Indonesian Journal of Biotechnology, December, 2013 Vol. 18, No. 2, pp.83-91

Characterization of *Streptomyces* spp. Producing Indole-3-acetic acid as Biostimulant Agent

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Abstract

Twenty six isolates of *Streptomyces* spp. obtained from *Cyperus rotundus* L. rhizosphere were tested for ability to produce *indole-3-acetic acid* (IAA) in *yeast malt extract* (YM) medium containing 2 mg/mL tryptophan. Screening of the isolates for ability to produce IAA was carried out by adding Salkowski reagent in bacteria culture and was measured quantitatively by spectrophotometer at λ 530 nm. Thin Layer Chromatography (TLC) method was used to determine IAA. To ensure the IAA production in *Streptomyces* isolates, gene involved in IAA biosynthesis was detected by amplifying *Tryptophan Monooxigenase* **(***iaaM***)** gene. The study of the effect of tryptophan on the production of IAA was measured at different concentrations of tryptophan (0, 1, 2, 3, 4, 5 mg/mL) in the bacterial culture. The result showed that there were two *Streptomyces* spp. isolates which could produce IAA, namely the isolates of *Streptomyces* sp. MS1 (125.48 µg/mL) and *Streptomyces* sp. BR27 (104.13 µg/mL). The TLC result showed that the compound in both isolates was identified to be IAA. The amplification results showed that *iaaM* gene was detected in both isolates. This results indicated that the IAM pathway is predicted involved in the biosynthesis of IAA in the selected isolates. Both of the isolates were able to produce IAA after 24 h incubation and the highest production was at 120 h incubation with the concentration of tryptophan was 2 mg/mL dan 1 mg/mL, respectively. Therefore, it is concluded that *Streptomyces* spp. isolates are able to produce IAA and potentially to be utilized as biostimulat agent.

Keywords: *Streptomyces* spp., indole-3-acetic acid (IAA), indole-3-acetamide (IAM), *Tryptophan Monooxigenase* gene **(***iaaM***)**

Introduction

Bacteria strain that provide advantageous to the plant growth is classified as Plant Growing Promoting Rhizobacteria (PGPR) (Kloepper, 1991). The PGPR could be used as biostimulant, which was able to produce or change the concentration of plant hormone, such as indole-3-acetic acid (IAA), gibberellic acid, cytokinin and ethylene (Fernando *et al*., 2005). Auxin is a compound containing indole ring on its structure which has the ability to increase the plant growth by stimulating the cell elongation, root initiation, and the seed growth (El-Tarabily, 2008). IAA is a natural auxin and a metabolism product of *L*-tryptophan in microorganism. As much as 80% of rhizosphere bacteria could secrete IAA (Bhavdish *et al*., 2003). *Streptomycess* in rhizosphere produce growth stimulating substances such as auxin, gibberellin (Brown, 1972; Aldesuquy *et al*., 1998) and cytokinin (Aldesuquy *et al*., 1998).

Research conducted by Tuomi *et al*. (1994) showed that *Streptomyces griseoviridis* produced IAA *in vitro* which might stimulate the plant growth. Several studies showed that several *Streptomyces* species such as *S. olivaceoviridis, S. rimosus, S. rochei* (Aldesuquy *et al*., 1998), *S. lydicius* WYEC108 (Tokala *et al*., 2002), and *Streptomyces* spp. (El-Tarabily, 2008) had the ability to produce IAA and increased the growth of wheat and legume of *Pisum sativum*. Khanna *et al*. (2010)

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reported that *Streptomyces* sp. CMU-H009 isolated from rhizosphere *Cymbopogon citrates* produce IAA and stimulate the growth and the elongation of corn's root and beans. This indicated that *Streptomyces* had great potential to produce IAA which could be employed as biostimulant agent.

In bacteria, there are different pathways in synthesizing IAA and several bacteria strain might perform more than one synthetic pathway (Costacurta and Vanderleyden, 1995). The two main biosynthetic pathways of IAA were indole-3-acetamide (IAM) and indole-3-pyruvate (IpyA). The former has been identified on *Bradyrhizobium japonicum* dan *Rhizobium fredii* (Sekine *et al*., 1989), *Azospirillum brasilense* (Bar and Okon, 1993), *Agrobacterium tumefaciens* and *Pseudomonas savastanoi* (Comai and Kosuge, 1982; Yamada *et al*., 1985) and *Streptomyces* spp. (Manulis *et al*., 1994). In this pathway, there were two enzymes involved. They are tryptophan monooxigenase (IaaM) coded by *iaaM* gene and IAM hydrolase (IaaH) coded by *iaaH* (Spaepen *et al*., 2007). Therefore, functional gene involved in the biosynthesis of IAA could be found on the isolate of *Streptomyces* spp. which could produce IAA.

This research was aimed to evaluate the ability of *Streptomyces* spp. isolated from *Cyperus rotundus* L. rhizosphere in producing IAA, to ensure the production of IAA on the isolate through *iaaM* gene detection, as well as to determine the effect of tryptophan on the ability of isolate to produce IAA.

Materials and Methods

Sub-culture of Streptomyces spp. isolate

As much as 26 *Streptomyces* spp. isolates employed in this study was cultivated on the mediums of yeast malt extract (YM) broth and starch nitrate agar (SNA). From each isolate, 0.1 mL was taken from the glycerol stock and was inoculated in 5 mL of YM medium at pH 7. The incubation was conducted for 3 days at room temperature. Then, 0.1 mL of culture was inoculated to the SNA medium using spread plate method and was incubated at room temperature. The observation was conducted from 4 days to 3 weeks, then it was streaked and stored on SNA medium as the stock.

Screening of Streptomyces spp. which might produce indole-3-acetic acid (IAA)

The ability of *Streptomyces* spp. isolates in producing IAA was conducted using method of Bano and Mussarat (2003). The isolate was suspended onto 0.5 mL of sterile aquadest and then was vortexed. As much as 0.1 mL of suspension was taken and was inoculated using spread plate method on the YM media and was incubated on the room temperature for 5 days. After the colony grew, the colony was taken using cylinder (diameter of 8 mm) and was inoculated onto 5 mL of YM broth containing 2 mg/mL of *L*-tryptophan on pH 7. The culture was incubated at 30°C and was shaken at 125 rpm for 7 days. The culture was centrifuged on 11,000 g for 15 min. The supernatant (1 mL) was mixed with 2 mL of Salkowski reagent and the mixture was left standing for 30 min. The production of IAA was indicated by the presence of pink color. The quantitative analysis of IAA was conducted using spectrophotometer at λ 530 nm.

Detection and identification of indole 3-acetic acid (IAA)

Identification of IAA was conducted using thin layer chromatography (TLC). The isolate was inoculated onto 200 mL of YM *broth* media which had been added with tryptophan at pH 7 (Khamna *et al*., 2010). Tryptophan was added with the concentration of 1 and 2 mg/mL for the cultures of *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27, respectively. The culture was incubated at 30°C and was shaken at 125 g for 5 days. The culture was centrifuged at 4,000 g for 15 min. The supernatant (150 mL) was then extracted with 100 mL of ethyl acetate. The organic layer was placed on the flask and was evaporated to give the solid phase. The phase was dissolved with 2 mL of methanol. Then,

10 µl of solution was spotted on the silica gel plate. As much as 0.5 mg of synthetic IAA was dissolved in 2 mL of methanol. 10 µl of solution was spotted on the same silica gel plate as the control. The TLC was performed with the eluent of chloroform:ethyl acetate: formic acid (5:4:1). The spot was visualized under UV transilluminator as blue spot at 254 nm.

Detection of iaaM gene on the Streptomyces spp. isolate

Isolation of chromosomal DNA was conducted using spooling with a glass rod method (Irawati, 2006) with a slight modification. The *Streptomyces* spp. isolate was inoculated on the YM *broth* medium (50 mL) at pH 7 for 3-4 days. The culture was centrifuged at 3,000 g for 20 min. The pellet was washed with 4 mL of 50 mM EDTA solution and was centrifuged at 3,000 g for 15 min. The obtained pellet was resuspended onto 2 mL of mixture (25% sucrose: 0.5 M EDTA: lysozyme 10 mg/mL 100 µL) and was incubated at 37°C for 1 h. Then, 1200 µL NaCl 5 M, 700 µL 0,5 M EDTA, 1500 µL 20% SDS were added and the mixture was incubated at 56°C for 1 h. Chloroform was then added and the mixture was slowly shaken for 20 min and was centrifuged at 3,000 g. The supernatant was placed into the new conical flask and absolute ethanol was added (2x volume). The DNA precipitation will appear. The centrifugation with slow speed was carried out to precipitate the DNA molecule. It was washed with ethanol 70% and was centrifuged. The pellet was dried and dissolved using TE buffer.

The amplification of *iaaM* was done using PCR thermal cycler method with the specific primer of *iaaM*F (5'CGACTTCTCCGACATGAACC'3) and *iaaM*R (5'AGTCGTCACAGGCGATCTTC'3). The condition of PCR was 1 cycle included the initial denaturation at 95°C for 3 min, 30 cycles included the denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min and 1 last cycle included extension at 72 °C for 10 min, cooling at 4 °C for 10 min. The amplification product was visualized using electrophoresis for 20 min (100 volt). The bacteria isolate which show DNA band with the size of 500 bp on the electrophoresis gel was considered has the functional gene.

The effect of incubation time on the production of indole-3-acetic acid (IAA) by the selected isolates

The selected *Streptomyces* isolate was suspended on the sterile aquadest and was mixed by vortexing. The suspension (0.1 mL) was taken and inoculated using spread plate method in the agar YM media and was incubated at the room temperature for 5 days. Then, the colony (10 disc with diameter of 8 mm) was taken and was cultivated in 50 mL of YM broth media containing 2 mg/mL *L*-tryptophan at pH 7. The culture was incubated at 30 °C and was shaked at 125 rpm for 7 days. The determination of IAA concentration was conducted each 24 h. The culture was centrifuged at 11,000 g for 15 min, then the supernatant (1 mL) was mixed with 2 mL of Salkowski reagent and was left standing for 30 min. The quantitative analysis was conducted by using spectrophotometer at λ 530 nm.

The effect of tryptophan concentration on the production of indole-3-acetic acid (IAA) by the selected isolates

The selected *Streptomyces* isolate was suspended on the sterile aquadest (0.5 mL) and was vortexed. The suspension (0.1 mL) was taken and was inoculated using spread plate method in the agar YM media and was incubated at the room temperature for 5 days. Then, the colony (10 disc with diameter of 8 mm) was taken and was cultivated in 50 mL of YM broth media containing *L*-tryptophan at different concentration (0, 1, 2, 3, 4, 5 mg/mL). The culture was incubated at 30 °C and was shaked at 125 rpm for 5 days. The determination of IAA concentration was conducted each 24 h. The culture was

centrifuged at 11,000 g for 15 min, then the supernatant (1 mL) was mixed with 2 mL of Salkowski reagent and was left standing for 30 min. The quantitative analysis was conducted by using spectrophotometer at λ 530 nm. Next, the centrifugation product was filtered. The pellet was dried on the oven at 70°C and was weighed at each 6 h to determine the weight of dry cell.

Results and Discussion

Screening of Streptomyces spp. which might produce indole-3-acetic acid (IAA)

The ability of 26 isolates of *Streptomyces* spp. from rhizosfer *Cyperus rotundus* L. in producing indole-3-acetic acid (IAA) was evaluated. The evaluation showed that there were 2 isolates (7.7%) which had the ability to convert amino acid of tryptophan to IAA, they were *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27. Bhavdish *et al.* (2003) reported that the ability of rhizosfer bacteria to produce IAA was due to the high input of organic material from the root. The amount of IAA produced from the isolates could be seen on Table 1.

Table 1. The ability of two *Streptomyces* isolates in producing IAA

Isolate	Concentration of IAA (µg/mL)
MS1	79.52
BR27	17.38

Rhizosphere area was plant area affected by the root and was identified by the high microbial activity due to the abundance of exudates (Fitter and Hay, 2002). The released exudates by the root contained various amino acid (Walker *et al.*, 2003), sugar, organic acid, phenolic compound and protein (Bais *et al.*, 2006).

The results showed that the two isolates produced IAA in the different amount. Tsavkelova *et al.* (2005) stated that the bacteria with the same genus might produce IAA in the different amount. The amounts of IAA produced by *actinomycetes* (Gangwar *et al.*, 2012) and *Streptomyces* sp. CMU-H009 (Khamna *et al.*, 2010) were 17-39, 300 µgmL^{-1}, respectively.

Detection and identification of indole-3-acetic acid (IAA) produced by Streptomyces MS1 and Streptomyces BR27 using thin layer chromatography (TLC)

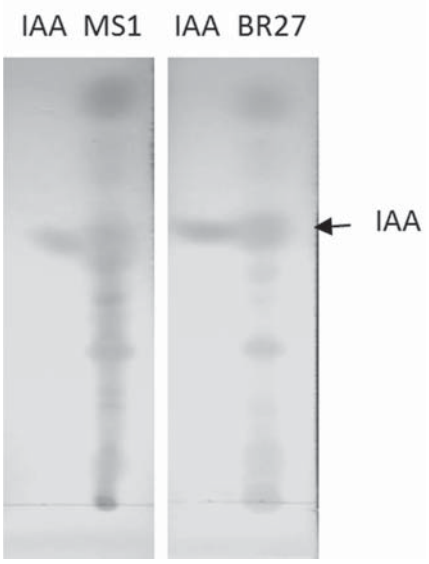


Figure 1. Thin Layer Chromatography profile of IAA produced by two isolates of *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27 under UV 254 nm. Syntetic IAA used as internal standard.

The thin layer chromatography (TLC) analyses at the wavelength of 254 nm (Figure 1) showed that IAA produced by two isolates of *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27 provide spot with same Rf value of synthetic IAA (0.55). This indicated that both isolates might produce IAA.

Detection of tryptophan monoxigenase (iaaM) gene on Streptomyces sp. MS1 and Streptomyces sp. BR27

The *iaaM* was a gene involved in the biosynthesis of IAA on the IAM pathway. Meanwhile, *iaaM* was detected in both isolates isolate which were shown by the presence of single band on the size of 500 bp (Figure 2). This result indicated that the production of IAA occurred via the IAM pathway.

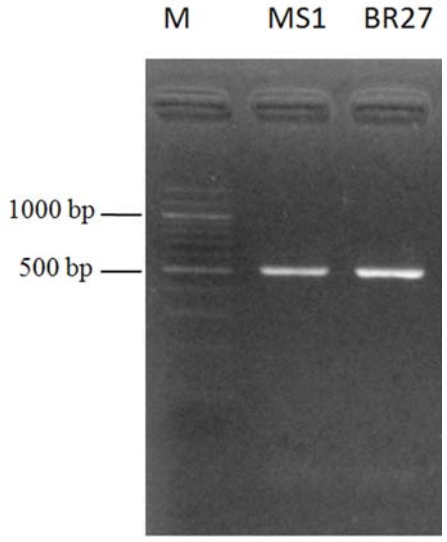


Figure 2. The *iaaM* involved in auxin biosynthesis pathway was detected in chromosomal DNA of both *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27. M : DNA marker 100 bp ladder

Two biosynthetic pathways of IAA that commonly found in the bacteria were IAM and IPyA pathways (Lambrecht *et al*., 2000). The production of IAA through the IAM pathway was controlled by different mechanism, including the input of tryptophan, the inhibition of the feedback of *iaaM* activity by IAA and IAM (Hutcheson and Kosuge, 1985). According to Hutcheson and Kosuge (1985), the biosynthesis of IAA through IAM pathway: Tryptophan + O_2 → IAM + CO_2 + H_2O and then IAM + H_2O → IAA + NH_4.

Manulis *et al*. (1998) reported that the expression of *iaaM* was high when the bacteria was in apoplast while expression of *ipdC* gene (key gene on the IPyA pathway) was high when the bacteria was found in the leave surface. Patten and Glick (1996) stated that if the microbial had several biosynthetic pathways to produce IAA, the pathway with the enzyme which was able to work at the environmental condition will dominantly occur in the biosynthesis of IAA. This indicated that the biosynthesis of IAA was adjusted for the expression on the different environment. Bacterias which employed IAM pathway in synthesizing IAA were *Agrobacterium tumefaciens* (Morris, 1995), *Bradyrhizobium* (Sekine *et al*., 1989), *Pseudomonas syringae*, *Pantoea agglomerans*, dan *Rhizobium* (Sekine *et al*., 1989; Theunis *et al*., 2004; Spaepen *et al*., 2007).

Effect of incubation time on the production of indole-3-acetic acid (IAA) by Streptomyces sp. MS1 and Streptomyces sp. BR27

The results showed that the production of IAA by *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27 was commenced after 24 h and reach the maximum point at 120 h (5 d) and decreased. The amount of IAA produced by two isolates from 0-168 h was presented in Figure 3. The results showed that the incubation time played significant role on the production of IAA by the two isolates. This effect gave different results for different strain of *Streptomyces* spp.. *Streptomyces viridis* CMU-H009 gave the maximum production of IAA after 72 h

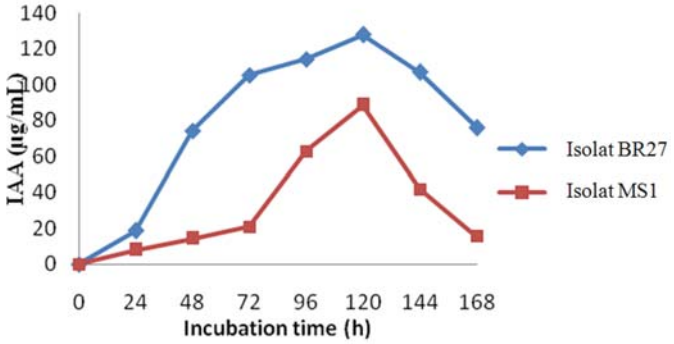
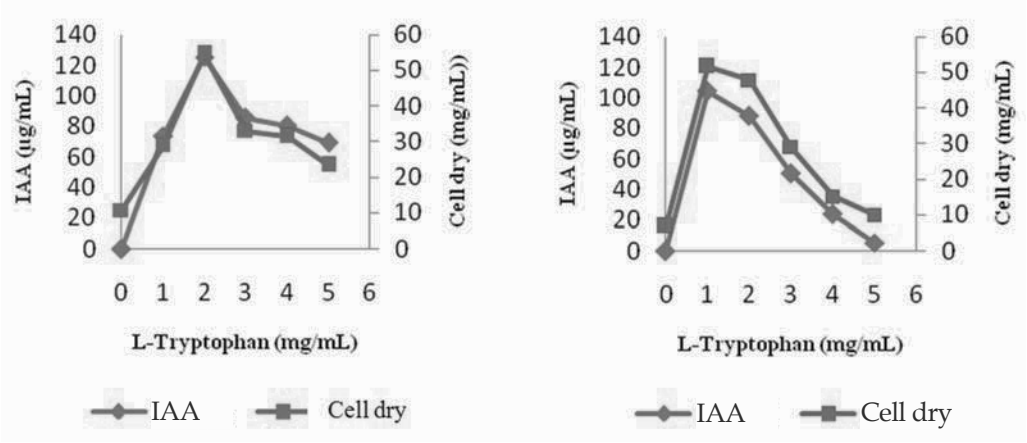


Figure 3. The effect of incubation time on the production of indole-3-acetic acid (IAA) by *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27.



(a) (b)

Figure 4. The effect of tryptophan concentration on the production of IAA and the cell dry weight on *Streptomyces* MS1 (a) and *Streptomyces* BR27 (b)

(3d) on the medium of YM *broth* containing 2 mg/mL of trypthophan (Khamna *et al*., 2010).

Walkinson *et al.* (1994) reported production of IAA from the bacteria isolate was affected by the incubation time. The decrease on the production of IAA might be due to the release of enzyme which might degrade IAA such as oxidase IAA and peroxidase produced by exudates as reported on *Rhizobum* sp. From *Cajanus cajan* (Datta and basu, 2000). Degradation of IAA could occur through the oxidation mechanim to produce dioxindole-3-acetic acid. The product was then oxidized into isatine which could be hydrolyzed into anthranilic acid in the acid condition (Jensen *et al*., 1995).

The effect of tryptophan concentration on the production of IAA and the cell dry weight on Streptomyces MS1 and Streptomyces BR27

The results showed that the production of IAA by the two isolates was linearly correlated to the cell dry weight. The production of IAA was increased by the increasing of the dry weight. The *Streptomyces* sp. MS1 (Figure 4a) and *Streptomyces* sp. BR27 (Figure 4b) might produce IAA with the tryptophan concentrations of 2 and 1 mg/L, respectively. On the higher concentration, tryptophan provides bad effect on the production of IAA and the dry weight.

The concentration of tryptophan strongly affected by the ability of isolate in producing IAA. The IAA could not be produced without the addition of tryptophan on the medium. Tryptophan had been identified as the main precursor for IAA and played important role in modulating biosynthetic level of IAA on bacteria (Spaepen *et al*., 2007). Besides, tryptophan could be employed as the source of carbon and energy which will be decomposed into several compounds which in turn will be involved in the metabolism pathway (Moat and Foster, 1995).

Khamna *et al*. (2010) showed that the highest IAA production by *Streptomyces viridis* CHU-H009 on the medium YM *broth* with the tryptophan concentration of 2 mg/mL. However, the utilization of tryptophan on higher concentration resulted in bad effect on the production of IAA and the dry weight. Ahmad *et al*. (2005) reported that rhizosphere bacteria of *Azotobacter* spp. and *Pseudomonas* spp. produced the highest IAA on the medium of nutrient broth with the tryptophan concentration of 2 and 5 mg/mL. Bar and Okon (1993) showed that concentration of tryptophan of 1.4 mg/mL or higher could inhibit the growth of *Az*ospirillum *brasilense* Sp7.

The application of the exogenous tryptophan was required to increase the production of IAA in various bacteria, such

as *Azospirillum*, *Pseudomonas putida* dan *Rhizobium* (Prinsen *et al*., 1993; Brandi and Lindow, 1996; Patten and Glick, 2002; Theunis *et al*., 2004). Tryptophan on rhizosphere could be obtained from the degradation of root cell and microbial cell and root exudates. This showed that IAA was produced during the late exponential and stationer phases (Omay *et al*., 1993).

Spaepen *et al*. (2007) mentioned that the role of IAA to the bacteria has not clearly known yet. However, IAA produced by the bacteria isolate might stimulate the growth of plant. The growth could initiate the exudation of root organic compounds which could be employed for bacteria growth.

Conclusion

This study showed that *Streptomyces* sp. MS1 and *Streptomyces* sp. BR27 produce IAA on the medium with the addition of tryptophan. In addition, the TLC analysis and amplification of *iaaM* gen showed that the two isolates produce IAA. Therefore, it is concluded that *Streptomyces* spp. isolates has potential as biostimulant agent.

Acknowledgement

This research was partly supported by Public Service Education Fund Management Institution (LPDP) master scholarship program 2012.

References

Ahmad, F., Ahmad, I. and Kha, M.S., 2005. Indole Acetic Acid Production by The Indigenous Isolates of *Azotobacter* and Fluorescent *Pseudomonas* in The Presence and Absence of Tryptophan. *Turkish J of Biol*., **29,** 29-34.

Aldesuquy, H.S., Mansour, F.A. and Abo-Hamed, S.A., 1998. Effect of The Culture Filtrates of *Streptomyces* on Growth and Productivity of Wheat Plants. *Folia Microbiologica*, **43,** 465-470.

Bais, H.P., Weir, T.E., Perry, L.G., Gilroy, S. and Vovanco, J.M., 2006. The Role of Exudates in Rhizosfer Interactions with Plants and Other Organism. *The Annual Rev of Plant Biol*., **57**, 233-266.

Bano, N. and Musarrat, J., 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Curr. Microbiol*., **46**, 324-328.

Bar, T. and Okon, Y., 1993. Tryptophan conversion to indole-3-acetic acid via indole-3-acetamide in *Azospirillum brasilense* Sp7. *Canadian J. of Microbiol*., **39**, 81–86.

Bhavdish, N., Johri, A., Sharma, J. and Virdi, S., 2003. Rhizobacterial diversity in India and its influence on soil and plant health. *Adv in Biochem Eng/Biotechnol*., **84**, 49-89.

Brandl, M.T. and Lindow, S.E., 1996. Cloning And Characterization Of A Locus Encoding An Indolepyruvate Decarboxylase Involved In Indole-3-Acetic Acid Synthesis In *Erwinia herbicola*. *Appl. and Environ. Microbiol*., **62**, 4121–4128.

Brown, M.E., 1972. Plant Growth Substances Produced by Microorganism of Soil and Rhizosphere. *J. of Appl. Bacteriol*., **35**, 443-451.

Comai, L. and Kosuge, T., 1982. Cloning characterization of *iaaM*, a virulence determinant of *Pseudomonas savastanoi*. *J. of Bacteriol*., **149**, 40-46.

Costacurta, A. and Vanderleyden, J., 1995. Synthesis of phytohormones by plant-associated bacteria. *Critical Rev. in Microbiol*., **21**, 1-18.

Datta, C. and Basu, P.S., 2000. Indole Acetic Acid Production By a *Rhizobium* Species From Root Nodules Of A Leguminous Shrub, *Cajanus cajan*. *Microbiol. Res*., **155**, 123-127.

El-Tarabily, K. A., 2008. Promotion of Tomato (*Lycopersicon esculentum* Mill.) Plant Growth by Rhizosphere Competent 1-aminocyclopropane-1-carboxylic Acid Deaminase-Producing *Streptomycete Actinomycetes*. *Plant and Soil*, **308**, 161-174.

Fernando, D., Nakkeeran and Zhang, Y. (2005) Biosynthesis Of Antibiotics By

PGPR And Its Relation In Biocontrol Of Plant Diseases. In: *PGPR: Biocontrol and Biofertilization* (Z.A. Siddiqui, ed.). pp 67-109. Springer, Dordrecht, The Netherlands.

Fitter, A.H. and Hay, R.K.M. (2002) *Environmental Physiology of Plants*. Academic Press. San Diego.

Hutcheson, S.W. and Kosuge, T., 1985. Regulation of 3-indole acetic acid production in *Pseudomonas syringae* pv. Savastanoi. *J. of Biol. Chem*., **260**(10), 6281-6287.

Ilic, S.B., Konstantinovic, S.S., Todorovic, Z.B., Lazio, M.L., Veljkovic, V.B., Jokovio, N. and Radovanovic, B.C., 2007. Characterization and Antimicrobial Activity of The Bioactive Metabolites in *Streptomyces* Isolates., *Microbiology*, **76**, 421-428.

Irawati, W. 2006. *Kajian Fisiologis dan Molekular Sifat Resistensi Bakteri Terhadap Tembaga*. Disertasi. Fakultas Biologi, Universitas Gadjah Mada. Yogyakarta.

Jensen, J.B., Egsgaard, H., Vanonckelen, H. and Jochimsen, B.U., 1995. Catabolism Of Indole-3-Acetic-Acid And 4-Chloroindole-3-Acetic And 5-Chloroindole-3-Acetic Acid In *Bradyrhizobium japonicum*. *J. of Bacteriol*., **177**, 5762–5766.

Khamna, S., Yokota, A., Peberdy, J. F. and Lumyong, S., 2010. Indole-3-acetic Acid Production by *Streptomyces* sp. Isolated From Thai Medicinal Plant Rhizosphere Soils. *EurAsian J. of BioSci*., **4**, 23-32.

Kloepper, J. W. 1991. Plant Growth-Promoting Rhizobacteria as biological control agents of soilborne diseases. Di dalam: Petersen JB, editor. *The Biological Control of Plant Diseases. Proceedings of The International Seminar 'Biological Control of Plant Diseases and Virus Vectors*; Tsukuba, 17-21 September 1990. Japan: NARC. 142-148.

Lambrecht, M., Okon, Y., Vande Broek, A. and Vanderleyden, J., 2000. Indole-3-Acetic Acid: A Reciprocal Signalling Molecule In Bacteria–Plant Interactions. *Trends in Microbiol*., **8**, 298–300.

Manulis, S., Shafrir, H., Epstein, E., Lichter, A. and Barash, I., 1994. Biosynthesis of indole-3-acetic acid via the indole-3-acetamide pathway in *Streptomyces* spp. *Microbiology.*, **140**, 1045-1050.

Manulis, S., Haviv-Chesner, A., Brandl, M.T., Lindow, S.E. and Barash, I., 1998. Differential Involvement Of Indole-3-Acetic Acid Biosynthetic Pathways In Pathogenicity And Epiphytic Fitness Of *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant–Microbe Interactions*, **11**, 634–642.

Maziyah, S. 2011. *Keanekaragaman dan potensi Streptomyces Rizosfer Rumput Teki* (*Cyperus rotundus* L.) *Daerah Tercemar Merkuri sebagai Agen Bioremediasi Cemaran Logam*. Tesis. Fakultas Biologi UGM. Yogyakarta.

Moat, A.G. and Foster, J.W. (1995) *Microbial Phsyology*, Third Ed. John Wiley and Sons. New York.

Morris, R.O., 1995. Genes Specifying Auxin And Cytokinin Biosynthesis In Prokaryotes. In: "*Plant Hormones: Physiology, Biochemistry, and Molecular Biology*" (Davies, P.J., ed.). pp 318–339. Dordrecht: Kluwer Academic Publishers.

Omay, S.H., Schmidt, W.A., and Martin, P., 1993. Indoleacetic Acid Production By The Rhizosphere Bacterium *Azospirillum brasilense* Cd Under In Vitro Conditions. *Canadian J. of Microbiol*., **39**, 187–192.

Patten, C.L. and Glick, B.R., 2002. Regulation Of Indoleacetic Acid Production In *Pseudomonas putida* GR12-2 By Tryptophan And The Stationary-Phase Sigma Factor RpoS. *Canadian J. of Microbiol*., **48**, 635–642.

Prinsen, E., Costacurta, A,, Michiels, K., Vanderleyden, J., and Van Onckelen, H., 1993. *Azospirillum brasilense* Indole-3-Acetic Acid Biosynthesis: Evidence for a Non-Tryptophan Dependent Pathway. *Mol. Plant–Microbe Interactions*., **6**, 609–615.

Sekine, M., Watabane, K., and Syono, K., 1989. Molecular Cloning of A Gene For Indole-3-acetamide Hydrolase From

Bradyrhizobium japonicum. *J. of Bacteriol*., **171**, 1718-1724.

Spaepen, S., Vanderleyden, J., and Remans, R., 2007. Indole-3-acetic Acid in Microbial and Microorganism-Plant Signaling. *FEMS Microbiol. Rev*., **10**, 1-24.

Theunis, M., Kobayashi, H., Broughton, W. J., and Prinsen, E., 2004. Flavonoids, NodD1, NodD2, and Nod-box NB15 Modulate Expression Of The y4wEFG Locus That is Required for Indole-3-acetic Acid Synthesis in *Rhizobium* sp. Strain NGR234. *Mol. Plant-Microbe Interactions*., **17**, 1153-1161.

Tokala, R. K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H., Deobald, L.A., Bailey, J.F., and Morra, M.J., 2002. Novel Plant-Microbe Rhizosphere Interaction Involving *Streptomyces lydicus* WYEC108 and the Pea Plant (*Pisum sativum*). *Appl. and Environ. Microbiol*., **68**, 2161-2171.

Tuomi, T., Laakso, S., and Rosenquist, H., 1994. Indole-3-acetic Acid Production by a Biofungicide *Streptomyces griseoviridis* A Strain. *Annales Botanici Fennici*, **31,** 59-63.

Walker, T.S., Bais, H.P., Grotewold, E., and Vivanco, J.M., 2003. Root Exudation and Rhizosphere Biology. *Plant Physiol*., **132**, 44-51.

Wilkinson, K.G., Sivasithamparam, K., Dixon, K.W., Fahy, P.C., and Bradley, J.K., 1994. Identification and Characterization of Bacteria Associated With Western Australian Orchids. *Soil Biol. and Biochem*., **26**, 137-142.

Yamada, T., Palm, C., Brooks, B., and Kosuge, T., 1985. Nucleotide sequences of the *Pseudomonas savastanoi* indoleacetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. *Proceedings of the National Academy of Sciences*, **82**, 6522-6526.