 2018. Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and comosite maize *(Zea mays L.). Microbiological Research* 214: 101-113

Matsuda F, Miyazawa H, Wakasa K. (2005). Quantification of Indole-3-acetic and Amino Acid Conjugates in Rice By Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. *Biosci Biotechol Biochem*: 69: 778-783.

Sachdev DP, Chaudhari HG, Kasture VM, Dhavale DD, ChopadeA(2009)Isolation and characterization of indole acetic acid (IAA) producing Klebsiella pneumoniae strains from rhizosphere of wheat (Triticum aestivum) and their effect on plant growth.Indian Journal of Experimental Biology.47:993 –1000.

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant Signaling. *FEMS Microbiol* Rev 31: 1-24

Silva, H. S. A., R. S. R. Romeiro, D. Macagnam, B. A. H. Viera, M. C. B. Pereira, A. Mounteer. 2004. Rhizobacterial induction of systemic resistance in tomato plants: nonspecific protection and increace in enzyme activities. *Biol Control* 29: 288-295.

Tangapo, Monalisa. 2020. Potensi Bakteri Endofit Ubi Jalar (*Ipomoea batatas* L.) dalam Menghasilkan Hormon Indole Acetic Acid (IAA) dengan Penambahan L-triptofan. *Jurnal Bios* Vol. 10 No 1.

Kunkel, B.N., Chen, Z., 2006. Virulence strategies of plant pathogenic bacteria. In: Dworkin, M., et al. (Eds.), The Prokaryotes. A Handbook on the Biology of Bacteria, Ecophysiology and Biochemistry, 3rd ed. pp. 421-440.

Celloto VR, Oliveira AJB, Jose´ EG, Watanabe CSF, Graciette M, Regina ACG (2012) Biosynthesis of indole-3-acetic acid by new klebsiella oxytoca free and immobilized cells on inorganic matrices. Sci World J 2012:7.

Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical bacterial isolates unidentifiable. *J Clin Microbiol* 38 (1): 3623-3630.