ISSN: 1412-4718, eISSN: 2581-138x

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Potential use of PGPR based biofertilizer for improving the nutrient availability in soil and agronomic efficiency of upland rice

Abstract. Present study aimed to perform literature review to investigate the current status and potential use of plant growth promoting rhizobacteria (PGPR) for enhancing the soil plant's health, plant growth and productivity in sustainable ways. The Systematic Literature Review (SLR) methods was applied according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) on published scientific literature from 2012-2022. Results revealed that genus diversity of PGPR (*Bacillus* sp., *Serratia* sp., *Streptomyces* sp., *Pseudomonas* sp., and *Burkholderia* sp.) produce siderophore and phytohormones such as indole-3-acetic acid (IAA), gibberellin (GAs), auxin, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, organic acids, biocontrol agent that contribute to the improvement of nutrient bioavailability (nitrogen fixing, phosphate solubilizing and Fe-uptake). The growth character of rice inoculated with biofertilizer and combined with 50% inorganic fertilizer produced no significant difference with pots that received 100% inorganic fertilizer. These results concluded that PGPR that produced phytohormone and siderophore could be developed as a potential bioagent or biofertilizer to improve the growth character and yield of upland rice (*Oryza sativa* L.) in dry environmental conditions.

Keywords: Biofertilizer · N2-fixer · PGPR producing phytohormone and siderophore · Systematic review

Potensi pemanfaatan pupuk hayati berbasis PGPR untuk meningkatkan ketersediaan unsur hara di tanah dan efisiensi agronomis padi gogo

Sari. Kajian tinjauan pustaka ini dilakukan untuk menyelidiki status saat ini dan potensi penggunaan rizobakteri pemacu pertumbuhan tanaman (PGPR) untuk meningkatkan kesehatan tanah, pertumbuhan tanaman, dan produktivitas secara berkelanjutan. Metode Systematic Literature Review (SLR) diterapkan sesuai dengan Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) pada literatur ilmiah yang diterbitkan dari 2012-2022. Mesin pencari Google Scholar dan ScienceDirect dengan kata kunci yang tepat digunakan untuk memilih dan mengumpulkan jurnal ilmiah yang terindeks bereputasi dan digunakan sebagai referensi utama database. Hasil penelitian menunjukkan bahwa keragaman genus PGPR (*Bacillus* sp., *Serratia* sp., *Streptomyces* sp., *Pseudomonas* sp., dan *Burkholderia* sp.) menghasilkan siderophore dan memproduksi phytohormon seperti indole-3-acetic acid (IAA), giberelin (GAs), auksin, dan 1-aminocyclopropane-1-carboxylic acid (ACC) aktivitas deaminase, asam organik, agen biokontrol yang berkontribusi pada peningkatan bioavailabilitas nutrisi (pengikatan nitrogen, pelarutan fosfat, dan penyerapan Fe). Karakter tumbuh padi yang diinokulasi pupuk hayati dan dikombinasikan dengan pupuk kimia dosis 50% menghasilkan perbedaan yang tidak nyata dengan pot yang menerima pupuk anorganik 100%. Hasil ini menyimpulkan bahwa PGPR penghasil pitohormon dan siderophore dapat dikembangkan sebagai bioagent atau pupuk hayati yang potensial untuk meningkatkan karakter tumbuh dan hasil padi (*Oryza sativa* L.) gogo pada kondisi lingkungan yang kering.

Keywords: Biofertilizer · Penambat-N2-fixer · PGPR produksi fitohormon dan siderofor · Systematic review

Manuscript received : 19 July 2022, Revision accepted : 13 December 2022, Published : 21 December 2022
DOI: http://dx.doi.org/10.24198/kultivasi.v21i3.40061

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Introduction

Rice (*Oryza sativa* L.) is one of the staple foods consumed by half the world's population especially in the Asian region. The Food and Agriculture Organization of the United Nations reports that rice is considered a vital crop for food security (Cavite *et al.*, 2021). The demand for rice is increasing in line with the increasing world population, but rice productivity is often constrained by biotic and abiotic factors (Nabila and Kasiamdari, 2021).

Abiotic components such as drought stress and salinity are constraints that are often found in rice cultivation. Research shows that giving PGPR inoculants under these unfavorable conditions shows positive results. According to research from Nautiyal *et al*. (2013), rice plants inoculated with *Bacillus amyloliquefaciens* SN13 with high salt stress showed better results when compared to no inoculant (Nautiyal *et al.*, 2013). Another result, namely the effectiveness of *Serratia* spp. in rice cultivation on upland obtained a significant increase in N uptake, shoot dry weight and grain yield (Nascente *et al.*, 2019).

Microorganism especifically microbes living in the rhizosphere of several plants and giving positive effects on the host plant through various mechanisms are usually termed plant growth-promoting rhizobacteria. In the rhizosphere, plant roots secrete a number of exudates that act as attractants for microbes, which eventually improve the physicochemical properties of the surrounding soil. These exudates maintain the function and structure of microbial communities near plant roots. Plant growth promoting rhizobacteria (PGPR) indirectly help plant symbionts by initiating induced systemic resistance, exerting an antibiosis effect, and potentially improving the content of plant cell metabolites (Saeed *et al.*, 2021).

Plant growth promoting rhizobacteria behave as a bacterium capable of inducing growth and increasing plant tolerance to allelochemicals left in the soil by previous crops. The use of microorganisms to increase plant tolerance to biotic and abiotic stresses is a sustainable cultivation system without excess fertilizer (Mishra and Nautiyal, 2013).

Rhizobacterial communities are involved in recycling of soil nutrients, promotion of plant health, and maintenance of soil structure. Each plant provides a unique rhizosphere environment that selects specific rhizomicrobial flora. Moreover, rhizobacterial communities quickly change their biomass, enzyme activity, diversity, abundance, and composition according to the environmental changes such as seasonal variation, application of fertilizers or pesticides, tillage, and crop rotation (Hahm *et al.*, 2017). Plant growth promoting rhizobacteria (PGPR) influence plant health and productivity by solubilization of mineral nutrients, stimulation of root growth and suppression of root diseases. These PGPR include *Azospirillum, Pseudomonas, Bacillus, and Agrobacterium* species. Plant growth promoting rhizobacteria also can protect plants from the deleterious effects of some environmental stresses including heavy metals, flooding, salt, and phytopathogens (Omar *et al.*, 2014).

Auxin, indole-3-acetic acid (IAA), is an important phytohormone produced by several strains of PGPR and it is well-known that treatment of IAA-producing rhizobacteria increases the plant growth. IAA released by rhizobacteria mainly affect the root system by increasing its size and weight, branching number, and the surface area in contact with soil. All these changes lead to an increase in its ability to probe the soil for nutrient exchange, therefore improving plant's nutrition pool and growth capacity. IAA also drives the differentiation of adventitious roots from stem as auxins induce stem tissues to redifferentiate as root tissue (Manimekalai and Kannahi, 2017).

Plants live in intimate association with microorganisms and their interaction in rhizosphere are responsible for plant health and soil fertility. Siderophore produced by rhizosphere bacteria may enhance plant growth by inhibiting the colonization of roots by the harmful microbes (Patel *et al.*, 2018).

Siderophore is organic compounds with small molecular weights and with powerful ferric ion-chelating molecules that produced by microorganisms to acquire the iron essential for growth. Siderophore is known to differ from one another in their chemical structure and properties. Hydroxamate siderophore are produced by bacteria and fungi, while catecholate only produced by bacteria. Carboxylates siderophore are produced by a few bacteria and exclusively by the fungi of the order Mucorales. The most commonly found siderophore is hydroxamate siderophore (Nabila and Kasiamdari, 2021).

The use of biofertilizers is expected to be an alternative system for sustainable agricultural cultivation in limited nutrient conditions in the soil. This is increasingly attractive because of its positive impact on plant growth and the environment (Kantachote *et al*., 2016). Increasing the use of microbial-based biofertilizers whose life span is time-bound in the future requires the quality control of biofertilizer its self. The harvesting process, formulation, shelf life of the final product are attributes of the quality control documentation used (Bharti and Suryavanshi, 2021).

Several studies have shown that there is a positive effect of fertilization with biological agents containing siderophores, IAA and ACC deaminase which are presented in the results and discussion of this review journal. The main purpose of this article is to provide scientific information of potential application PGPR based biofertilizer to improve the upland rice growth and agronomic traits.

Materials and Methods

The research method was used a Systematic Literature Review with journal searches based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow guidelines. Preferred Reporting Items for Systematic Reviews and Meta-Analyses is one of the guidelines used in assessing systematic reviews and meta-analyses. This method focuses on the ways in which authors can ensure transparent and complete reporting of this type of research (Sastypratiwi and Nyoto, 2020). The data sources used in this study refer to databases and indexes that can be accessed on Google Scholar and ScienceDirect. The search was conducted on April 16th, 2022 with the keywords used in Google Scholar "Rhizobacteria producing siderophore and phytohormones, rhizobacteria for upland rice, beneficial of siderophore. In a ScienceDirect search using the keywords, Rhizobacteria producing siderophore, phytohormones of rice. Briefly, the flowchart of algorithm is shown in Figure 1.

The selection criteria used are the year of publication from 2012–2022 with the criteria for scientific publications in the form of research journals and selected based on the title and abstract. Contents of all screened journals with appropriate criteria, shows the siderophore and IAA test results and presenting data on rice responses to PGPR treatment.

The results of the search for journals using Google Scholar at the beginning of the search with the manual system found 1,210 then screened for relevance to the desired theme, obtained 222 journal titles. The search with Science Direct resulted in 1,048 then screened for the time of publication of the journal, the type of article (research article) and the type of access used was open access, resulting in 107 research articles. Subsequently, 18 journal duplication titles were removed, leaving 311 titles. Screening from the title approach using plants, 157 journal titles were published, followed by specific titles using upland rice, 15 relevant journal titles were published. After being screened for full text, the journals containing information according to the criteria were obtained five titles.

Result and Discussion

The results showed that the Rhizobacteria capable of synthesizing IAA and producing siderophore gave a positive response to plant growth parameters with variables: plant height, root length, shoot length and rice germination.

Plant growth promoting rhizobacteria play a role in plant growth through several mechanisms: (1) increasing the dissolution of mineral nutrients and nitrogen fixation, (2) inhibiting soil pathogens by producing HCN, siderophores, antibiotics, (3) improving plant tolerance to drought, salinity, metal toxicity and P dissolution, (4) produce phytohormones (Figuiredo *et al*., 2011).

Provision of *Bacillus* sp. isolates. as a siderophore-producing bacteria using rice husk biochar media is able to chelate Fe in rice fields in an oxidized state. This Fe chelation can indirectly release Fe-fixed phosphate and have an impact on increasing soil pH from 4.51 to 5.6 (Tiara *et al*., 2019). Another study using biofertilizers in rice cultivation showed that isolates capable of producing siderophores gave better agronomic yields for rice when compared to using 100% chemical fertilizers. The treatment of biofertilizers and a dose of 50% chemical fertilizer recommendations resulted in a weight of 100 grains of dry grain 3.29 grams, whereas with a 100% chemical fertilizer treatment it was only 2.70 grams (Cavite *et al*., 2021). Plant growth promoting rhizobacteria can be synthesized phytohormones to stimulate plant cell growth. Physiologically IAA is the most active auxin in plants. The results of research by Dimpka (2009) studying various plant species when inoculated with IAA-producing bacteria were shown to increase root growth, increase lateral roots and root hair formation (Dimpka, 2009: Vandana, 2020). Research by Bal *et al.* (2013) also showed that the treatment of PGPR isolates that produced IAA and DCC aminase in rice plants had a root length of 15.25 cm. This root length was 2.89 cm longer and the plant height was 2.63 cm higher in the germination phase when compared to controls and isolates that did not produce IAA and DCC aminase at the same time (Bal *et al.*, 2013).

Detection of siderophore compounds. Analysis of rhizobacteria which are capable of producing siderophores generally uses Chrome Azurol Sulfonate agar media (CAS) (Schwyn and Neilands, 1987). Siderophores are positive if the bacterial colonies form an orange circle in the middle or a clear zone around the bacterial colonies after incubating 3 days to 1 week (Cavite *et al.*, 2021; Priyanka *et al.*, 2017).

Detection of hydroxamate type siderophore was carried out by using the tetrazolium test. The direct formation of a dark red color indicates the presence of a hydroxamate siderophore. Briefly, the ability to produce the phytohormone (auxine or IAA) indicates by production of pink to red color, holozone for the phosphate solubizing phosphate (PSB), and the production of siderophore indicates by the orange halos around colony like in Figure 2 (Cavite *et al.*, 2021).

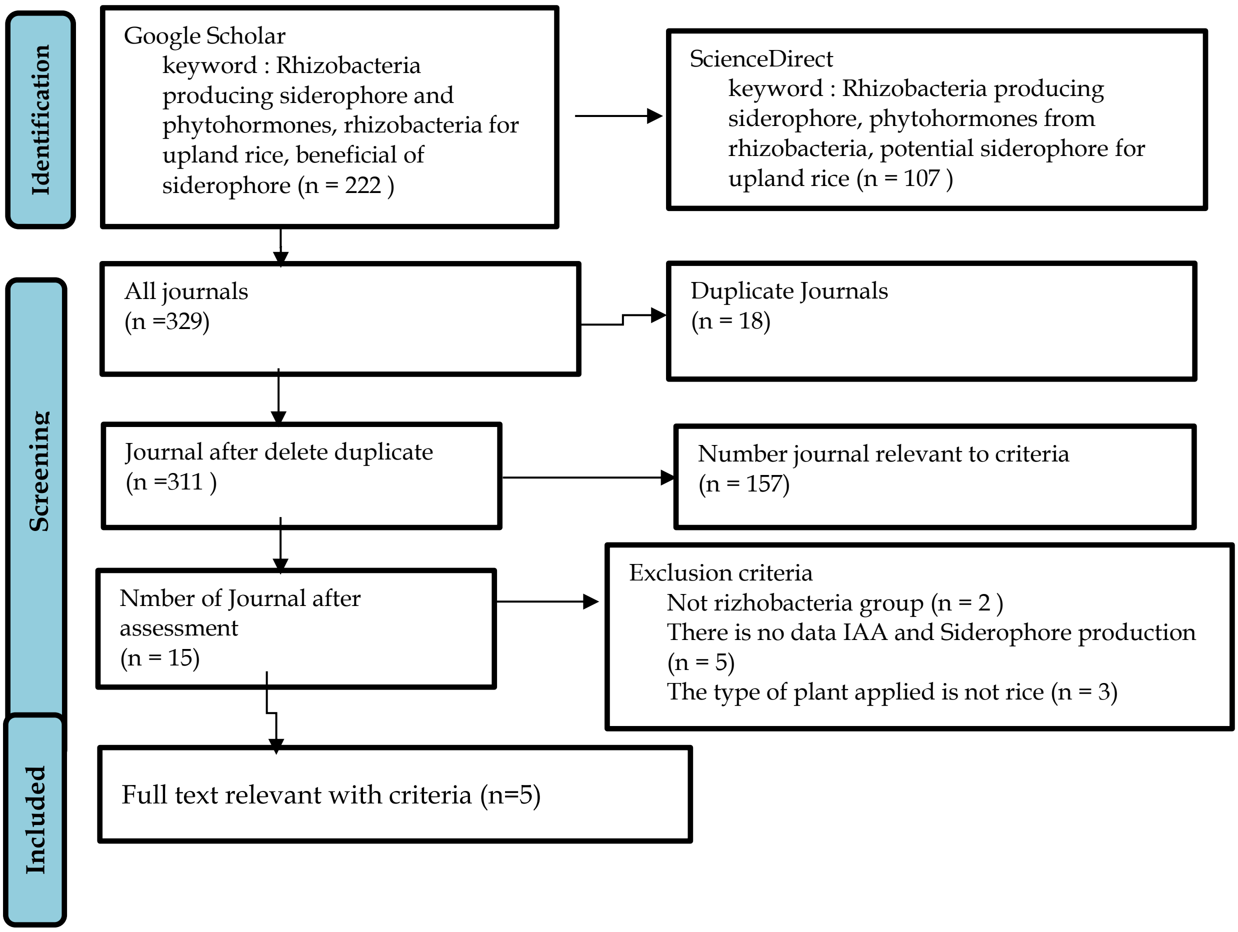

Figure 1. Flow chart Systematic Literature Review: Rhizobacteria producing siderophore and phytohormone improving uptake upland rice

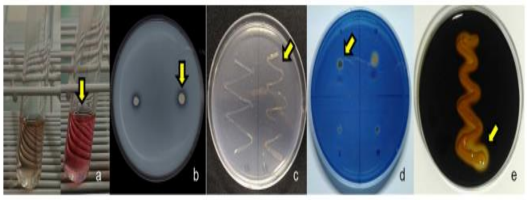

Figure 2. Positive indicator of the presence of phytohormon and siderophore from Cavite *et al.* (2021) research

ACC deaminase was determined according to Penrose and Glick (2003) with several modifications. Determination of indole-3-aceticacid (IAA)**.** Bacterial isolates were grown on Nutrient Broth (NB) media. The amount of IAA produced from isolates can be determined by calculated using a spectrophotometer (UU/VIS) with a wavelength of 530 nm. The existence of IAA can be seen from the standard curve (Omar *et al.*, 2014). In addition to using NB, the media commonly used is Nutrient Agar (NA) using the Glickmann and Dessaux (1995) method.

Rhizobacteria has different genera of bacteria, both indigenous and strain-characterized. Not all bacterial isolates were able to produce IAA and siderophore. The bacterial isolates that have been produced were tested whether they contain siderophore or not by using Chrome Azurol Sulphonate (CAS) as shown in Figure 2. Siderophore characteristic is determined by the orange color around the bacteria and this can be used as a qualitative analysis. The ability of siderophore to bind iron (Fe^{3+}) into siderophore-iron bonds can inhibit the growth of pathogens. The size of the clear zone or purple and orange zone indicates the strength of Rhizobacteria in the size of the clear zone or purple and orange zone indicates the strength of rhizobacteria in producing siderophore (Prihatiningsih *et al.*, 2017).

Plant growth promoting rhizobacteria activities and produce metabolites that affect plant health (Figure 3.). Phosphorous solubilization and siderophore production by PGPR improve plant growth by increasing plant nutrient uptake. Furthermore, certain PGPR can produce phytohormones, especially IAA, and stimulate root and shoot elongation. Production of ACC deaminase by PGPR may indirectly enhance plant growth. When plants severe stress, they synthesize ethylene from ACC taken up from the soil (Hahm *et al.*, 2017).

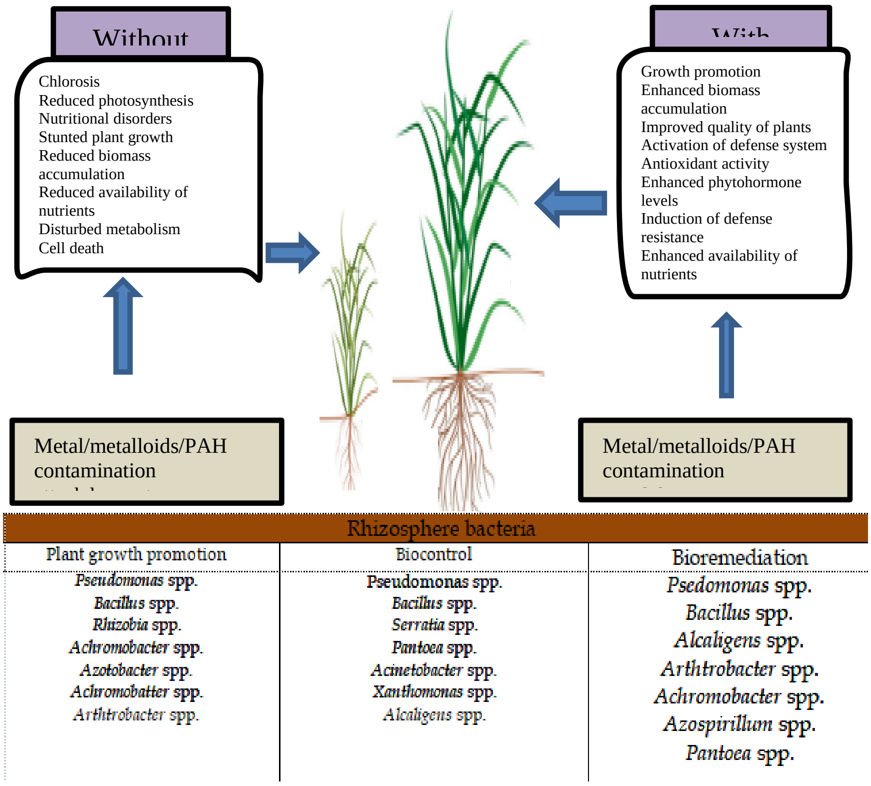

Figure 3. Difference responses of plants to stress between with PGPR and without PGPR

Jurnal Kultivasi Vol. 21 (3) Desember 2022
ISSN: 1412-4718, eISSN: 2581-138x

The number of siderophore formed quantitatively can be determined by using a spectrophotometer at 630 nm wavelength. The percentage of siderophore can be calculated by the formula:

$$[(Ar - As)/Ar] \times 100$$

where Ar and As are the absorbance of reference solution (mixture of CAS reagent and un-inoculated broth) and absorbance of sample (mixture of CAS reagent and supernatant of sample), respectively. Siderophore have two types, namely hydroxamate and catecholate. The hydroxamate type can be tested with tetrazolium and produces a dark red color as a sign of the hydroxamate siderophore. Siderophore of catecholates were tested by Arnow's test (Nabila and Kasiamdari, 2021).

In the picture above, it can be ascertained that the plants given PGPR grew better than plants without PGPR, because they were tolerant of drought and salinity. Apart from being a plant growth promoter, rhizosphere bacteria also function as biocontrol and bioremediation. Illustration of repeated images from journal sources (Saeed, 2021).

Phytohormone production. Phytohor-mones or plant growth regulators are organic substances, which at low concentrations (< 1 mM), promote, inhibit, or modify growth and development of plants. Ironically, production of these phytohormones can also be induced by certain microbes, such as PGPR, in plants (Figure 4). Common groups of phytohormones include gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, and auxins that the root cell can proliferate by overproducing lateral roots and root hairs with a consecutive increase in nutrient and water uptake. Plant growth regulators are also called exogenous plant hormones, as they can be applied exogenously as extracted hormones or synthetic analogues to plants or plant tissues (Gouda *et al.*, 2018).

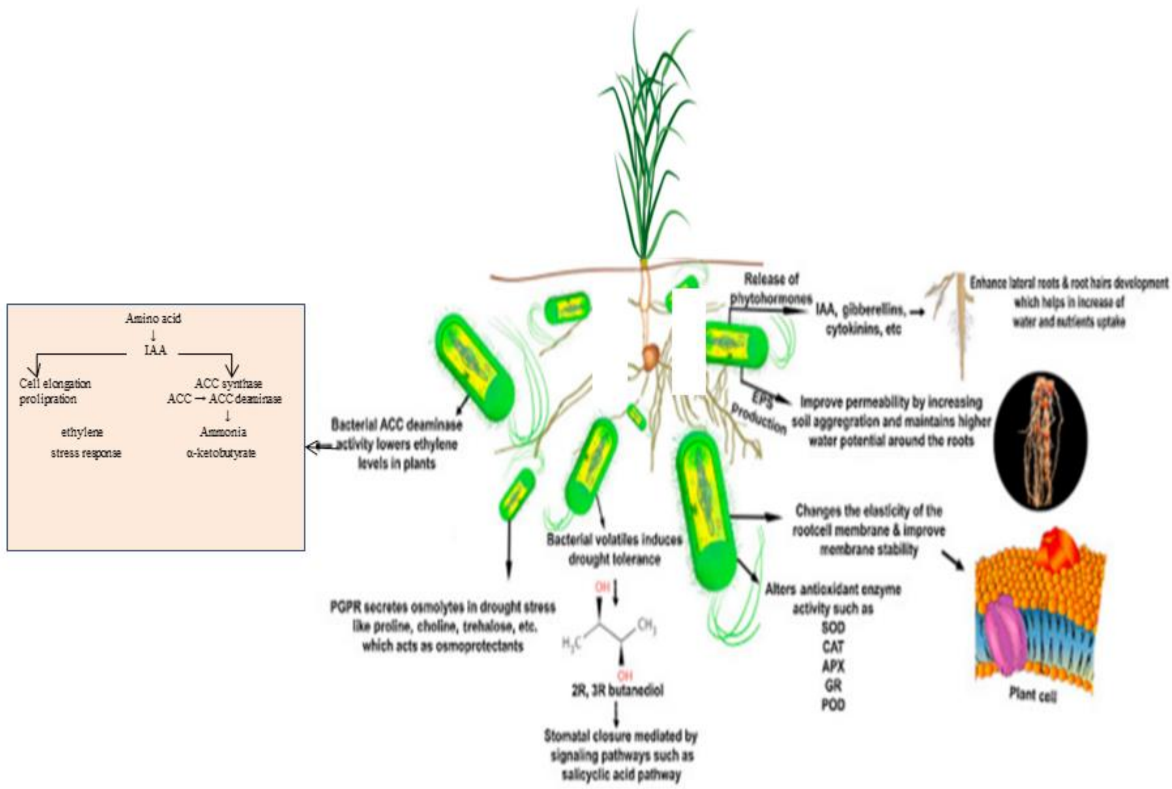

Figure 4. Mechanism of plant drought tolerance induced by PGPR producing phytohormones

In the Figure 4, PGPR synthesizes ACC into ACC deaminase which is derived from amino acids, then this ACC deaminase will produce ammonia and -ketobutyrate in response to drought. In addition to ACC deaminase, biofilm and IAA, giberalin and cytokinins are also produced. The role of PGPR in plant growth is very important in nutrient management and biocontrol activities by colonizing the rhizosphere. Rhizobacteria is increasingly important in regulating biotic and abiotic stresses by various mechanisms because of their potential in ameliorating environmental stress, and PGPR in the modification of phytohormones activity. Plants produce phytohormones such as IAA, gibberellin, ethylene, abscisic acid (ABA) and cytokinins which have been shown to have tolerance abiotic stress. In addition, phytohormones can be synthesized by PGPR which can stimulate plant cell growth. Auxin has been shown to increase root growth and increase lateral root and roots hair formation. Dehydration in cells results in the induction of ABA biosynthesis which is a stress hormone released during water deficit conditions (Vandana *et al.*, 2020).

Plant growth promoting rhizobacteria can restructure the root system and root tissue structure which will affect the hormonal balance. The ability to produce IAA is one of the important properties of PGPR. Generally, the IAA test uses the Salkowsky reagent (Table 1) and is tested with a spectrophotometer (Patel *et al.*, 2018).

IAA biosynthesis by bacteria occurs with tryptophan identified as the main precursor compound in several pathways, there are: (1) indole-3-pyruvate, (2) indole-3-acetonitrile (IAN), (3) tryptamine, and (4) indole-3-acetamide. Tryptophan is generally considered a precursor in the formation of IAA, because the addition of tryptophan to bacterial cultures stimulates an increase in IAA synthesis. Identification of IAA product by rhizobacteria was determined using a spectrophotometer at a wavelength of 535 nm and then compared with the IAA standard curve. A bacterial strain capable of producing IAA thereby stimulating plant growth of *Pseudomonas putida* was investigated and reported to be able to produce IAA in a faster time (Patel *et al.*, 2018).

Table 1. Isolation of Rhizobacteria producing siderophore and phytohormones

Author, Year	Bacteria	Siderophore and/or phytohormone analysis scheme
Bal *et al.*, 2013	Rhizobacteria	ACC : isolate gowed in sterile DF salt containing 3 mM ACC as the nitrogen source, shaker at 200 rpm and temperature 30 °C for 24 h. Incubation for 48 hours.
Omar *et al.*, 2014	Rhizobacteria from dry rhizosfer	IAA : isolate were grown in Nutrient Broth (NB) on an incubator shaker (150 rpm) at room temperature for 24 h, incubation 1 ml bacterial culture into 100 L of sterile NB B amended with 5 ml L-tryptophan solution and allowed to grow for 48 h.
Priyanka *et al.*, 2017	*Pseudomonas sp.*	Sideropor : Isolates were inoculated on King's B medium supplemented with a strong iron chelater 8-Hydroxyquinoline (50 mg/L), incubated at 28 ± 2^0 C for 48-72 h ACC : Isolat suspended into 2 mL of modified DF medium with 2 mM concentration of ACC incubated at 28^0C for 36-72 hours
Tiara *et al.*, 2019	*Baccilus Sp.*	Purification of bacterial isolates Bacillus Sp. using the line method and then grown on TSA agar media. Propagation on Luria-Bertaini (LB) media, shaken for 2x24 hours
Cavite *et al.*, 2021	*Burkholderia pyrrocinia*	IAA : isolate was inoculated in nitrogen-free broth which consists of 10.65 g Burks medium and 500 mL distilled H2O supplemented with 0.05 g L−1 tryptophan. After seven days of incubation, cultures were centrifuged for 10 min at 13,000 rpm in 4 °C. ACC : isolates were grown in nitrogen-free salts agar medium (DF-ACC agar) supplemented with 0.3 g L−1 ACC, Plates were incubated in the dark for 7 days Siderophore : The isolates were grown on Crom Azurol S (CAS) media, which consisted of 4 solutions namely, blue dye solution, MM9/PIPES solution, casamino acid solution and glucose stock, sterilized (1210C for 20 minutes) and then inoculated on the agar surfacee

Role of Siderhopores. Siderophores and phytosiderophores is a specific Fe^{3+} chelating agent. The strong bond between Fe^{3+} and the siderophore protects the complex against hydrolysis and environmental enzymatic degradation (Ferreaira *et al.*, 2019). Siderophore may stimulate the biosynthesis of antimicrobial compounds by increasing the availability of Fe minerals for bacteria so that compound play an important role in the host plant resistance system. Rhizobacteria that produce siderophore can increase the ability of bacteria to colonize roots (Agustiyani, 2016). The formation of siderophore can be detected by the production of an orange circle or a clearing zone around the bacterial colony after incubation for 1 week at room temperature (Table 1)

Plants take advantage of the secretion from microorganisms that produce siderophores. Siderophore is produced by gram negative and gram positive bacteria with different mechanism. In Gram-negative bacteria, outer membrane transporters (OMT) bind Fe^{3+}-siderophores complexes, moving them to the periplasm through the protein TonBExbBD later bind to periplasmic siderophore-binding proteins (SBPs), crossing the peptidoglycan layer, and delivered to a siderophore-permease-adenosine triphosphatase (ATPase) system in the cytoplasmic membrane that will release them into the cytoplasm. In Gram-positive bacteria, lipoprotein SBPs, anchored to the cell membrane, bind extracellular Fe-siderophore complexes and import them by a siderophore-permease-ATPase system. (Ferreaira *et al.*, 2019).

Siderophore-producing bacteria bind Fe elements outside the cell wall and then Fe is transported into the cell membrane through specific receptors. The main of siderophore properties is hydroxyamic acid, a compound that is able to bind ferrous metal ions and can indirectly release Fe-fixed phosphate and is available for plants. Phosphorus is needed by plants as a source of energy and root growth (Tiara *et al.*, 2019).

Bacterial isolation comes from rice roots in saline soil. The selected isolates were studied for their effect on germination and ethylene emission under salt stress. The initial value of rice germination decreased by 20%. All selected isolates were able to have a positive impact on plant growth variables. As isolates have the ability to produce both ACC deaminase and IAA they promoted root, shoot and other growth indices of rice to a greater extent. It is likely that IAA and ACC deaminase stimulate root growth in a coordinated. The isolates with the highest ACC product gave the longest root and shoot length results.

Input from combination of half-dose chemical fertilizers with biological agents gave the most significant results in up taking of N nutrients. Full-dose chemical fertilizers increased K uptake, but the value was not significantly different from the application of 50% chemical fertilizers with inoculants. Grain yields with only one selected isolate did not significantly increase yields compared to full-dose recommended fertilizers (Table 2). Generally the genus and species of rhizobacteria isolated from rice roots are *Serratia* sp., *Streptomyces mutabilis*, *Pseudomonas fluorescens*, and *Burkholderia pyrrocinia* which were mostly reported to show plant growth-promoting activities in both screenhouse and feld conditions. Bacterial inoculants that capable of producing IAA can promote lateral roots and root hair formation which able to increase plant tolerance to salinity and stress (Cavite *et al.*, 2021).

The percentage of germination with the highest value (98.4%) was also produced from biological agents that had the highest ACC value. Treatment of biological agents containing siderophore was able to increase plant growth variables. Plant growth promoting rhizobacteria having ACC deaminase activity help plants to withstand stresses (biotic or abiotic) by reducing the level of stress ethylene. Inoculation with the PGPR isolates also increased the fresh and dry weight of both root and shoot. It was assumed that higher dry weight would mean longer and stronger roots and shoots as well as plants that would be able to better withstand salt stress (Bal *et al.*, 2013).

High of Fe solubility has the potential to cause rice poisoning. The physical characteristics of paddy fields that have high Fe levels are characterized by oily and yellow rice water. In put the siderophore-producing bacteria isolates and added rice husk biochar was able to significantly reduce the total Fe and Fe^{3+} content in volcanic paddy soils compared to isolates that did not contain siderophore.

In the soil, plant roots usually coexist with bacteria and fungi which can produce siderophores capable of alienating that available soluble iron and hence interfere with plants growth plants and facilities. Siderophore production provides a competitive advantage for PGPR which can colonize roots and eliminate the unfavorable impact of pathogenic bacteria (Priyanka *et al*., 2017).

Bacteria with IAA product can stimulate plant growth because of the growth hormone it produces. This strain produced the highest amount of IAA in culture media supplemented with L-tryptophan. Indole acetic acid affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Manimekalai and Kannahi, 2017).

IAA production is believed to be crucial for promotion of root elongation and root hair development production and showed that several high IAA-producing bacteria increased shoot and root length of rice, while producing the highest number of shoots per plant and shoot height in paper germination on petridish and in pot experiment (Tan *et al*., 2014). Plants height as one of the tested agronomic elements shows a very significant effect on fertilization with biofertilizer and soil treatment. Plant height with treatment of 100% inorganic fertilizers gives results that are not significantly different from the provision of 50% inorganic fertilizers and biological fertilizers (Cavite *et al*., 2021).

Table 2. Indicators and effects of siderophore and phytohormon on plants

Author, Year	Identification of Siderophore and IAA	Result
Bal *et al*., 2013	IAA: Salkowski method, isolates were screened to produce HCN Siderophore: formation of an orange circle around the incubated bacterial colony (at room temperature for 24 h)	The highest percentage of rice seed germination in selected isolates that produce ACC, IAA and siderophore compared to controls. This treatment also reduces plant stress to salt.
Omar *et al*., 2014	IAA: Salkowski method, isolates were screened to produce HCN Siderophore: formation of an orange circle around the incubated bacterial colony (at room temperature for 24 h)	Rhizobacteria containing IAA can dissolve phosphate, increase shoot growth, density and root hair length, increase rice seed germination and increase growth.
Priyanka *et al*., 2017	Sideropor: Colonies show orange color after 3 days incubation (28 ± 2^{0}C), Measure the diameter of the orange circle. ACC : Measure the amount of α-ketobutyrate and ammonia from ACC cleavage	Isolates containing siderophore and ACC at the same time with a higher percentage resulted in higher plant height and root length than control.
Tiara *et al*., 2019.	Siderophore pure isolates were grown in LB media and shaken for 2x24 hours	Increased of the soil's pH (from an average of 4 to an average of 5), decreased levels of oxidized Fe^{3+} in the soil with a higher volume of isolate.
Cavite *et al*., 2021	IAA: Colorimetric method using Salkowski reagent. Discolortion from pint to red, IAA positive Siderophore: formation of orange halos or clearing zones around bacteria after 7 days	Bio agent that producing IAA, ACC and siderophore had the highest root length and plant height compared to inoculants containing only one of them. However, the average grain yields (g plant^{-1}) using a single inoculant was still less than 50% the treatment with biological agents + 50% chemical fertilizers. Treatment of 100% chemical fertilizers still gives the best results.

The combined bacterial inoculation and fertilization showed significant effects in N uptake. Half fertilization with bioagent *A. delafeldii* showed the highest shoot N uptake (61.46 mg plant^{-1}), which is significantly (138%) higher than the untreated plants. In terms of P uptake, lower values than N were observed across all treated plants which ranged from 0.033 to 0.124 mg plant^{-1}. Potassium uptake data revealed significantly higher values (by 62%) in plants grown in sterilized soil. Application of full inorganic fertilizer significantly increased shoot K uptake over the untreated plants. Substituting 50% of inorganic fertilizer with consorssium bacteria (*R. pickettii, B. pyrrocinia, and A. delafeldii*) resulted to comparable shoot K uptake values relative to full inorganic fertilizer treated plants (Cavite *et al.*, 2021).

The other research report that inoculant treatment by soaking the rice seeds in PGPR isolates suspension before germination or after being transferred in culture bottles shows significant effect on plant growth through the parameters of seed height, root length, plant wet weight and plant dry weight observed compared to control. Some physiological factors of PGPR isolates are very influential in the growth and development of plants with specific mechanisms which are the ability to produce plant hormones that increase root length so that plant nutrient uptake also increases, nitrogen fixation, phosphate solubilizing while the non-specific mechanism, by suppressing plant pathogen toxicity and increase the induction of systemic resistance by its ability to produce siderophore or HCN (Sudewi *et al.*, 2020).

Conclusion

Biodiversity of beneficial bacteria in rhizosphere contribute to production of siderophore and growth factor or substance (phytohormones) that are highly need to improve the plant growth and productivity. This rhizobacteria produce the phytohormone and growth substance such as IAA, gibberellic acid, organic acid produce, ACC deaminase, siderophore and as well as increase the bioavailability of essentially nutrients through N_2-fixation, phosphate and potassium solubilization and chelating process (N, P, K and Fe). The application of this beneficial microbes as a biological agent could increase the rice productivity and fertilizers efficiency. The rice yield of inoculated crops combined with 50% dose of chemical fertilizer resulted a non-significant different with plot received 100% inorganic fertilizer. These results suggest that siderophore and plant growth booster (growth factors) producing bacteria can be developed as a potential bioagent or biofertilizers for increasing the growth, yield upland rice and promoting the climate resilient sustainable agriculture.

Testing PGPR isolates in pots on several plants, namely rice, wheat and bottlegourd also had a significant effect on the root length of the three plants. Isolates have the potential to stimulate plant growth both coleoptile elongation and or root length in plants. This treatment also had a significant effect on the height of wheat and mustard plants but had no significant effect on the height of rice plants.

Acknowledgements

The authors a deeply thanks to acknowledge Universitas Padjadjaran Through Academic Leadership Grant (ALG) program for supporting this research.

References

Agustiyani, D. 2016. Penapisan dan karakterisasi rhizobakteria serta uji aktivitasnya dalam mendukung perkecambahan dan pertumbuhan benih jagung (Zea mays L.). Jurnal Biologi Indonesia, 12(2) : 241–248.

Bal, H.B., L. Nayak, S. Das, and T.K. Adhya. 2013. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant and Soil, 366(1–2): 93–105.

Bharti, N. and M. Suryavanshi. 2021. Quality control and regulation of biofertilizer: current scenario and future prospects. Advances in BioInoculant: 133-141.

Cavite, H.J.M., A.G. Mactal, E.V. Evangelista, and J.A. Cruz. 2021. Growth and yield response of upland rice to application of plant growth-promoting rhizobacteria. Journal of Plant Growth Regulation, 40(2): 494–508.

Ferreaira, M.J., H. Silva, and A. Cunha. 2019. Siderophore-producing rhizobacteria as a promising tool for empowering plants to cope with iron limitation in saline soils: A review. Pedosphere, 29(4): 409–420.

Figuiredo, M.V.B., L. Seldin, F.F. Araujo, and R.L.M. Mariano. 2011. Plant Growth Promoting Rhizobacteria: Fundamental and Aplication, Microbiology Monograph 18: 21-43. Berlin: Springer Verlag Berlin Heidelberg.

Glickmann, E. and Y. Dessaux. 1995. A critical examination of the specificity of the Salkowski reagent for indolic compouds produced by phytopathogenic bacteria. Applied Enviroment Microbial, 61: 793-796.

Gouda, S., R.G. Kerry, G. Das, S. Paramithiotis, H.S. Shin, and J.K. Patra. 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological Research, 206: 131–140.

Hahm, M.S., J.S. Son, B.S. Kim, S.Y. Ghim. 2017. Comparative study of rhizobacterial communities in pepper greenhouses and examination of the effects of salt accumulation under different cropping systems. Archives of Microbiology, 199(2): 303–315.

Kantachote, D., T. Nunkaew, T. Kantha and S. Chaiprapat. 2016. Biofertilizers from Rhodopseudomonas palustris strains to enhance rice yields and reduce methane emissions. Appl. Soil. Ecol., 100:154–161. https://doi.org/10.1016/j.apsoil.2015.12.05

Manimekalai and G. Kannahi. 2017. Adaptable benefits of selected rhizospheric isolates from rice and legumes. Asian Journal of Science and Technology, 8: 5443-5449.

Mishra, S. and C.S. Nautiyal. 2012. Reducing the allelopathic effect of Parthenium hysterophorus L. on wheat (Triticum aestivum L.) by Pseudomonas putida. Plant Growth Regulation, 66: 155–165.

Nabila and R.S. Kasiamdari. 2021. Antagonistic activity of siderophore-producing bacteria from black rice rhizosphere against rice blast fungus Pyricularia Oryzae. Microbiology and Biotechnology Letters, 49(2): 217–224.

Nascente, A.S., A.C. Lanna, T.P. de Sousa, A.A. Chaibub, A.C.A. de Souza, and M.C.C. de Filippi. 2019. N fertilizer dose-dependent efciency of Serratia spp. for improving growth and yield of upland rice (Oryza sativa L.). Int. J. Plant Prod., 13:217–226. https://doi.org/10.1007/42106-019-00049-5.

Nautiyal, C.S., S. Srivastava, P.S. Chauhan, K. Seem, and K.S. Sopory. 2013. Plant growth promoting bacteria Bacillus amyloliquefaciens NBRISNI13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol. Biochem., 66: 1-9.

Omar, M.H., Z. Berahim, N. Ariffin, M.R. Ismail, H.M. Saud, N. Amalina, and S.H. Habib. 2014. Improved water use efficiency in rice under limited water environment through microbial inoculation. Journal of Food, Agriculture and Environment, 12(3–4): 149–154.

Patel, P., Trivedi, G., Saraf, M. 2018. Iron biofortification in mungbean using siderophore producing plant growth promoting bacteria. Environmental Sustainability, 1(4), 357–365.

Penrose, D.M. and B.R. Glick. 2003. Methods for isolating and characterizhing ACC deaminase-containing plant growth promoting rhizobacteria. Physol Plant, 118: 10-15.

Prihatiningsih, N., H.A. Djatmiko, and P. Lestari. 2017. Aktivitas siderofor Bacillus Subtilis sebagai pemacu pertumbuhan dan pengendali patogen tanaman terung. Jurnal Hama Dan Penyakit Tumbuhan Tropika, 17(2): 170.

Priyanka, A.T., A.S. Kotasthane, A. Kosharia, R. Kushwah, and N. Zaidi. 2017. Crop specific plant growth promoting effects of ACCd enzyme and siderophore producing and cynogenic fluorescent pseudomonas. 3 Biotech, 7(1): 1–11.

Saeed, Q., W. Xiukang, F.U. Haider, J. Kučerik, M.Z. Mumtaz, J. Holatko, and Naseem. 2021. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. International Journal of Molecular Sciences, 22(19).

Sastypratiwi, H. and R.D. Nyoto. 2020. Analisis Data Artikel Sistem Pakar Menggunakan Metode Systematic Review. Jurnal Edukasi Dan Penelitian Informatika (JEPIN), 6(2), 250.

Schwyn, B. and J.B. Neilands. 1987. University chemical assay for detection and determination of siderophore. Biochem, 160: 46-52.

ISSN: 1412-4718, eISSN: 2581-138x

Sudewi, S., U.A. Palu, B. Baharuddin, U. Hasanuddin, A.R. Saleh. 2020. Screening of plant growth promotion rhizobacteria (PGPR) to increase local aromatic rice plant growth. International Journal of Pharmaceutical Research, 13(01).

Tan, K.Z., O. Radziah, M.S. Halimi, A.R. Khairuddin, S.H. Habib, and Z.H. Shamsuddin. 2014. Isolation and characterization of rhizobia and plant growth-promoting rhizobacteria and their effects on growth of rice seedlings. American Journal of Agricultural and Biological Science, 9(3): 342–360.

Tiara, C.A., F.D. Rahmatina, R. Fajrianeldi, and L. Maira. 2019. Sido-Char sebagai pembenah keracunan Fe pada tanah sawah. Jurnal Tanah Dan Sumberdaya Lahan, 6(2), 1243–1250.

Vandana, U.K., B. Singha, A.B.M. Gulzar, and P.B. Mazumder. 2020. Molecular mechanisms in plant growth promoting bacteria (PGPR) to resist environmental stress in plants. In Molecular Aspects of Plant Beneficial Microbes in Agriculture. Academic Press, 221-223. https://doi.org/10.1016/B978-0-12-818469-1.00019-5.