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Increasing Plant Health using Plant Growth Regulator from Rice Rhizobacteria

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Abstract: Plant health is a very important factor in the agriculture system. The presence of plant hormones such as Auxin, gibberellic acid, and siderophores with variation numbers in the plant gives different effects on plant health. Related to the environment rich with rhizobacteria, they have a unique function because rhizobacteria can produce a chemical compound known as Plant Growth Promoting Rhizobacteria (PGPR). The purpose of the research is to study the production of auxin, gibberellic acid, and siderophore from rice rhizobacteria as the source of potential hormones for plant growth. Ten rhizobacteria isolates have been isolated from the rice rhizosphere. They are potential candidates as biofertilizers and biopesticide agents. The hormone production from each isolate was tested by spectrophotometric methods: Auxin at a wavelength of 535 nm with Salkowski's reagent, gibberellic acid at 254 nm, and siderophores at 560 nm with Hardaway's reagent. The results showed the highest auxin hormone was obtained from isolate 10 (3.374 ppm), the highest gibberellic acid from isolate 4 (3.960 ppm), and the highest siderophores hormone from isolate 2 (2.910 ppm). The conclusion of the research is, plant growth regulator from rice rhizobacteria has the potential increasing plant health.

Keywords: Auxin, Gibberellic Acid, Siderophores, Rhizobacteria, Rhizosphere

Introduction

In recent years, the global issue related to sustainable agricultural systems still developing around the world. Using of microbes as soil biofertilizers attracted many countries applying in their agricultural areas. People's high demand for healthy agricultural products is the trigger for farmers producing food materials based in an eco-friendly environment.

We know rhizobacteria is the root-associated bacterial living around the plant base and playing important role in providing nutrients. The rhizobacteria including those in the Plant Growth Promoting Rhizobacteria (PGPR) group, directly or indirectly promote plant growth and development. PGPR application is considerably important in improving plant growth, plant health, and yields (Bhattacharyya and Jha, 2012; Sharma *et al.*, 2014a).

Inoculation of rice plants used Plant Growth Promoting Rhizobacteria (PGPR) as the one important approach of eco-friendly technology. Commonly rice fields are induced by salinity causing ion toxicity, osmotic stress, ion imbalance, mineral deficiencies, and reducing quality and total yield of the affected crop (Rady *et al.*, 2021; El-Mageed *et al.*, 2022).

Hormones as the organic compound stimulating plant growth could be formed naturally and chemically. Plant hormones have the function to promote, inhibit or alter growth and development in a very small concentration. The kind of plant hormones such as Auxin (indole acetic acid), Gibberellic Acid (GA3), and siderophores, but are insufficient amounts supporting plant growth and development. Some PGPR strains can synthesize auxin from precursor present in the root exudates or organic matter. The previous study showed rhizobacteria from the rice plant rhizosphere producing auxin about 1.39 and 15.74 g/mL^{-1} (Javorekova *et al.*, 2020).

Auxin can spur cell prolongation, affecting healthy plant development. The impact of auxin on the plant showed the few parts of the plant that are not exposed to light will have faster growth, in contrast to other parts exposed to sunlight. Auxin is an easy find in the seed embryos, meristems of apical buds, and young leaves of the plant. Another function

of auxins is to stimulate flowering, increase enzyme activity and stimulate the formation of new roots. Also, auxin affects the induction of flowering (Haim *et al.*, 2021).

Gibberellic acid is a hormone similar to auxin, produced by plants in the meristem of apical buds, roots, young leaves, and embryos as the growth hormones in plants. The hormones greatly affect genetic traits (genetic dwarfism), flowering, ripening process or fruit ripening, mobilization of food materials during the germination phase, stimulating cambium activity and xylem development, preventing seed and shoot dormancy, and other physiological aspects. In addition, in the response to gravitational stimuli, gibberellic acid playing role in the early stages of cambium formation and stem gravitropism in the *Mangium* seedling (Hedden and Sponsel, 2015; Nugroho *et al.*, 2012).

Siderophores contain in plants influence genetic traits, stimulate flowering and fruit ripening, and are responsible for cell division (Patel and Minocheherhomji, 2018). It plays a role in the biological control of plant diseases with a very high affinity for iron and soluble in water (Aguado-Santacruz *et al.*, 2012). The main function of siderophores is to chelate ferrous iron/Fe(III) from various terrestrial and aquatic habitats, thereby making the compound available to microbial and plant cells (Ahmed and Holmstrom, 2014).

The potential of rhizobacteria as the trigger of plant growth through their ability to produce growth hormones is a desirable characteristic of rhizobacteria. Therefore, the use of rhizobacteria as the bio-stimulator agents must be carried out in consortium to optimize the production of growth hormones. Rhizobacteria can live synergistically then potentially increasing plant health. The purpose of the research is to study the production of auxin, gibberellic acid, and siderophore from rice rhizobacteria as the source of potential hormones for plant growth. In the future, studies focusing on plant health and the impact of growth hormone production from several potential rhizobacteria need more effort to explore it.

Materials and Methods

Isolates Preparation

Ten potential rhizobacteria were isolated from rice rhizosphere at three regencies in South Sulawesi, Indonesia (Takalar, Gowa, and Maros). The isolates were coded isolates 1 to 10. All of the isolates were prepared and reproduced using the scratch-and-spray method on the media.

Auxin Production Test

The production of auxin (indole acetic acid) test was performed with Salkowski's reagent in the spectrophotometric method. The isolates were cultured on the Nutrient Broth medium enriched with 0.1 ppm L-Tryptophan as the precursor, then incubated at 28ºC in the dark for 5 days. Furthermore, 5 mL of culture was taken and put into the test tube, followed by centrifugation at 10 min at 8000 rpm. The supernatant was taken at 1 mL, added 4 mL of Salkowski's reagent (150 mL H_2SO_4, 250 mL distilled water, and 7.5 mL $FeCl_3.6H_2O$ 0.5 M) was inside a test tube. There was incubated for 24 h at 28°C in the dark. The observations were made based color change of the culture to pink indicating the isolates contain auxin. The absorbance of all of the isolates was measured at a wavelength of 535 nm. Auxin production was measured based on the auxin standard curve.

The concentration of auxin in the analysis used a spectrophotometric method based on the isolates absorbed by the UV-Visible spectrophotometer on Lambert-Beer law, the absorbance value is proportional to the sample concentration. The wavelength used is 535 nm in the visible region. The wavelengths were selected based on the color produced by the interaction between Salkowski's reagent and auxin which produced pink color.

Gibberellic Acid Production

Gibberellic Acid (GA3) production test was carried out based on standard methods. Firstly, the isolates were cultured on a liquid Nutrient Broth medium, then incubated at 37°C for 5 days. The culture was centrifuged for 10 min at a speed of 8000 rpm. About 15 mL of culture was added to 2 mL of zinc acetate, and after 2 min, added 2 mL of potassium Ferrocyanide solution was then centrifuged again for 10 min. Finally, 5 mL of the supernatant was added to 30% hydrochloric acid, mixed, and incubated at 27°C for 75 min. Blank was prepared using 5% hydrochloric acid. The absorbance was measured at wavelength 254 nm. The average of gibberellic acid produced is measured by the gibberellic acid standard curve.

Production of Siderophores

The siderophores production test was carried out using Hathway's reagent method. The isolates were cultured on liquid Nutrient Broth medium then incubated at 37°C for 5 days. After incubation, the culture was centrifuged for 10 min at a speed of 8000 rpm. About 20 mL of supernatant was taken and pH was adjusted using an HCl solution. About 20 mL of the supernatant was added into 20 mL of ethyl acetate, then extraction was carried out twice. Finally, 5 mL of the test solution was added to 5 mL of Hathway's reagent (1 mL of 0.1 m ferric chloride and 1 mL of 0.1 N HCl were added to 100 mL of distilled water mixed with 1 mL of 0.1 m potassium ferricyanide). The absorbance was measured at a wavelength of 560 nm. The average of siderophores production was measured by the siderophores standard curve.

Results and Discussion

Production of Auxin

Auxin is an important growth hormone basic need in plant development. Table 1 showed the results of absorbance

value and auxin production. The highest production of auxin was obtained from isolate 10 (3.375 ppm) and the lowest was shown by isolate 5 (1.734 ppm).

The results of Table 1 about the auxin hormone test showed that all isolates were able to produce auxin. There indicated color changes in the culture after adding Salkowski's reagent and incubation for 24 h. The color of the culture before the test was a cloudy yellow changed to pink color (Fig. 1). Salkowski's reagent containing $FeCl_3$ and $HClO_4$, reacted with auxin to be a pink color.

According to Kholida and Zulaika (2016), the color change of the isolate to pink after adding Salkowski's reagent was due to the reaction between Salkowski's reagent with auxin or Indole-3-Acetic Acid (IAA) forming compounds from tris-(indole)-3-acetate) iron (III) complex gives a reddish to red color. Therefore, rhizobacteria as the main source produced auxin turn to pink color when reacted with Salkowski's reagent.

The genetic factors of each isolate strongly influenced the production of auxin by rhizobacteria. On the other hand, PGPR mediates biological control directly by eliciting induced systemic resistance against several plant diseases (Jetiyanon and Kloepper, 2002) and increasing many hormones produced by the plant (Ashrafuzzaman *et al.*, 2009). The nutrient content of rhizobacteria in the growth media also greatly influences auxin production, especially media containing L-Tryptophan as the precursor. Commonly rhizobacteria produced optimum levels of auxin in the presence of sucrose and tryptone as carbon and nitrogen sources, respectively (Suliasih and Widawati, 2020). Auxin produced by rhizobacteria are phytohormones able to increase plant growth. Various types of plant growth-promoting rhizobacteria produce various types of phytohormones. For example, indole 3-acetic acid is the plant growth hormone that is responsible regulates physiological processes (Herlina *et al.*, 2017).

Differences in the auxin concentration are also due to the strain, growth phase, and age of the isolate. Patil *et al.* (2011) stated that rhizobacteria started producing auxin at the beginning of growth and maximum at the stationary phase. Production of auxin reaches the maximum when growth conditions decrease, with limited carbon availability and acidic conditions (Dewi *et al.*, 2015). These conditions occur when rhizobacteria enter the stationary phase. The differences in auxin production could be due to the supply of L-tryptophan as the precursor of auxin synthesis. L-tryptophan is a precursor in auxin biosynthesis in many plants and microbes (Patil *et al.*, 2011). Sometimes auxin production decreases because rhizobacteria re-consume the auxin from their production when the growth medium has inadequate nutrients (Lestari *et al.*, 2007).

Production of Gibberellic Acid

The result of Table 2 showed all of the isolates produced gibberellic acid in a very number. It was indicated by the occurrence of color changes in the culture after adding zinc acetate and potassium Ferrocyanide solution and then incubating for 24 h. The color of the isolates was cloudy yellow, and after adding zinc acetate and potassium ferrocyanide solution formed pale yellow (Fig. 2).

Gibberellic acid production of each rhizobacterium showed the highest production from isolate 4 (3.960 ppm) and the lowest from isolate 5 (3.849 ppm).

As we know, auxin is an important plant hormone, and gibberellic acid also plays an important role as a growth regulator that promotes cell prolongation, seed germination, flowering, and fruit ripening. Based on the induced systemic, in nature, the unique hormone also producing by organisms such as plants, fungi, and bacteria (Kloepper *et al.*, 2004). The role of Gibberellic Acid (GA3) in the plant has many benefits because it can break dormancy, increase flowering, and spur the process of seed germination and cell elongation.

The ability of each isolate to produce gibberellic acid is influenced by several factors: The biochemical characteristics of each isolate and environmental factors (temperature, light, nutrients in the culture media, humidity, pH, and incubation time) (Kumar *et al.*, 2014). In addition, the gibberellic acid plant growth-promoting hormones, playing important role in seed germination (Urbanova and Leubner-Metzger, 2016), response to abiotic stress (Colebrook *et al.*, 2014) stem elongation (Wang *et al.*, 2017) flowering (Muñoz-Fambuena *et al.*, 2012) and other physiological effects that occur in interaction with other phytohormones (Hedden and Sponsel, 2015).

Commonly gibberellic acid or GA3 producing by plants, fungi, and bacteria (Camara *et al.*, 2018). The microbe strain and the growth media composition greatly affected the production of siderophores in each rhizobacterium isolate. Siderophores are secondary metabolites produced by microbes in the form of organic compounds with low molecular mass. The main function of the siderophores is to chelate iron/Fe(III) from various terrestrial and aquatic habitats, making they are available to microbes and plant cells. Siderophores are compounds that playing role in the biological control of plant diseases with a very high affinity for iron, soluble in water, and rapidly diffuse (Habazar and Yaherwandi, 2006).

Production of Siderophores

The result in Table 3 showed all of the isolate's potential to produce siderophores. The color change of the culture was indicated after adding Salkowski's reagent and 1 mL 0.1 m potassium ferrocyanide, then incubation for 24 h. Figure 3 was showed the culture color was cloudy yellow and changed to blue color.

Besides the color change in isolates after added reagent, the highest production of siderophores showed in isolate 2 (2.910 ppm), and the lowest showed in isolate 10 (0.005 ppm) (Table 3).

Table 1: The isolates code and absorbance of auxin production from rhizobacteria

Isolates code	Absorbance	Number of auxin production (ppm)
1	0.131	1.906
2	0.183	2.718
3	0.149	2.187
4	0.193	2.875
5	0.120	1.734
6	0.187	2.781
7	0.136	1.984
8	0.165	2.484
9	0.212	3.171
10	0.225	3.375

Table 2: The isolates code and absorbance of gibberellic acid production from rhizobacteria

Isolates code	Absorbance	Number of gibberellic acid production (ppm)
1	3.899	3.894
2	3.949	3.950
3	3.888	3.881
4	3.958	3.960
5	3.859	3.849
6	3.890	3.884
7	3.944	3.944
8	3.889	3.882
9	3.895	3.889
10	3.881	3.873

Table 3: The Isolates Code and Absorbance of Siderophores Production from Rhizobacteria

Isolates code	Absorbances	Number of siderophores production (ppm)
1	0.448	2.351
2	0.548	2.910
3	0.348	1.793
4	0.338	1.737
5	0.460	0.106
6	0.036	0.050
7	0.366	1.893
8	0.342	1.759
9	0.135	0.603
10	0.028	0.005

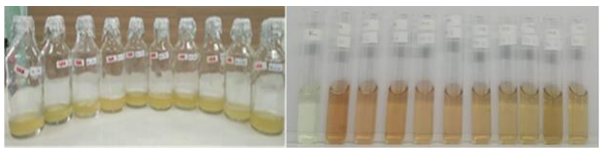

Fig. 1: The color of auxin before and after treatment (pictures of result research by Maimuna Nontji, 2021)

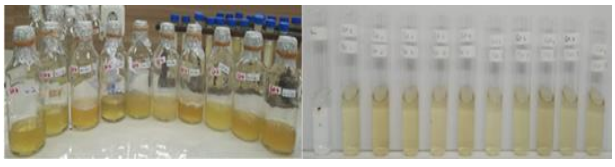

Fig. 2: The color of gibberelic acid before and after treatment (pictures of result research by Maimuna Nontji, 2021)

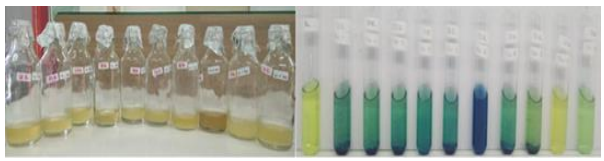

Fig. 3: The color of siderophores before and after added reagent (pictures of result research by Maimuna Nontji, 2021)

Several studies reported that siderophores produced by bacterial such as *Azotobacter* (16.22%), fluorescent *Pseudomonas* (11.11%), and *Bacillus* (10%) increased shoot and dry wheat biomass by 23 and 45%, respectively (Fischer *et al.*, 2007). This useful compound also playing important role in controlling plant pathogens.

Related to the color change of siderophores from rhizobacteria, (Sharma *et al*., 2014b; Mina *et al*., 2013) reported that the medium color was changed by

rhizobacteria from blue to orange. The difference in color changes in the medium plate (orange, purple, or purplish-red) recommends the production of siderophores of a different nature by the variety of microorganisms isolated and the color intensity can be a consequence of siderophore concentration. Many siderophores-producing microorganisms suppress some soil-borne fungal pathogens through a direct role as the biocontrol ability. A maximum number of siderophores was shown by *Pseudomonas* sp. in the of range, 20-21 mm in diameter of the orange color zone.

In addition, siderophores-producing rhizobacteria effectively plant root disease infections causing annual losses in tobacco plants (Motta *et al.*, 2004; Haas and Défago, 2005). Furthermore, there also explained that 75% of isolates contain siderophores around 40-60%, such as *Pseudomonas*, could be potentially applied to low-iron soils to prevent plant soil-borne fungal pathogens (Tian *et al.*, 2009; Koche *et al.*, 2012; Deng *et al.*, 2016). New knowledge in plant health always develops over time. For the future, the highest rice rhizosphere microbes production plant growth hormones recommended for application plant growth based ecosystem friendly.

Conclusion

Ten isolates of rhizobacteria isolated from rice rhizosphere produced plant-growth-promoting hormones resulting in many varieties. The potential highest production of hormones: Isolate 10 produced auxin hormone (3.375 ppm), isolate 4 produced gibberellic acid (3.960 ppm), and isolate 2 produced siderophores (2.910 ppm), respectively. Based on the research, using a plant growth regulator from rice rhizobacteria has the potential increasing plant health and reduce apply of a chemical compound in nature.

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Author's Contribution

Maimuna Nontji: Conception and design, analysis and interpretation of data, drafting the manuscript, final approval of the manuscript.

Ayu K. Parawansa: Translator and final approval of the manuscript.

Saida Saida: Interpretation data and final approval of the manuscript.

Suriyanti Suriyanti and Ida Suryani: Organizing the research and final approval of the manuscript.

Mulianty Galib: Study of literature and final approval of the manuscript.

Anwar Robbo and Lailatul Qadar: Laboratory assistant.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read, and approved the manuscript and that no ethical issue is involved.

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