Asmiaty Sahur *et al*, International Journal of Advances in Agricultural Science and Technology,
Vol.4 Issue.1, January- 2017, pg. 07-15 **ISSN: 2348-1358**
Impact Factor: 6.057

Isolation and Characterization of Indigenous Rhizosphere Bacteria Producing Gibberellic Acid and Indole Acetic Acid from Local Soybeans in South Sulawesi

Asmiaty Sahur[1], Ambo Ala[2], Baharuddin Patanjengi[3], Elkawakib Syam'un[4]

[1]*Department of Agronomy, Faculty of Agriculture Hasanuddin University, Indonesia*
asmiaty_sahur@yahoo.com
[2]*Department of Agronomy, Faculty of Agriculture Hasanuddin University, Indonesia*
amboala_aa@yahoo.com
[3]*Center for Biotechnology, Research Center (PKP) HasanuddinUniversity, Makassar 90245 Indonesia*
baharunhas@yahoo.com
[4]*Department of Agronomy, Faculty of Agriculture Hasanuddin University, Indonesia*
elkawakib@yahoo.com

Abstract: ***This study aimed to isolate and characterize the indigenous rhizosphere bacteria producing Gibberellic Acid and Indole Acetic Acid as plant growth regulators isolated from local soybean of South Sulawesi, Indonesia. Several root samples and soil samples of soybean plants were collected from the rhizosphere of local soybeans in three different areas of South Sulawesi such as Soppeng, Bone and Takalar. There were fifty six isolates of bacteria taken from Soybean roots and soil and grouped into gram-positive bacteria and gram negative bacteria. There were 35 isolates that produced a thick slime or felt slimy when cultured on Natrium Broth media and the remaining 21 produced spores. The results showed considerable potential for the bacterial isolated to produce Gibbrelic Acid in high concentration. The best isolates of thick slime producing Gibberellin Acid was RK 30 (4.670 mg/ml), RK 17 (3.797 mg/ml), RK 15 (3.703) and RK 35 (3.222 mg/ml). There were 4 isolates that produced high concentrations of Indole Acetic Acid including RK 32 (2.794 mg / ml) followed by RK 8 (1.810 mg/ml), RK 23 (1.714 mg / ml), and RK 30 (1.678mg / ml) respectively.***

Keywords: ***Rhizosphere bacteria, Gibberellic Acid, Indole Acetic Acid, Soybeans***

1. Introduction

Soybean is one of the legumes that contain vegetable protein that is high enough range around 34% to be comparable with animal protein for human nutrition (Ditjentan, 2004). Soybeans are both a source of vegetable protein, and serve as a functional food to prevent the onset of degenerative diseases, such as coronary heart disease and hypertension. Substances called iso flavones contained in soy products function as an antioxidant. Now soybeans are widely used as a source of alternative energy (biofuels). As a source of vegetable protein, soybeans are generally consumed in the form of processed products, such as: tofu, tempeh, soy sauce, tauco, soy milk and other forms of snacks (Sudaryanto and Swastika 2007).

However, traditional agricultural practices have relied on extensive cultivation of land and the use of pesticides and chemical fertilizers to produce high soybean production. As a result, some of side effects on this practice have emerged. They include erosion and loss of topsoil and soil structure damaged from cultivation of the soil, the use of highly hazardous pesticides in the food chain, which can cause contamination and eutrophication of fresh water and the marine environment. Therefore there is a need to find solution to solve those problems. One solution in order to solve those problems is to use bacteria as bio-fertilizer.
According to Klooper *et al.*, (1996), the existence of bacteria in the rhizosphere can lead to invasion of the root system and result in the colonization process helping the plants to grow as well as supplying nutrients to the host plants. However, those bacteria may not always be synergistic and become pathogens. In addition, Klooper concluded that these bacteria have the potential to support and increase the availability of many nutrients including Nitrogen Phosphorus as well as performing as a plant growth hormone.

One of the plant growth hormones that can be produced by rhizosphere bacteria is Gibberellic Acid. It is one of the hormones promoting the growth of plants economically and industrially. As a hormone plant growth promoter, Gibberellin Acid can be produced by higher plants around the rhizosphere area by bacteria (Pandya and Desay, 2014). A common structural element of the Gibberelic is the tetracyclic diterpenoid acids which are produced in the growth and metabolism process of plants, such as at the germination process of seed, promoting seedling processes, and leaf as well as stem of plant growth (Bottini, Cassan, Piccoli, 2004).

The aim of the study is to isolate and characterize the physiological ability of rhizosphere bacteria isolated from local soybeans in South Sulawesi. They might have potential to be used as bio-stimulants or as bio-fertilizer which can be applied to the plants and confer protection against soil borne pathogens.

2. Materials and Methods

2.1 Location of Bacteria Site.

Soil samples have been taken at a depth of 0-20 cm in the three areas of soybean rhizosphere in South Sulawesi, Indonesia. In each area, one healthy soybean were taken and placed in the plastic bag for isolation process. The samples of soil around the rhizosphere area were collected in one bag and labeled those with the criteria of the site where the samples have been collected.

2.2 Isolation soil samples from rhizosphere soybean

Isolation of rhizosphere bacteria carried out by a serial dilution method. Ten grams of rhizosphere soil was weighed and dissolved in 90 ml of sterile water, then shake for 30 minutes. One ml of rhizosphere soil suspension was added to a reaction tube containing 9 ml of sterile water to get suspension with 10^{-2} concentration of the dilution. The same process were done for several level of concentration such as 10^{-3}, 10^{-4}, and then 10^{-6} respectively.

The next step is took 0.1 ml of the suspension and it was grown on media specific such as Yeast Mannitol Agar and Tap Water Yeast Extract in a petri dish Both medium which already contains rhizosphere bacteria were incubated for 24 hours at room temperature. Every single colonies were grown to reisolate and made as pure culture and planted on the Nutrient Agar media 10^{-8} suspension. Subsequently 0.1 ml of the suspension was grown on NA medium in a petri dish. NA medium which already contains rhizosphere bacteria were incubated for 24 hours at room temperature. Every single colony were grown to reisolate and made as pure culture.

2.3 Characterization of Rhizosphere Bacteria Isolated from Soybean Plants

2.3.1 Analyzing Gibberellic acid (GA3)

The production of Gibberellic acid was tested by culturing the bacteria on to nutrient broth media (Borrow *et al.*, 1995). Furthermore one ml of bacterial isolates were added to the media and incubated at 37˚C for seven days. The cultures then were centrifuged at 8000 g for 10 min to remove the bacterial cells. Fifteen cultures were added to 5 ml of zinc acetate. Account after 2 minutes was added 2 ml of potassium Ferro cyanide solution and centrifuged at 8000 g for 10 min. Five ml of the supernatant was added to five ml of 30

per cent hydrocloric acid and the mixture was incubated at 20 ^{0}C for 75 minutes. The blank cuvette was prepared with five percent hydrocloric acid. Absorbance was measured at 254 nm in the UV-VIS spectrophotometer. From a standard curve prepared by using gibberellic acid solution of known quantities, produced of GA by the culture was calculated and expressed as mg/l.

2.3.2 Analyzing Indole Acetic Acid (IAA)

Production of indole -3-acetic acid (IAA) by bacteria was tested using nutrient broth and Salkowski reagent (Gutierrez *et al.*, 2009). PGPR isolates cultured in NB is equipped with L-tryptophan (0,1g/l) at room temperature in the dark for five days, and the supernatant was taken after centrifugation. One ml of the supernatant was added to one ml of Salkowski reagent (12 g l-1FeCl3 in 429 ml/l H_2SO_4) (Glickman *et al.*, 1995) and incubated in dark for 24 hours at room temperature. The intensity of pink color developed was read at 535 nm using a UV-VIS spectrophotometer. From a standard curve prepared with known concentration of IAA, the quantity in the culture filtrate was determined and expressed as mg/l.

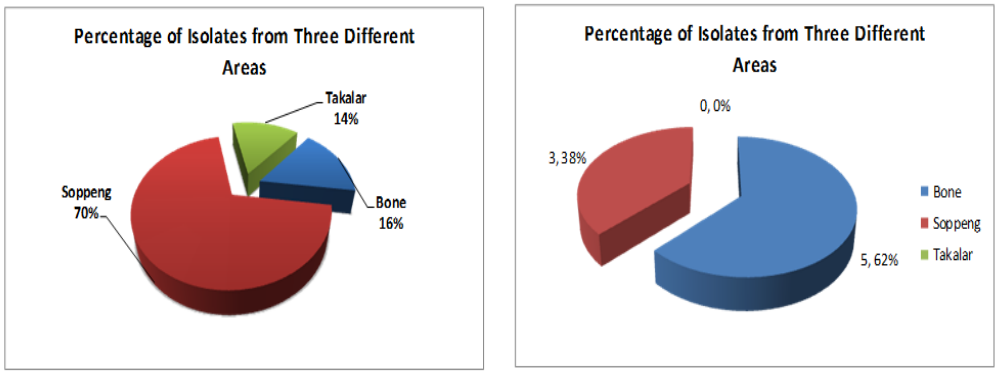
3. Results and Discussion

3.1 Isolated Rhizosphere Bacteria from Three Different Areas

Observation of several isolates indicated that between three different source of Soybean grown, Rhizosphere bacteria from Soppeng is the highest amount of bacteria isolated, and some of them can be isolated and grown from Bone and then Takalar. The result also gave indication that the bacteria from rhizophere most commonly grown compare to bacteria isolated from root of soybean plants.

Our result showed that the rhizosphere bacteria isolated from three different areas of south Sulawesi is rhizosphere bacteria producing plant growth and development is also regulated by phytohormones. The production of phytohormone such as Gibberellin, Indole Acetic Acid which produced by PGPR's have been reported by many researchers, however not many evidence regarding production of gibberellins by the plant growth promoting rhizobacteria has been reported (Amar *et al*.,2013) . Yet, it has been reported to be produced by certain rhizosphere bacteria's such as *Bradyrhizobium japonicum* (Boiero *et al*, 2007). Gibberellins also can alter the plant morphology by the elongation of stem tissues. (Amar *et al*., 2013).

The results showed in the figure one presented the source of isolates producing Gibberellin acid and Indole Acetic Acid. The highest numbers of isolates were successfully isolated from rhizosphere from Soil in Soppeng area followed by Bone and then Takalar. The isolated of root rhizosphere mainly from Bone and then Soppeng. None of bacteria of the plant's root was found in Takalar area.



(1a) (1b)

Figure 1: (1a) Isolates Producing Gibberellin and Indole Acetic Acid (IAA) which isolated from Rhizosphere of Soybean Plants, (1b) Isolates Producing Gibberellin and Indole Acetic Acid (IAA) which isolated from Root of Soybean Plants.

The observation of the colour of colonies (after 24 hours) incubation in selective media of rhizosphere bacteria, it was shown that fifty-six isolates were generally dominated by yellow, white, cream, and orange (Table 1). The colour of colonies was shown in Figure 2.

Table 1: Characteristic of Bacteria Isolated from Soybean Plants Which Has Gram Negative

Isolates Code	Varities	Age (Days)	Location	Colours	Gram Reaction
Rk 1	Anjasmara	80	Soppeng	Yellow	-
Rk 2	Anjasmara	80	Soppeng	White	-
Rk 3	Anjasmara	80	Soppeng	White	-
Rk 4	Anjasmara	80	Soppeng	Yellow	-
Rk 5	Anjasmara	80	Soppeng	Cream	-
Rk 6	Anjasmara	80	Soppeng	Yellow	-
Rk 7	Anjasmara	80	Soppeng	Cream	-
Rk 8	Anjasmara	80	Soppeng	White	-
Rk 9	Anjasmara	80	Soppeng	White	-
Rk 10	Anjasmara	80	Soppeng	Cream	-
Rk 11	Anjasmara	80	Soppeng	Cream	-
Rk 12	Anjasmara	80	Soppeng	White	-
Rk 13	Anjasmara	80	Soppeng	White	-
Rk 14	Anjasmara	80	Soppeng	White	-
Rk 15	Anjasmara	80	Soppeng	Cream	-
Rk 16	Anjasmara	80	Soppeng	White	-
Rk 17	Anjasmara	80	Soppeng	White	-
Rk 18	Anjasmara	80	Soppeng	Cream	-
Rk 19	Anjasmara	80	Soppeng	White	-
Rk 20	Anjasmara	12	Soppeng	Yellow	-
Rk 21	Anjasmara	12	Soppeng	Yellow	-
Rk 22	Anjasmara	12	Soppeng	Orange	-
Rk 23	Anjasmara	12	Soppeng	Yellow	-
Rk 24	Anjasmara	12	Soppeng	White	-
Rk 25	Wilis	12	Takalar	White	-
Rk 26	Anjasmara	12	Takalar	White	-
Rk 27	Anjasmara	12	Takalar	White	-
Rk 28	Anjasmara	12	Takalar	White	-
Rk 29	Anjasmara	12	Takalar	Cream	-
Rk 30	Anjasmara	12	Takalar	White	-
Rk 31	Anjasmara	12	Takalar	Yellow	-
Rk 32	Anjasmara	12	Takalar	White	-
Rk 33	Anjasmara	12	Bone	White	-
Rk 34	Anjasmara	12	Bone	White	-
Rk 35	Anjasmara	1i	Bone	White	-

Table 2: Characteristic of Bacteria Isolated from Soybean Plants Which Has Gram Positive

Isolates Code	Varities	Age (Days)	Location	Colour	Gram Reaction
AK 1	Anjasmara	80	Soppeng	Putih	+
AK 2	Anjasmara	80	Soppeng	Putih	+
AK 3	Anjasmara	80	Soppeng	Krem	+
AK 4	Anjasmara	80	Soppeng	Putih	+
AK 5	Anjasmara	80	Soppeng	Kuning	+
AK 6	Anjasmara	80	Soppeng	Krem	+
AK 7	Anjasmara	80	Soppeng	Krem	+
AK 8	Anjasmara	80	Soppeng	Putih	+
AK 9	Anjasmara	14	Soppeng	Putih	+
AK 10	Anjasmara	14	Soppeng	Putih	+
AK 11	Anjasmara	14	Soppeng	Krem	+
AK 12	Anjasmara	14	Soppeng	Krem	+
AK 13	Anjasmara	14	Soppeng	Krem	+
AK 14	Anjasmara	14	Soppeng	Putih	+
AK 15	Anjasmara	14	Soppeng	Putih	+
AK 16	Anjasmara	14	Soppeng	Putih	+
AK 17	Anjasmara	14	Soppeng	Putih	+
AK 18	Anjasmara	14	Soppeng	Putih	+
AK 19	Anjasmara	14	Soppeng	Putih	+
AK 20	Anjasmara	14	Soppeng	Putih	+
AK 21	Anjasmara	14	Soppeng	Putih	+

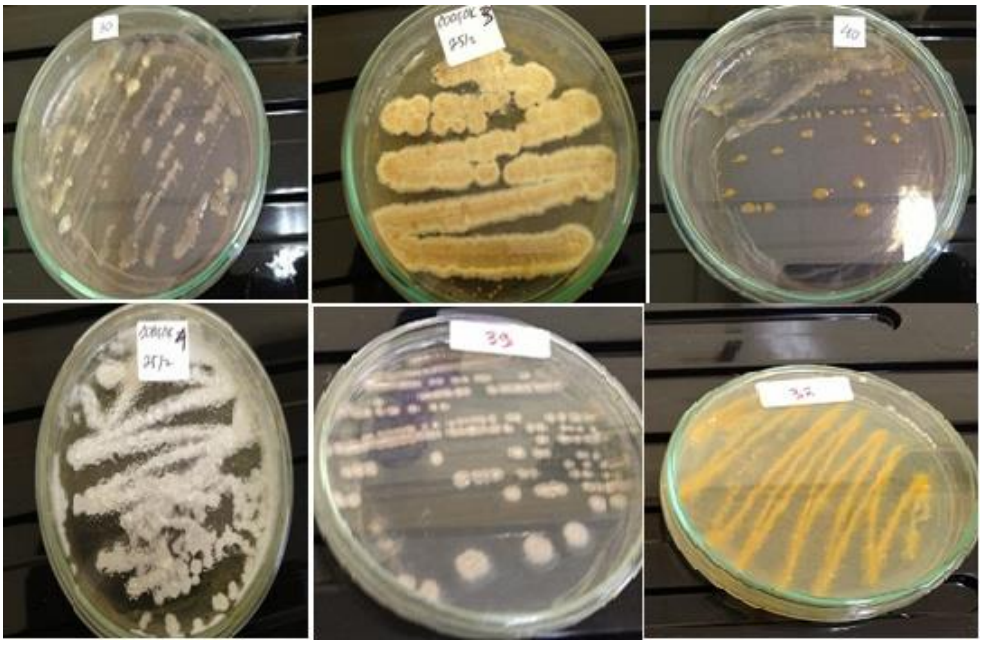


Figure 2: Some of Isolates Producing GA and IAA

3.2 Production of Indole Acetic Acid (IAA)

The ability of the bacterial isolates to produce IAA was detected by the development of pink color after the addition of salkowski reagent to the culture. Some species of bacteria have the ability to produce IAA. Many evidence suggests that PGPR can affect plant growth and development as it can produce phytohormones. Phytohormones such as auxin (IAA) is known to stimulate cell elongation and cell division differentiation (Achmad *et al*., 2005), and gene regulation (Ryu *et al*., 2008). Indole acetic acid is the common natural auxin that shows all auxin activity and extensively affects plants physiology (Etesemi *et al*., 2009). Indole acetic acid is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement (Glickman *et al*., 1995). In our study, all 35 isolate were able to produce IAA growing in medium addition of tryptophan. Maximum IAA production was recorded in isolate RK 32 (2.794 mg l-1) as compared to other isolates. The minimum amount of IAA production was recorded in RK 4 (0.190 mg l-1). Furthermore, the highest production of IAA which identified as gram positive bacteria is AK 7 (3.208 mg l^{-1}) and the lowest is AK 17 (0.034 mg l^{-1})

3.3 Production of GA3

A number of isolates was isolated from soybean crop and it is expected to have a potential in producing gibberellin, because gibberellin can give positive influence on the germination of plants in improving the cell elongation.

The analysis is using standard methods (Borrow *et al*., 1995) shows that the fifty six isolates can produce GA3 at different concentrations (Table 3). The results of the analysis of the capability of producing GA3 concentration is in the range of 0.034 mg l^{-1} sampai 3.222 mg/l. Isolates RK 35 (3.222 mg/l) showed high concentrations while the lowest concentration shown by isolates AK 21(0.084 mg/l).

According to Bottini *et al.*, (2004), bacteria can increase the levels of GA in cultured isolates because there is a process conjugation of root exudates of Gibberellin. Gibberellin plays an important role in controlling the developmental processes of plants that includes germination, cell elongation, and the development of flowers and seed (Lakitan, 1996) .It was also expressed by King and Evans (2003), that gibberellin has a very useful processes associated with the induction of flowers and the growth of flowers and fruits. The capability of gibberellin in promoting plants growth is stronger than the AIA (Reinoso *et al*., 1993). Gibberellin is also involved in stimulating root growth, abundance of root hairs, and inhibition of differentiation of flower buds on angiosperms, setting dormant vegetative buds and generative phase, and inhibiting senescence process in many organs at various species of plants (Bottini and Luna, 1993; Fulchieri *et al.*, 1993; Tanimoto (1987) in Bottini *et al.*, 2004).

Table 3: (a) Level of Concentration Isolates Producing IAA and Gibbrellin Acid that has gram negative
(b) Level of Concentration Isolates Producing IAA and Gibbrellin Acid that has gram positive

3a

Isolates	IAA (mg l^{-1})	GA (mg l^{-1})
RK1	1.714	2.902
RK2	0.651	2.512
RK3	0.698	2.389
RK4	0.19	2.628
RK 5	1.54	2.573
RK 6	1.365	2.539
RK 7	0.921	2.687
RK 8	0.968	2.455
RK 9	0.571	2.521
RK 10	0.571	2.858
RK 11	0.714	2.949
RK 12	0.328	2.439
RK 13	0.413	2.535
RK 14	0.937	2.856
RK 15	1.333	2.645
RK 16	0.889	2.743
RK 17	1.095	3.797
RK 18	0.905	3.176
RK 19	0.683	2.628
RK 20	0.365	2.588
RK 21	0.889	2.538
RK 22	0.968	3.135
RK 23	1.698	2.622
RK 24	0.635	3.293
RK 25	1.079	3.703
RK 26	0.857	2.419
RK 27	0.302	2.502
RK 28	0.841	2.698
RK 29	0.73	2.658
RK 30	1.429	4.67
RK 31	1.222	2.654
RK 32	2.794	2.476
RK 33	0.429	2.571
RK 34	0.825	2.588
RK 35	0.638	3.222

3b

Isolates	IAA (mg l^{-1})	GA (mg l^{-1})
AK1	2.890	0.099
AK2	2.982	0.049
AK3	2.751	0.050
AK4	2.610	0.037
AK5	2.664	0.061
AK6	2.685	0.071
AK7	3.208	0.059
AK8	2.899	0.127
AK9	2.786	0.077
AK10	2.806	0.097
AK11	3.066	0.072
AK12	2.898	0.096
AK13	2.714	0.057
AK14	2.898	0.060
AK15	3.001	0.068
AK16	0.127	0.088
AK17	0.034	0.104
AK18	0.062	0.088
AK19	0.028	0.250
AK20	0.051	0.214
AK21	0.072	0.080

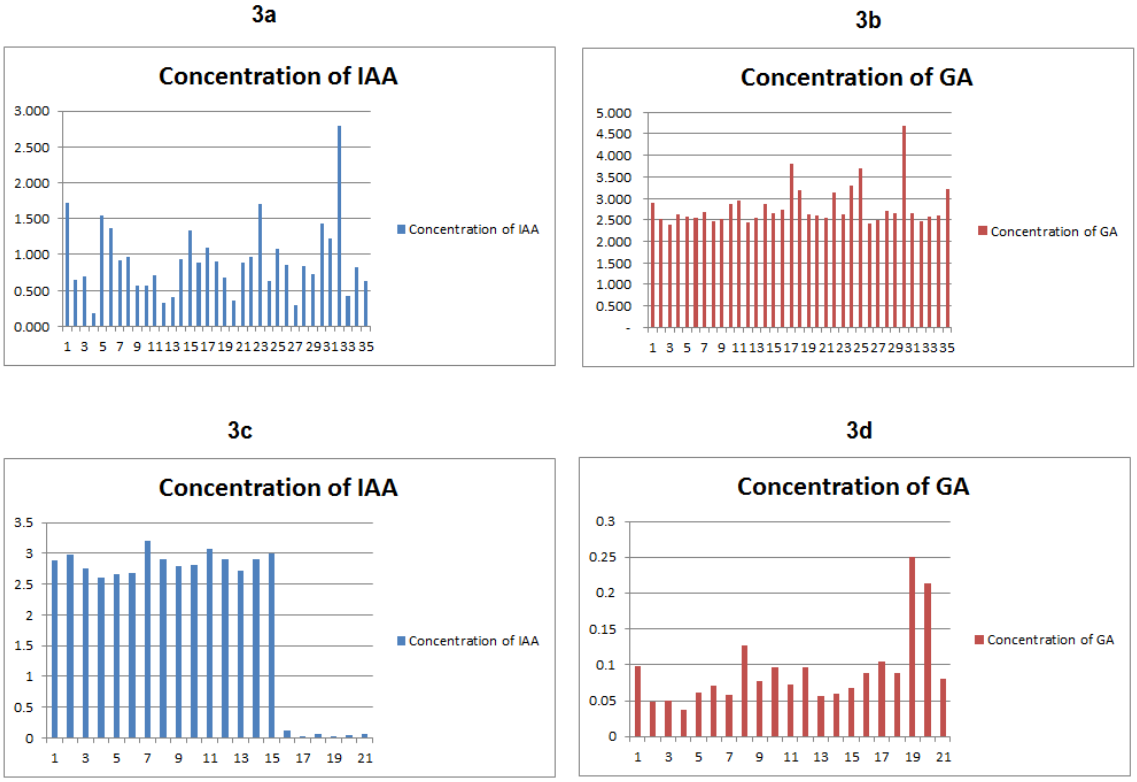


Figure 3: (a) Level concentration of Indole Acetic Acid produced by Rhizosphere Bacteria that has negative gram Isolated from Soybean plants.
(b) Level concentration of Gibrellic Acid produced by Rhizosphere bacteria that has negative gram isolated from Soybean Plants.
(c) Level concentration of Indole Acetic Acid produced by Rhizosphere Bacteria that has positive gram Isolated from Soybean plants.
(d) Level concentration of Gibrellic Acid produced by Rhizosphere bacteria that has positive gram isolated from Soybean Plants

Our results indicated that the comparison between two different types of rhizosphere bacteria has different pattern in the number of phytohormone they produced such as Indole Acetic Acid and Gibberellin Acid. As shown in figure 3a and 3b rhizosphere bacteria with negative gram has produced Gibberellin Acid highest than Indole Acetic Acid compare to bacteria with positive gram has produced Gibberellin Acid lower than Indole Acetic Acid as indicated in figure 3c and 3d.

4. Conclusions

The vast majority of bacteria isolated from three different areas of South Sulawesi was successfully grown from Takalar. In terms of characterization Bacteria isolated from rhizosphere of Indigenous soybean of South Sulawesi had more than one physiological characters such they can produce Gibberellin acid and Indole Acetic Acid. Isolates RK 32 (2.794 mg/l) produced the highest level of IAA concentration and RK 30 (4.670 mg/l) secreted the highest number of GA concentration. Furthermore the isolates with positive gram that produced the highest concentration of GA was AK 20 (0.214 mg/l) and produced IAA AK 11 (3.066 mg/l).

5. Acknowledgements
The author thanks to those who already helped in doing the isolation process also the student who helps took several plants samples from three different areas of South Sulawesi. Also the author acknowledged Dr.Malcolm Wagener, The University of Queensland, Australia, for his valuable inputs and comments into this manuscript.

References

[1] Achmad F., Ahmad I., Khan M.S., 2005, Indole acetic acid production by indogeneous isolates of Azotobacter and fluoresecent Pseudomonas in the presence and absence of trypthopan. Turk. J.Biol 29, pp.29-34.
[2] Amar J. D., Kumar M., Kumar R.,2013, Plant Growth Promoting Rhizobacteria (PGPR): An Alternative of Chemical Fertilizer for Sustainable, Environment Friendly Agriculture. Res. J. Agriculture and Forestry Sci. 2013; 1(4), pp.21-23
[3] Bottini R, Cassan F, Piccoli P. (2004). Gibberellin Production by bacteria and its involvement in plant growth promotion and yield increase. Appl. Microbiol Biotech. 65, pp.497-503.
[4] Borrow A., Brain P.W., Chester U.E., Curtis P.J., Hemming H.G., Jeffereys E.C., Lloyd R.B., Nixon I.S., Norris G.L.F., and Radley N., 1995, Gibberellic acids a metabolic product of the fungus *Gibberella fujikuroi* some observations on its production and isolation. J. Sci. Food. Agric 6, pp.340-348.
[5] Cassán F., Lucangeli C., and Piccoli P., 2001b, *Azospirillum spp.* Metabolize [17, 17-2H2] Gibberellin A20 to [17, 17-2H2] Gibberellin A1 in vivo in dry rice mutant seedlings. *Plant Cell Physiol.* 42, pp.763-767.
[6] Ditjentan, 2004. Profil Kedelai (Glycine max). Buku 1. Direktorat Kacang-Kacangan dan Umbi-Umbian. Departemen Pertanian. Jakarta.
[7] Etesami H, Hossein A., Abolfazl A., 2009, Evaluation of plant growth hormones production (IAA) ability by Iranian soils, rhizobial strains and effects of superior strains application on wheat growth indexes. World Appl Sci J. 6(11), pp.1576–1584.
[8] Kloepper J.W., Beauchamp C.J., 1992, A review of issues related to measuring of plant roots by bacteria. Canadian Journal of Microbiology 38, pp.1219–1232
[9] King R.W., Evans L.T., 2003, Gibberellins and flowering of grasses and cereals: prizing open the lid of the "florigen" black box. Annu Rev Plant Biol 54: pp.307–328
[10]Lakitan, B., 1996. Fisiologi Pertumbuhan dan Perkembangan Tanaman. PT Raja Grafindo Persada. Jakarta.
[11]Boiero D.P., Masciarelli O., Penna C., and Luna V., 2007, Phytohormone production by three strains of Bradyrhizobium japonicum and possible physiological and technological implications. Application Microbiol Biotechnologi (2007) 74: pp.874–880.
[12]Pandya.N.D. and Desai P.V., 2014, Screening and characterization of GA3 producing Pseudomonas monteilii and its impact on plant growth promotion. Int.J.Curr.Microbiol.App.Sci. 3(5), pp.110-115
[13]Glickman, 1995, Contribution of arbuscular mycorrhizas to biological protection of micropropagated pineapple (Ananas comosus (L.) Merr) against Phytophthora cinnamomi Rands. Agric Sci Finl 3: pp.241-251.
[14]Gutierrez C.K., Matsui G.Y., Lincoln D.E., and Lovel C.R., 2009, Production of the phytohormone indole-3 acetic acid by the estuarine species of the genus Vibrio. Appl Environ Microbiol 75, pp.2253-2258.
[15]Ryu R., and Patten C.L., 2008, Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by 4 TyrR in Enterobacter cloacae UW5. American Society for Microbiology 190 (Suppl 21), pp.1-35
[16]Sudaryanto T., dan Swastika D.K.S., 2007, Ekonomi Kedelai di Indonesia. Badan Penelitian dan Pengembangan Pertanian. Pusat Penelitian dan Pengembangan Tanaman Pangan. Bogor.

