p-ISSN 0854-9028 e-ISSN 2549-9343
AGRIC
JURNAL ILMU PERTANIAN
Fakultas Pertanian dan Bisnis Universitas Kristen Satya Wacana
Jl. Diponegoro 52-60 SALATIGA 50711 - Telp. 0298-321212 ext 354
email: jurnal.agric@adm.uksw.edu, website: ejournal.uksw.edu/agric
Terakreditasi Kementrian Riset, Teknologi dan Pendidikan Tinggi berdasarkan SK No 200/M/KPT/2020

SIDEROPHORE PRODUCTION OF THE RHIZOBACTERIA ISOLATED FROM LOCAL "KAMBA" RICE PLANTS, POSO REGENCY IN CENTRAL SULAWESI

Sri Sudewi[1], Baharuddin Patandjengi[2], Ambo Ala[3], Muh. Farid BDR[3], Abdul Rahim Saleh[4], and Ratnawati[1]

[1]Department of Agrotechnology Faculty of Agriculture, University of Alkhairaat Palu 94221
[2]Department of Plant Pest and Disease, Faculty of Agriculture, University of Hasanuddin Makassar 90245
[3]Department of Agrotechnology, Faculty of Agriculture, University of Hasanuddin Makassar 90245
[4]Department of Agrotechnology Faculty of Agriculture, University of Sintuwu Maroso Poso, 94619
email: srisudewirahim@gmail.com

Received: 16 April 2022 | Accepted: 21 September 2022

ABSTRACT

Rhizobacteria isolated from plant roots have the ability to produce siderophore compounds. These compounds play a role in inhibiting the growth of pathogens by binding to iron (Fe^{3+}) which is needed by pathogens in their development. This research was aimed to find out the siderophore-producing bacteria isolated from local Kamba rice plants and their potential as biocontrol agents. Among the 28 isolates that were successfully isolated from the Kamba local rice rhizosphere, 10 isolates had the potential to produce siderophores with different morphological characters. The test was carried out to see the bacterial isolates capabilities to produce siderophores from two types of siderophores namely catechol and salicylate types. For the catechol type, the highest concentration of siderophore was found in the KBA8 bacterial isolate with 10.990 mg L^{-1}, while the lowest was in the KBA1 bacterial isolate with only 5.876 mg L^{-1}. The salicylate type siderophore with the highest concentration produced 9.493 mg L^{-1} was from the RKGU15 isolate and the lowest was found in KBU14 isolate which produced only 2.994 mg L^{-1}. The isolates included in the Gram-positive group were 4 isolates while the Gram-negative group were 6 isolates and 90% isolates were able to produce the enzyme catalase. The results of this study indicate that all bacterial isolates can produce siderophores so that they have the potential as biocontrol agents to support environmentally friendly and sustainable agriculture.

Keyword: Siderophore, rhizobacteria, kamba, biocontrol

INTRODUCTION

One of the inhibiting factors for increasing the productivity of local rice plants is the attack of Plant Pest Organisms (OPT). Therefore, an effort is needed so that local rice varieties in Central Sulawesi, especially local Kamba rice, can be protected from pests and diseases. Efforts can be made by utilizing rhizobacteria that have the ability to produce siderophores. Isolation of rhizobacteria is important because it is an asset in providing iron needed by plants. Rhizobacteria that produce siderophore compounds are able to chelate iron from the environment so that it cannot be accessed by pathogens for growth, ultimately resulting in a decrease in the ability of pathogens to infect disease in host plants.

The Poso Regency, a region in Central Sulawesi, is an area where most people cultivate local rice plants for generations. One type of local rice varieties found in this region is known as Kamba. The specialty of Kamba local rice is that it has a fragrant aroma that can arouse appetite and is also delicious and has a fluffier texture.

Local Kamba rice cultivated by farmers in isolated areas of Poso Regency still applies traditional cultivation systems, according to the customs and traditions of the local communities without using inorganic fertilizers, pesticides, and other chemical herbicides. Such well maintained and natural ecosystems condition creates a natural growth environment and increases the diversity of microorganisms, one of which is bacteria that have the potential as a biocontrol that can produce siderophore compounds.

Siderophore is a small organic molecular compound weighing between 500 to 1500 Da or is a specific chelating Fe^{3+} compound that is produced by microorganisms in limited Fe^{3+} conditions to increase the absorption capacity of iron by these microorganisms (Ahmed and Holmström, 2014; Saha et al., 2016). Therefore, siderophore-producing bacteria play a role in increasing plant growth directly and controlling phytopathogens indirectly (Kumar et al., 2018). Siderophore-producing bacteria indirectly have chelating properties of iron which make iron inaccessible to pathogens, thus limiting its growth.

The three main groups of siderophore are hydroxamate, catecholate and carboxylate (Ali and Vidhale, 2013), but certain siderophores also have ligands with a mixture of lysine, ornithine, and histamine derivatives (Sah and Singh, 2015). There are 270 reported siderophores have been structurally marked out of 500 known siderophores (Cornelis, 2010) and several hundred chelators of active iron compounds have been characterized and purified (Hider and Kong, 2010).

Microorganisms that can produce siderophores are bacteria and fungi, and some graminaceous plants. Generally, hydroxamate siderophores are produced by fungi, with catecholate is only produced by bacteria, and carboxylates are produced by plants (Khan et al., 2018; Pecoraro et al., 2021). Siderophore from the catechol group produced by bacteria has a stronger affinity for the element of iron than hydroxamate siderophore, which is generally produced by fungi so that siderophores have the potential as an efficient source of iron for plants (Rehan et al.,2022). The Catechol siderophore type has a phenolic or 2,3-*Dihydroxy Benzoate* (DHB) binding group. Catechol, also known as *Pyrocatechol* or 1,2-

Dihydroxybenzene, is an organic compound with the molecular formula $C_6H_4(OH)_2$ (Sah and Singh, 2015; Rai et al., 2020).

Siderophore production by microbes is mainly influenced by the presence of iron in the environment and complex environmental factors such as pH, metal contamination, and interactions between microbes and Fe^{3+} (Dimkpa, 2016). Some research results mentioned some bacteria that have the capability to produce siderophore, such as *Azotobacter* (Dewedar et al., 2018; Baars et al., 2016; Zhang et al., 2019), *Bacillus* (Rizzi et al., 2019; Yu et al., 2017; Sinha et al., 2019), *Pseudomonas* (Sasirekha and Srividya, 2016; Kousser et al., 2019; Yu et al., 2017), *Azospirillum* (Vijayalakshmi et al., 2019; Pedraza, 2015), *Enterobacter* (Kumar et al., 2017), *Rhizobium* (Ferreira et al., 2019), *Paenibacillus* (Chen et al., 2016; Wang et al., 2019; Liu et al., 2017). Genus *Bacillus* and *Pseudomonas* high potential as a controlling agent biological because capable of producing secondary metabolites (Cosntantia and Ferniah, 2020; Sudewi et al., 2021). Some research results also showed the use of siderophore-producing rhizobacteria combined with other biological fertilizers has the potential to increase crop production. Siderophore-producing rhizobacteria can be used as a biocontrol agent that is very promising in supporting environmentally friendly and sustainable agriculture.

Add the novelty and benefit of this research to science and technology

MATERIALS AND METHODS

Study area

Sampling area for the isolation of siderophore-producing rhizobacteria was located in the paddy fields of the residents in the Bada Valley, Poso Central Sulawesi. The rhizosphere soil samples were taken randomly on 3 healthy rice plants in each rice field location (5 sample locations) and then composited. The rice plants were gently removed, then the plant samples were transferred into labeled A3 brown envelopes and then is put into a cooler box and taken to the Laboratory for further isolation.

Isolation and morphological characterization of bacterial isolates

Siderophore-producing rhizobacteria were isolated from the rhizosphere of Kamba local rice plant in the Bada Valley in Poso Regency, Central Sulawesi. A total 10 g of soil particles attached to plant roots were

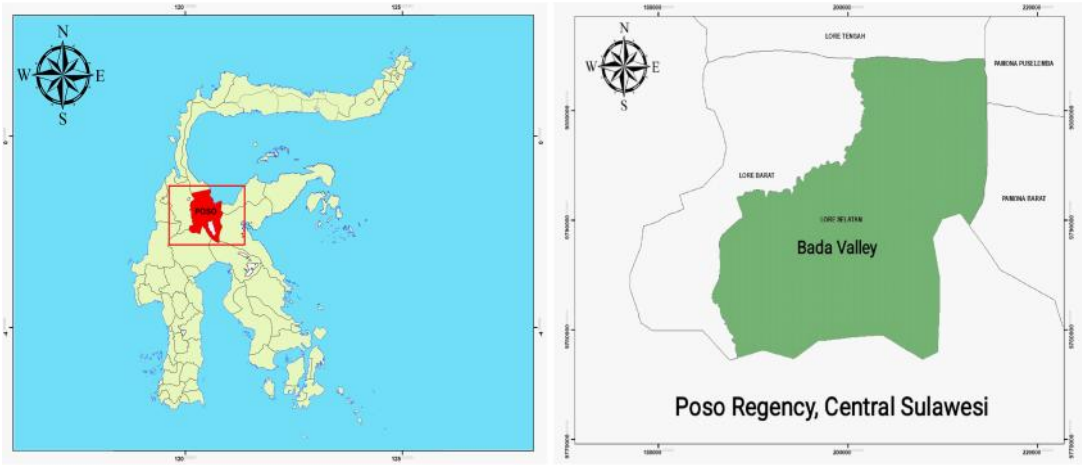


Figure 1 Sampling Area Rhizobacteria in Bada Valley, Poso Central Sulawesi

taken and then crushed to be suspended in an erlenmeyer containing 100 ml of sterile distilled water. Furthermore, the suspension was shaken using a shaker at a high speed of 120 rpm for 15 minutes. The suspension obtained is taken as much as 1 mL for The dilution was carried out in stages up to a dilution level of 10^{-9}.

Suspensions were taken from dilutions of 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9} using only 0.1 ml each dilution and is then spread on agar nutrient media, incubated for 24 hours at 28°C (Ben-David and Davidson, 2014).

The single colony that has grown on the NA media's morphology is observed with the aim to determine the characterization of each bacterial isolate which includes size (*pinpoint, small, moderate, large*), pigmentation (colony color), colony shape (*circular, irregular, rhizoid, filamentous*), borders of the colonies (*entire, lobate, undulate, serrate, curled, filamentous/erose*), and elevation (f*lat, raised, convex, umbonate, pulvinate*). For further testing purposes, every single colony that grows is isolated until pure isolate culture is obtained (Cappucino and Sherman, 2008).

Hypersensitive Response (HR) Test

The hypersensitive response occurs in plants as a reaction to infection of fungi, viruses, and plant pathogenic bacteria. The existence of a hypersensitivity reaction of bacterial isolates was qualitatively tested based on the modified Atkinson et al., 1985 method. The test aims to determine the response of siderophore-producing rhizobacteria isolates that are potentially pathogenic to plants. Brown necrosis spots and dryness in tobacco leaf tissue indicate a positive hypersensitivity reaction, otherwise, if necrosis does not occur, the resulting reaction is a negative hypersensitivity reaction. Tests carried out used healthy tobacco leaves (*Nicotiana tabacum* L.) aged ± 3 months. Bacterial isolates were previously cultured on NA media for 24 hours at room temperature of 28^0C. As much as 1 ml of bacterial isolate suspension is injected into leaf tissue with a density of 10^8 CFU/mL through secondary leaf bone using a 1 ml syringe (without needle). Control isolates in this test used aquadest PA as a negative control, and the isolate *Burkholderia glumae* as a positive control (pathogenic bacteria which is a collection of the Biotechnology Laboratory of Hasanuddin University). After 24 hours, it was observed up to 72 hours to see the hypersensitivity reaction in tobacco plants that had been injected.

Gram test and catalase test

A simple method to determine the nature of Gram bacterial isolates is by using 3% *Potassium Hydroxide* (KOH) (Suslow et al., 1982). Pure single colonies that were previously cultivated on NA media were taken in one loop then dissolved in 3% KOH. Bacterial isolates that produce mucus when removed from the suspension surface indicate that the bacterial isolates belong to the Gram-negative group (-), whereas bacterial isolates that do not produce mucus when removed from the suspension surface are included in the Gram-positive (+) group.

The production of catalase from bacterial isolates can be proven by adding one loop (full loop) of pure single colony culture then smeared on a slide that has been given two drops of *hydrogen peroxide* (H_2O_2) 3%.

Thorugh this method of the catalase test, it aims to test the ability of the catalase-producing microbes to degrade H_2O_2. The emergence of gas bubbles from free oxygen when 3% *hydrogen peroxide* solution is dropped is an indicator of a positive reaction (+), whereas a negative reaction does not indicate this (Reiner, 2010).

Test the ability of bacterial isolates to produce siderophore

Bacterial isolates capable of producing siderophore refer to the method (Sivasakthivelan and Stella, 2012), which is modified. Qualitative measurements of bacterial isolates produce siderophores are characterized by changes in the color of the solution after the addition of *Hathway reagents* (1 ml 0.1 M *Iron chloride*, 1 ml of 0.1 N *hydrogen chloride* was added to 100 ml of aquadest and added 1 ml of 0.1 M *kalium ferricyanide*). A total of 100 ml of sterile *Nutrient Broth* (NB) media was prepared, then bacterial isolates were inoculated into the NB media and then incubated for 7 days at 37^0C then centrifuged for 20 minutes at 10.000 rpm. Add 20 ml of *ethyl acetate* ($CH_3CH_2OC(O)CH_3$) into 20 ml of the supernatant extraction twice then add 5 ml of the *Hathway reagent* test solution. A total of 20 ml of ethyl acetate ($CH3CH2OC(O)CH_3$) was added to 20 ml of the supernatant, extracted twice and then added 5 ml of *Hathway's reagent* test solution. Quantitative concentration measurement was by measuring optical density using a UV-VIS spectrophotometer at a wavelength of 560 nm for the *salicylat*e type and 700 nm for the *catechol* type. *Sodium salicylate* is used as a standard to measure the concentration of salicylic siderophores. The standard is made from dilution with $C_7H_5NaO_3$ (*sodium salicylate*) concentration ranging from 0 to 2.5 mg L^{-1}. The regression equation is Y = 0.1792x + 0.0332, where R2 = 0.9598. catechol-type siderophore was measured using 2.3 DHBA as a standard, with concentrations ranging from 0 to 2.0 mg L^{-1}. The standard curve of the regression equation Y = 0.2097x + 0.0371 where R2 = 0.9584. Concentrations in a culture filtrate were determined and expressed as mg L^{-1}.

RESULTS AND DISCUSSION

Isolation and morphological characterization of bacterial isolates

Among the 28 isolates that were successfully isolated from the Kamba local rice rhizosphere, which had the potential to produce siderophores as many as 10 isolates. The observation of morphological characterization of the bacterial isolates generally has a circular shape. Tests showed nine bacterial isolates varying in sizes, such as pinpoint-sized, small-sized, medium-sized, and large-sized. The elevation of these bacterial isolates showed as many as eight isolates were flat, the others were elevated or had convex elevations where as many as eight isolates had flat borders and two corrugated isolates. The colors of bacterial isolate colonies vary between white, beige, yellow, and purplish black. Based on these morphological observations, it is known that the ten bacterial isolates have different morphologies. It is suspected that one isolate with another is not the same bacteria. Morphological characterization results from the bacterial isolates can be seen in (Table 1, Fig 3).

The difference in characterization seen in each bacterial isolate was thought to be due to differences in the origin of the samples obtained, the cultivation techniques used, the availability and different nutritional requirements that affect the presence of microorganisms that exist in the soil.

Table 1 Results from the morphological characterizations of the bacterial isolates

Isolate Code	Colony Morphology				
	Size	Coloni Form	Colony Edge	Elevation	Colony Colour
KBA 1	Small	Circular	Entire	Flat	Yellow
KBA 8	Small	Circular	Entire	Flat	White
KBA14	Small	Circular	Entire	Umbonate	Cream
KBK3	Moderate	Circular	Undulate	Flat	Cream
KBU14	Moderate	Circular	Entire	Raised	White
KBU25	Moderate	Circular	Entire	Flat	Yellow
KGU3	Large	Irregular	Undulate	Flat	White
KLE13	Moderate	Circular	Entire	Flat	Cream
RKGU15	Pinpoint	Circular	Entire	Flat	Purplish Black
RKLE11	Small	Circular	Entire	Flat	Yellow

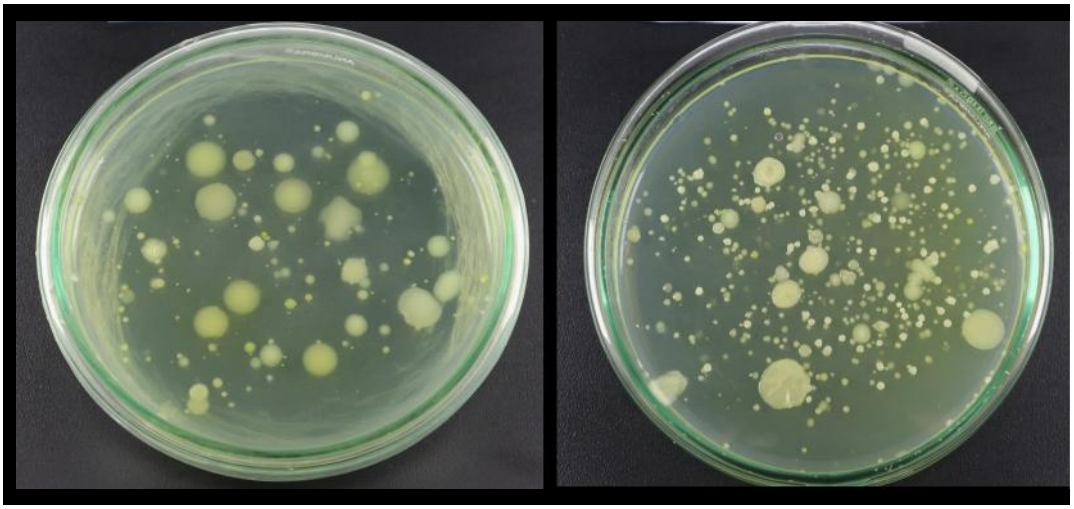


Figure 2 Single colony of soil bacteria isolated in NA (Nutrient Agar) plate

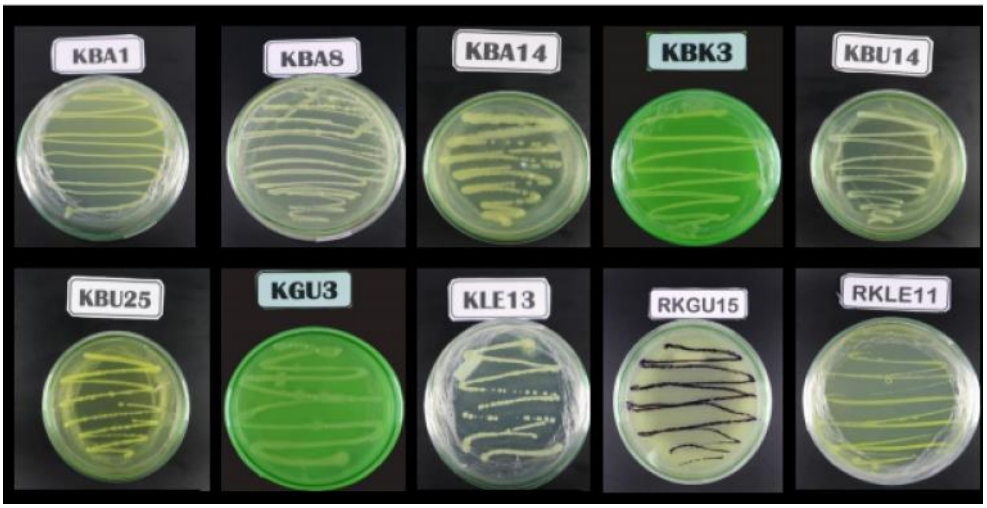


Figure 3 Morphology from the colony isolates of the potential siderophore-producing bacteria

Hypersensitivity reaction, gram test, and catalase isolate of siderophore-producing bacteria

Bacterial isolates that do not cause negative effects or phytotoxicity are one of the main conditions that allow the isolates to be utilized as biocontrol agents. A simple way that can be done to determine which bacterial isolates are not pathogenic is to conduct a Hypersensitive reaction (HR) test. Isolates with siderophore-producing bacteria are selected based on their pathogenicity. From the Hypersensitive reaction test results from 10 siderophore-producing bacterial isolates, the results showed negative Hypersensitive reaction properties, seen in Table 2. The data proves that the negative HR bacterial isolates have the potential to be biocontrol agents and vice versa where positive HR bacterial isolates cannot be used as a biocontrol agent because the bacteria are pathogenic and can cause diseases in plants.

The Gram test carried out showed results that isolates with siderophore-producing bacteria which belonged to the Gram-negative group consisted of six isolates while four bacterial isolates were included in the Gram-positive group (Table 2). One of the differences between gram-negative and gram-positive bacteria lies in the cell wall, where the cell wall of Gram-negative bacteria is thinner than the cell wall of Gram-positive bacteria, this affects antibacterial activity (Pulingam et al., 2019).

The ability of bacterial isolates in catalase activity is one indicator of the biocontrol characterization of these bacterial isolates. Based on the results of the study, nine isolates of the Siderophore-producing bacteria showed catalase activity which can be seen in Figure 4.

One important component in the biocontrol mechanism is the activity of catalase produced by bacterial isolates, which is related to the increase in plant resistance towards pathogens because it shows its ability to withstand the pressure of environmental, mechanical, and chemical conditions. The catalase enzyme which can be produced by some bacteria can break

Table 2 Hypersensitive response (HR), gram, and catalase isolate test results from the siderophore producing bacteria

Isolate Code	Hypersensitive response (+/-)	Gram test (+/-)	Catalase test (+/-)
KBA 1	(-)	(+)	(+)
KBA 8	(-)	(-)	(+)
KBA14	(-)	(+)	(+)
KBK3	(-)	(-)	(+)
KBU14	(-)	(-)	(+)
KBU25	(-)	(+)	(-)
KGU3	(-)	(+)	(+)
KLE13	(-)	(-)	(+)
RKGU15	(-)	(-)	(+)
RKLE11	(-)	(-)	(+)

Note : (+) = positive, (-) = negative

down hydrogen peroxide into water and oxygen so that it will form a defense system from the toxic hydrogen peroxide that it produces itself (Murali and Patel, 2017).

Production of siderophore bacteria isolates from Kamba local rice plants

Testing the capabilities of the bacterial isolates to produce siderophore qualitatively results showed the change in suspension color after the *Hathway reagent* was administered compared with the control. Siderophore-producing bacterial isolates showed the ability to chelate Fe metal which is characterized by a change in suspension color from light green to dark blue shown in Figure 5.

One of the important components in the *Plant Growth Promotion Rhizobacteria* mechanism is the ability of bacterial isolates to produce siderophores which can be antagonistic to pathogenic bacteria and fungi so that they have the potential to become biocontrol agents (Kesaulya et al., 2015). Siderophore can bind iron (Fe^{3+}) into siderophore-iron bonds that become available to plants. The results of testing on bacterial isolates capable of producing siderophore resulted in 10 isolates with varying concentrations between one isolate and another. Bacterial isolates that produced the highest siderophores for catechol types were found in the KBA8 isolates with 10.990 mg L^{-1}, while the lowest amounted with 5.876 mg L^{-1} was found in

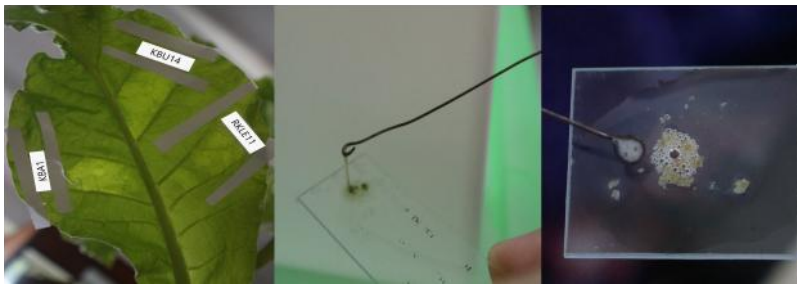


Figure 4 (a) Test results of Hypersensitive reaction isolates from the siderophore-producing bacteria that show negative reactions (-) observed after 24 hours to 72 hours (b) Gram tests, colonies with a slimy appearance are marked as gram-negative, whereas gram-positives are marked with a thin and non-slimy colony. (c) positive reaction Catalase test (+) show the presence of gas bubbles as of the result of the reaction between *Hydrogen Peroxide* and bacterial isolates, otherwise, the negative reaction (-) shows the absence of gas bubbles formed.



Figure 5 Color changes from the siderophore-producing bacteria isolates after adding the *Hathway reagent* test solution

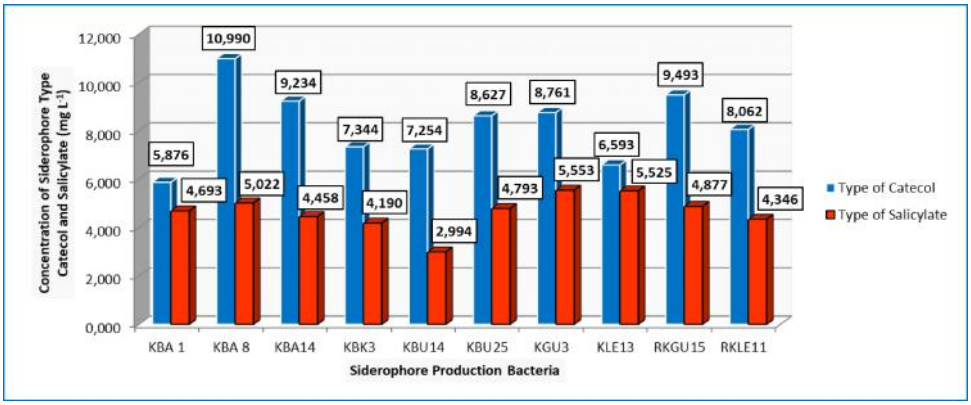


Figure 6 Production of bacterial siderophore isolates using UV-VIS spectrophotometry at wavelengths of 700 nm (catechol types) and 560 nm (salicylate types)

the KBA1 isolates. The highest salicylic siderophore type was found in KGU3 bacterial isolate with 5.553 mg L^{-1} and the lowest was in the KBU 14 bacterial isolate which was only 2.994 mg L^{-1} (Fig 6). The use of siderophore-producing PGPR isolates as antagonistic agents is a promising approach in agricultural production systems because it can increase crop production and protect plants from pathogens (Kuswinanti et al., 2014).

The mechanism of endophytic bacterial isolates in inducing plant resistance is by colonizing plant tissue so that it will stimulate plants to increase the production of salicylic acid, peroxidase, and phenol levels that play a role in plant resistance (Harni and Sinaga, 2015). Acid and phytohormone produced by bacterial isolates can play an important role in giving signals to the activity of responding to plant defenses against pathogens (Fragnire et al., 2011).

Microorganisms that can produce siderophores can provide benefits for plants because they can inhibit the growth of pathogens. Pathogens will lack the Fe^{3+} they need to survive because the Fe^{3+} is already bound by the siderophores (Fgaier and Eberl, 2011). The binding of iron with siderophores will limit the pathogen's movement because it is less able to infect due to lack of the elements it needs so that by itself can inhibit the development of diseases. Isolated bacterial isolates have the potential as biocontrol agents because they can produce siderophores (catechol and salicylic acid) and catalase enzymes.

Bacterial isolates that have the potential as biocontrol agents can indirectly increase plant growth by various mechanisms including the ability to compete in root nutrition with other organisms. Also, bacterial isolates must be able to produce siderophores, synthesize and release antibiotics in the rhizosphere. Iron must be in the form of Fe^{2+} and Fe^{3+} to be utilized by plants and microorganisms. Siderophore-producing bacteria can bind Fe^{3+} so that these bacteria are competitors for other microorganisms to obtain these compounds. The mechanism of siderophore-producing bacteria is by colonizing plant roots and then removing iron in the surface area by creating conditions suitable for root growth and not suitable for rhizoplane microbial growth (Budzikiewicz, 2001).

CONCLUSION

The highest concentration of siderophores was found in bacterial isolate KBA8 with 10.990 mg L^{-1}, and siderophores of salicylate type with the highest concentration produced 9.493 mg L^{-1} were from isolate RKGU15. Both isolates were not pathogenic, based on the results of the Hypersensitivity Reaction (HR) analysis. As many as 90% of isolates were able to produce catalase enzyme with 4 isolates of Gram positive group while 6 isolates of Gram negative group. The ability of bacteria to produce siderophore plays a role in overcoming the limitations of the element of Fe in the environment so that it potentially become a biocontrol agent to be developed as a biological agent.

ACKNOWLEDGEMENTS

The author would like to thank profusely to Kemenristekdikti-LPDP (Institute of Education Fund Management) who have fully funded this research, as well as all parties involved to help and facilitate all processes from research sampling, to testing in the Biotechnology Laboratory at the Center for Research Activities Hasanuddin University, Makassar.

REFERENCES

Ahmed E and Holmström S J M. 2014. *Siderophores in Environmental Research: Roles And Applications Microb.* Biotechnol 7, p. 196–208

Ali, S.S., and N.N. Vidhale. 2013. *Review Article Bacterial Siderophore and their Application/: A review*. Int.J. Curr. Microbiol.App.Sci 2(12) p. 303-312.

Atkinson, Merelle, M., J. Huang, and A. Knopp, James. 1985. *Hypersensitivity of Suspension-Cultured Tobacco Cells to Pathogenis Bacteria*. Physiol. Biochem. 75(11) p. 1270–1274.

Baars, O., X. Zhang, F.M.M. Morel, and M.R. Seyedsayamdost. 2016. *The Siderophore Metabolome of Azotobacter vinelandii*. Appl. Environ. Microbiol. 82(1) p. 27-39. doi: 10.1128/AEM. 03160-15.

Ben-David, A., and C.E. Davidson. 2014. *Estimation method for serial dilution experiments*. J. Microbiol. Methods 107 p. 214-221. doi: 10.1016/j.mimet. 2014.08.023.

Budzikiewicz, H. 2001. *Siderophore-Antibiotic Conjugates Used as Trojan Horses Against Pseudomonas aeruginosa.* Curr. Top. Med. Chem. 1(1) p. 73–82. doi: 10.2174/1568026013395524.

Cappucino, J.G., and N. Sherman. 2008. *Microbiology: a laboratory manual-10th ed*. (K. Churchman, editor). 10th ed. United States of America.

Chen, C., K. Xin, M. Li, X. Li, J. Cheng, et al. 2016. *Paenibacillus sinopodophylli sp. Nov., a siderophore-producing endophytic bacterium isolated from roots of Sinopodophyllum hexandrum (Royle) ying*. Int. J. Syst. Evol. Microbiol. 66(12) p. 4993–4999. doi: 10.1099/ijsem.0.001458.

Cornelis, P. 2010. Iron Uptake and Metabolism nn Pseudomonads. Appl. Microbiol. Biotechnol. 86(6) p. 1637–1645. doi: 10.1007/s00253-010-2550-2.

Dewedar, S., M.S. Abdel-Hamid, D. EL-Ghareeb, A.A. Haroun, A.F. ElBaz, et al. 2018. *Statistical Optimization and Chemical Characterization of Newly Extracted Siderophore From Azotobacter chroococcum*. Biosci. Res. 15(2) p. 1163–1177.

Dimkpa, C. 2016. Microbial siderophores: Production, Detection and Application In Agriculture and Environment. Endocytobiosis Cell Res. 27(2) p. 7-16.

Ferreira, C.M.H., H.M.V.M. Soares, and E. V. Soares. 2019. *Promising Bacterial Genera For Agricultural Practices: An Insight on Plant Growth-Promoting Properties and Microbial Safety Aspects*. Sci. Total Environ. 682 p. 779–799. doi: 10.1016/j.scitotenv. 2019.04.225.

Fgaier, H., and H.J. Eberl. 2011. *Antagonistic Control of Microbial Pathogens Under Iron Limitations By Siderophore Producing Bacteria In A Chemostat Setup*. J. Theor. Biol. 273(1) p. 103-114. doi: 10.1016/j.jtbi.2010.12.034.

Fragnire, C., M. Serrano, E. Abou-Mansour, J.P. Métraux, and F. L'Haridon. 2011. *Salicylic acid and its Location In Response to Biotic and Abiotic Stress*. FEBS Lett. 585(12) p. 1847–1852. doi: 10.1016/j.febslet.2011.04.039.

Harni, R., and M.S. Sinaga. 2015. *Mekanisme Bakteri Endofit Mengendalikan Nematoda Pratylenchus brachyurus pada Tanaman Nilam*. Bul. Penelit. Tanam. Rempah dan Obat 23(1) p. 102–114. doi: 10.21082/bullittro. v23n1.2012.

Hider, R.C., and X. Kong. 2010. *Chemistry and biology of siderophores*. Nat. Prod. Rep. 27(5) p. 637–657. doi: 10.1039/b906679a.

Kesaulya, H., Baharuddin, B. Zakaria, and S.A. Syaiful. 2015. *Isolation and Physiological Characterization of PGPR from Potato Plant Rhizosphere* in Medium Land of Buru Island. Procedia Food Sci. 3 p. 190–199. doi: 10.1016/j.profoo.2015.01.021.

Khan, A., P. Singh, and A. Srivastava. 2018. *Synthesis, Nature and Utility of Universal Iron Chelator – Siderophore: A review*. Microbiol. Res. 212-213(October) p. 103-111. doi: 10. 1016/j.micres.2017.10.012.

Kousser, C., C. Clark, S. Sherrington, K. Voelz, and R.A. Hall. 2019. *Pseudomonas aeruginosa inhibits Rhizopus Microsporus Germination Through Sequestration of Free Environmental Iron*. Sci. Rep. 9(1): 1-14. doi: 10.1038/s41598-019-42175-0.

Kumar, P., S. Thakur, G.K. Dhingra, A. Singh, M.K. Pal, et al. 2018. Inoculation of siderophore producing rhizobacteria and their consortium for growth enhancement of wheat plant. Biocatal. Agric. Biotechnol. 15(June) p. 264–269. doi: 10.1016/ j.bcab.2018.06.019.

Kumar, S.V., Menon, S., Agarwal, H and Gopalakrishnan, D., 2017. Characterization and Optimization of Bacterium Isolated From Soil Samples For The Production of Siderophores. Resour. Technol. 3(4) p. 434–439. doi: 10.1016/ j.reffit.2017.04.004.

Kuswinanti, T., B. Baharuddin, and S. Sukmawati. 2014. *Efektivitas Isolat Bakteri dari Rizosfer dan Bahan Organik Terhadap Ralstonia solanacearum dan Fusarium oxysporum pada Tanaman Kentang*. J. Fitopatol. Indones. 10(2) p. 68–72. doi: 10. 14692/jfi.10.2.68.

Liu, D., Q. Yang, K. Ge, X. Hu, G. Qi, et al. 2017. *Promotion of Iron Nutrition And Growth on Peanut by Paenibacillus Illinoisensis and Bacillus Sp. Strains In Calcareous Soil*. Brazilian J. Microbiol. 48(4) p. 656–670. doi: 10.1016/j.bjm.2017.02.006.

Murali, A., and S. Patel. 2017. *The Effect of Different Heavy Metal Acetate Solutions on the Inhibition of Catalase Enzyme*. J. South Carolina Acad. Sci. 15(2) p. 13.

Pedraza, R.O. 2015. *Siderophores Production by Azospirillum: Biological Importance, Assessing Methods and Biocontrol Activity. In: (eds.), F.D.C. et al., editor, Siderophores Production by Azospirillum: Biological Importance, Assessing Methods and Biocontrol Activity*. Springer International Publishing Switzerland, Argentina. p. 251-262.

Pulingam, T., K. Lin, E. Ali, J. Nelson, I. Julian, et al. 2019. *Colloids and Surfaces B: Biointerfaces Graphene oxide exhibits differential mechanistic action towards* Gram- positive and Gram-negative bacteria. 181(January) p. 6-15.

Reiner, K. 2010. Catalase-Test-Protocol.pdf. (November 2010) p. 1–9. doi: 66.208.62.130.

Rizzi, A., S. Roy, J.P. Bellenger, and P.B. Beauregard. 2019. *Iron homeostasis in Bacillus subtilis Requires Siderophore Production and Biofilm Formation. Appl.* Environ. Microbiol. 85(3). doi: 10.1128/AEM.02439-18.

Sah, S., and R. Singh. 2015. *Siderophore: Structural and Functional Characterisation - A Comprehensive Review*. Agriculture 61(3) p. 97–114. doi: 10.1515/agri-2015-0015.

Saha, M., S. Sarkar, B. Sarkar, B.K. Sharma, S. Bhattacharjee, et al. 2016. *Microbial Siderophores and Their Potential Applications: A Review. Environ*. Sci. Pollut. Res. 23(5) p. 3984–3999. doi: 10.1007/s11356-015-4294-0.

Sasirekha, B., and S. Srividya. 2016. *Siderophore Production By Pseudomonas aeruginosa FP6, A Biocontrol Strain for Rhizoctonia solani and Colletotrichum gloeosporioides Causing Diseases In Chilli*. Agric. Nat. Resour. 50(4) p. 250–256. doi: 10.1016/j.anres. 2016. 02.003.

Sinha, A.K., B. Parli Venkateswaran, S.C. Tripathy, A. Sarkar, and S. Prabhakaran. 2019. *Effects of Growth Conditions on Siderophore Producing Bacteria and Siderophore Production from Indian Ocean Sector of Southern Ocean*. J. Basic Microbiol. 59(4) p. 412-424. doi:10.1002/jobm. 201800537.

Sivasakthivelan, P., and D. Stella. 2012. *Studies on the Phytohormone Producing Potential of Agriculturally Beneficial Microbial (ABM) Isolates from Different Rhizosphere Soils of Sunflower in Tamil Nadu*. Int. J. Pharm. Biol. Arch. 3(5): p. 1150-1156.

Suslow, T., M. Schroth, and M. Isaka. 1982. *Application of a Rapid Method for Gram Differentiation of Plant Pathogenic and Saprophytic Bacteria Without Staining*. Am. Phytopathol. Soc. 72(7) p. 917–918.

Vijayalakshmi, N.R., M. Swamy, and N. Naik. 2019. *In Vitro Screening and Production of Plant Growth Promoting Substances by Azospirillum Isolates from Rhizoplane of Foxtail Millet*

Setaria italica (L.) Beauv. Int. J. Pure Appl. Biosci. 7(1) p. 224-229. doi: 10.18782/2320-7051.7340.

Wang, X., Q. Li, J. Sui, J. Zhang, Z. Liu, et al. 2019. *Isolation and Characterization of Antagonistic Bacteria Paenibacillus jamilae HS-26 and Their Effects on Plant Growth*. Biomed Res. Int. 2019. doi: 10.1155/2019/3638926.

Yu, S., C. Teng, J. Liang, T. Song, L. Dong, et al. 2017. *Characterization of Siderophore Produced By Pseudomonas syringae BAF.1 and Its Inhibitory Effects on Spore Germination and Mycelium Morphology of Fusarium oxysporum*. J. Microbiol. 55(11) p. 877–884. doi: 10.1007/s12275-017-7191-z.

Yu S, Teng C, Bai X, Liang J, Song T, Dong L, Jin Y and Qu J, 2017. *Optimization of Siderophore Production By Bacillus Sp. PZ-1 and Its Potential Enhancement of Phytoextration of PB From Soil.* J. Microbiol. Biotechnol. 27 p. 1500.

Zhang, N., X. Ma, Y. Yin, Y. Chen, C. Li, et al. 2019. *Synthesis of CuO-CdS Composite Nanowires and Their Ultrasensitive Ethanol Sensing Properties*. Inorg. Chem. Front. 6(1) p. 238-247. doi: 10.1039/c8qi00951a.

***