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# A Review of Plant Growth Promoting Rhizobacteria and Their Characteristics as Potential Biofertilizer

**Zakiah Mustapha[1], Khamsah Suryati Mohd[1], Radziah Othman[2], Nik Nurnaeimah Nik Muhammad Nasir[1], Mohammad Moneruzzaman Khandaker[1], Hafizan Juahir[1] and Mohd Fahmi Abu Bakar[1]***

*[1]Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia*

*[2]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Malaysia*

## ABSTRACT

The growing demand for agricultural products for food requirements caused the use of excessive inorganic chemical fertilisers, insecticides, fungicides, and pesticides for a quick and simple way to maximise and boost crop yield. This practice harmed food safety and caused the degradation of environmental, physical, and biological conditions. It has become alarming, and now is the time for a greener approach to increase agricultural output while minimising the use of inorganic chemical fertilisers. It was proven through many previous studies that using environmentally friendly biofertilisers has managed to increase crop yield while reducing the usage of chemical fertilisers. Plant growth-promoting rhizobacteria (PGPR) are mostly used in biofertiliser production because these types of microbes will enhance plant growth and yield by mobilising the available nutrients through several biological mechanisms, including fixation of atmospheric nitrogen, solubilisation, and mobilisation of phosphate and potassium, phytohormones production, disease suppression, and stress protection. Understanding their characteristics, biological mechanisms of action, and the nutritional and physical requirements for growth is important for successfully formulating and applying PGPR as a biofertiliser. The selection of the right PGPR with the desired characteristics, the ability to adapt to the environment, and the ideal formulation of the biofertiliser are the main criteria that should be emphasised when determining the success of biofertiliser. Knowledge and awareness regarding the

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E-mail addresses:
zakiahmustapha@unisza.edu.my (Zakiah Mustapha)
khamsahsuryati@unisza.edu.my (Khamsah Suryati Mohd)
radziah@upm.edu.my (Radziah Othman)
naeimah92@gmail.com (Nik Nurnaeimah Nik Muhammad Nasir)
moneruzzaman@unisza.edu.my (Mohammad Moneruzzaman Khandaker)
hafizanjuahir@unisza.edu.my (Hafizan Juahir)
mohdfahmi@unisza.edu.my (Mohd Fahmi Abu Bakar)
*Corresponding author

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use, benefits, and production of PGPR as a potential biofertiliser are important and should be explored to fulfil the crop's nutritional requirements more economically and sustainably.

*Keywords*: Biofertilizer, microbes, nutrient, PGPR, plant, soil

## INTRODUCTION

Agriculture is one of the most powerful tools in the country's economic development. Its activities are important in achieving rapid economic growth, poverty reduction, and structural transformation, thus playing an important role in food security to feed the growing population. A tremendous increase in the world population has led to the increase of high demand and production of agricultural products year by year. However, the pandemic, economic instability, and climate variability have threatened agricultural growth and put food security at risk. Unfortunately, in achieving the goal of feeding the expanding population, the use of intensive off-farm inputs such as chemical fertilisers and pesticides to increase crop productivity was also increased. The excessive and indiscriminate use of these chemical inputs for enhancing agricultural production has caused a lot of negative impacts on humans, the environment and biodiversity and risks to food security.

Amidst the current situation, there is a growing awareness of mitigating the agricultural sector and improving agricultural sustainability, which suggests regenerative methods that make the best use of naturally occurring processes and locally available resources. Biofertilizer is an organic fertiliser formulated using beneficial microorganisms such as the plant growth-promoting rhizobacteria, better known as PGPR. This microbial inoculant can be applied to plants and soil to enhance plant growth and yield by mobilising the available nutrients through a biological process. PGPR, through its several mechanisms, such as the synthesis of antibiotics, enzymes, and siderophores, can also be exploited as a successful strategy for protecting plants against the deleterious effects caused by biotic and abiotic stresses (Govindasamy et al., 2008). The application of biofertiliser on seed, plant surfaces, or soil caused the beneficial microorganisms to colonise the plant's rhizosphere or the interior to promote plant growth by increasing the availability of nutrients to the host plant (Fasusi et al., 2021). It also helps to build up the micro-flora biological activity and enhance soil fertility (Fasusi et al., 2021).

The rising awareness of the hazardous effects and increasing cost of chemical fertilisers have given momentum to the use of biofertiliser. Moreover, the production cost of biofertiliser is lower, with tremendous potential as an additional, sustainable, and green source of plant nutrients. Biofertilisers have now become an important component of integrated nutrient management (INM) and integrated plant nutrition systems (IPNS) (Sangeeth & Suseela Bhai, 2015). A wide range of PGPR, either in single species or in combinations, are used to

supply different kinds of nutrients to the soil with different modes of action. It produces a higher yield while being safe for both the environment and people, which promotes more sustainable economic growth for farmers, agriculture, and the nation.

Compared to chemical fertiliser, biofertiliser will ensure constant and sustainable nutrient supplies and prevent nutrient leaching through the microorganism's activities. However, in certain cases, biofertilisers sometimes require longer to show their real effects. This mostly occurs in newly applied areas or problem areas that have long been used for agricultural or other purposes. It also requires frequent application of biofertilisers for the beneficial microbes to dominate a place and be effectively functional due to adaptation factors and competition with other microorganisms in that applied area (A. Sharma & Chetani, 2017). Nevertheless, the right timing and frequent application of biofertiliser can partially substitute, enhance the function, and then subdue the application quantities of chemical fertilisers and still maintain the same yield for the application of cash or other types of crops (Lyu et al., 2023; Mustapha et al., 2017).

Studies have demonstrated that PGPR inoculation on the soil/plant ecosystem can enhance soil health, soil quality, crop development, yield, and quality. PGPR is frequently and widely used in organic farming and natural agriculture since it helps to solve issues related to the usage of chemical pesticides and fertilisers. Biofertilisers have improved and increased the number of beneficial microbes in the soil, thereby promoting a healthy environment for plants. Numerous field and greenhouse trials indicate the benefits of PGPR as a biofertiliser in crop production. The application of PGPR was proven to enhance crop growth and yield, giving crops protection and, at the same time, conserving natural resources for ultimately sustainable agriculture and environmental systems (García-Fraile et al., 2012). The prospects for improved agriculture using PGPR are particularly impressive because they have lower costs, give better yield, and reduce dependence on chemical substances. The role of PGPR as a biofertiliser is an added dimension that, if used properly, can enhance and optimise the best soil and crop management practices.

## PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) AND ITS FUNCTIONAL CHARACTERISTICS

Most beneficial or effective microbes (EM) in biofertilisers have a close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, while plant growth-promoting rhizobacteria (PGPR) refers to any beneficial bacteria that colonise the region under the influence of the plant's roots, known as the rhizosphere. These beneficial soil bacteria flourish in the plant's rhizosphere by growing in, on or around plant tissues and stimulate plant growth via direct or indirect means. Numerous species of PGPR have been studied, and among them are strains from genera such as *Bacillus*,

*Pseudomonas*, *Rhizobium*, *Burkholderia*, and *Enterobacter* (Khandelval et al., 2023).

Generally, PGPR functions in three different ways to enhance plant growth. As stated above, for biofertiliser, PGPR can synthesise compounds for plants, such as hormones and enzymes. They are also responsible for lessening or preventing plants from diseases and facilitating the uptake of certain nutrients from the soil. Plant growth promotion and development by PGPR are carried out by both direct and indirect mechanisms (Figure 1). Symbiotic and non-symbiotic PGPR showed direct plant growth promotion through nitrogen fixation, solubilisation of minerals such as phosphate and potassium, and production of plant hormones have been reported for several bacterial genera (Ashraf et al., 2004). Indirect plant growth promotion includes preventing the deleterious effects of phytopathogenic organisms by producing siderophores, antibiotics, and enzymes (S. B. Sharma et al., 2013).

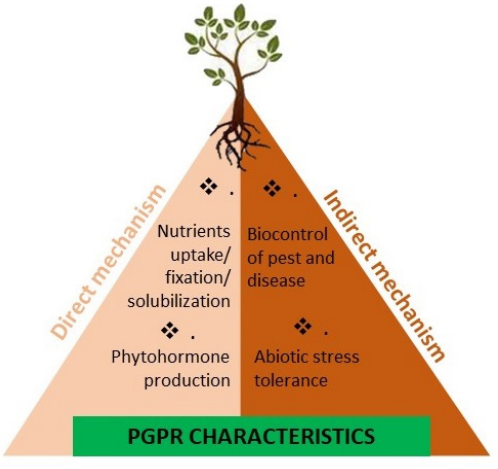


*Figure 1*. Plant growth-promoting rhizobacteria (PGPR) characteristics as biofertiliser

Biological nitrogen fixation (BNF), phosphate-solubilisation, potassium solubilisation, and phytohormone production are frequently cited as the main mechanisms of PGPR in enhancing crop growth and production. The inoculation of single or multiple strains of PGPR, which have multiple beneficial characteristics, is critical because this technique will reduce the amount of chemical fertiliser inputs while increasing crop growth and production. Thus, using PGPR in biofertiliser production is the current area of interest in developing sustainable agriculture. It is emphasised with the intention of obtaining further cumulative effects from the specific strains in the prepared inoculum without having any negative effects on the environment or plants.

## Nitrogen Fixing Bacteria

Nitrogen is the most important and commonly considered one of the foremost restrictive nutrients for plant growth. Nitrogen in the biosphere is available in the form of atmospheric nitrogen ($N_2$), which cannot be utilised by plants (Mustapha et al., 2018). The natural process of biological nitrogen fixation (BNF) is to make the unavailable form of nitrogen from the atmosphere accessible to plants. The process has been regarded as the main plant growth-promotion effect by soil microorganisms. It involves a specific enzyme called nitrogenase to convert nitrogen to an accessible form of ammonia ($NH_3$). The BNF process is only mediated in nature by bacteria and certain species of actinomycetes through

symbiotic or non-symbiotic relationships with plants (Soumare et al., 2020). The *Rhizobium*, which has a high degree of host specificity when infecting the roots of leguminous plants, is the best illustration of the symbiotic relationship between nitrogen-fixing microbes and plants. Whereas only a few groups of microorganisms, including free-living bacteria and blue-green algae, can fix nitrogen without symbiotic relationships (Soumare et al., 2020).

The inorganic chemical fertilizer N, such as urea, is widely used by farmers because of its immediate effect in supplying nitrogen to plants. However, many studies have shown that the application and increment of chemical fertilizer N only give a marginal yield increment on plants. Due to the very low only 30% nutrient uptake efficiency by plants, the remaining 70% of the applied fertiliser is typically lost through a variety of processes, including leaching, evaporation, and surface runoff to the natural water supply (Anas et al., 2020). This process will eventually cause the problem of eutrophication and result in the emission of nitrous oxide ($N_2O$), carbon dioxide ($CO_2$), and greenhouse gas (GHG) that are harmful to the atmosphere (Kusin et al., 2015). Moreover, the application of chemical fertiliser might lead to a decline in the community of beneficial soil microorganisms and soil fertility (Zainuddin et al., 2022).

Therefore, to be utilised as a biofertiliser on plants, the selection of PGPR with $N_2$-fixation capability is essential and necessary (Bakar & Othman, 2022). The use of N-fixing PGPR in biofertiliser is significant in reducing the use of synthetic nitrogen fertilisers. It could also increase the nitrogen uptake efficiency of the crops, thus conserving the environment. Biofertilisers with N-fixing bacteria are formulated because of their successful ability to fix free atmospheric nitrogen into the soil and enter the plant roots. The use of N-fixing biofertiliser has been proven effective in reducing the use of chemical fertilisers, thus reducing the harmful effects on soil and environmental health.

**Phosphate Solubilising Bacteria**

Phosphorus is the second most important macronutrient required by plants after nitrogen. Phosphorus is widely distributed in nature, both in organic and inorganic forms, in a bound state that is not readily available to plants. This element is still one of the major plant-limiting nutrients due to its availability and low solubility in the soil. It mostly remains in insoluble phosphates of iron, aluminium, and calcium in the soil (S. B. Sharma et al., 2013). The main problem with the application of mineral or organic phosphates fertiliser is the fact that a large portion of P-fertilizer is unavailable to plants because it is bound to the soil, creating a pool of residual P, or is lost via leaching, runoff, and/or erosion to the surface water creating eutrophication (Conijn et al., 2018). Thus, the important aspect of increasing soil phosphorus availability is the release of insoluble and fixed forms of phosphorus into the form accessible to plants.

Many PGPR communities known as phosphate solubilising bacteria (PSB) were identified, especially from the genus *Bacillus* and *Pseudomonas* (Illmer & Schinner, 1992; Wani et al., 2007). These groups of microorganisms are capable of hydrolysing organic and inorganic phosphorus compounds from insoluble compounds. The use of PSB can optimise crop production by increasing P uptake by the plant and minimise P losses from soils by various approaches, including lowering the soil pH, chelation, and mineralisation to make phosphorus accessible for plants to absorb (Ismail et al., 2016; Kalayu, 2019). PSB will produce organic acids or releases of protons that lower the soil pH (Kaur, 2019). It was proven in the P-solubilization test that a strong positive correlation had been reported between the solubilisation index and organic acids produced. The hydroxyl and carboxyl groups from the organic and inorganic acids produced by PSB will chelate the cations bound to phosphate, thereby converting them into soluble forms. Production of phosphatases enzyme by PSB will mineralise the soil organic P by hydrolysing organic forms of phosphate compounds, thus releasing inorganic phosphorus that will be immobilised by plants (Kalayu, 2019).

**Potassium Solubilising Bacteria**

In soils, potassium can be found in four main forms: water-soluble, mineral, exchangeable, and non-exchangeable (Kaur, 2019). These forms are not uniformly distributed throughout soils, but they are all in a state of dynamic equilibrium with one another and are often governed by the physicochemical characteristics of the soil. The readily available K in soil is usually very low, at 1–2% of total K, and exists in soluble and exchangeable forms (Lalitha & Dhakshinamoorthy, 2014). Most soil mineral potassium can be found in silicate minerals, including mica and K-feldspar, even though they make up more than 90 to 98% and are unavailable for direct plant uptake (Goldstein, 1994). Release of non-exchangeable K to the exchangeable form occurred when levels of exchangeable and soluble decreased due to crop uptake or leaching and perhaps by the increase in microbial activity (Sparks, 1999).

The potassium solubilising bacteria (KSB) can make up approximately 1–10% of available soil potassium, which contributes significantly to plant uptake (Memon et al., 1988). A few mechanisms involved in the potassium solubilising process by KSB include the secretion of organic acids and inorganic acids and polysaccharides, acidolysis, complexolysis, chelation, and exchange responses (Meena et al., 2015). Since there is abundant insoluble K in the soil, converting them into a form of K that plants can absorb may be more economically feasible. Studies have shown that a variety of KSBs can cause soluble K to be released from K-bearing minerals such as mica, illite, and K-feldspar by producing organic acid that will dissolve rock and chelate silicon ions to release K ions into the soil, which could uptake by plants (Zhang & Khong, 2014). PGPR such as *Bacillus mucilaginosus*, *Bacillus edaphicus*, and

*Bacillus circulans* have been explained as effective K solubilisers, while other PGPR such as *Burkholderia*, *Acidithiobacillus ferrooxidans*, and *Enterobacter hormaechei* have been described to effectively solubilise the silicate rocks to produce an available K for plant uptake (Etesami et al., 2017; Meena et al., 2015).

Potassium is usually added as an inorganic fertiliser source to optimise crop yield. However, intensive application of inorganic fertiliser has several negative impacts on the environment as not all fertilisers will be absorbed by plants. One possible alternative could be to exploit the reservoir of K in the soil fully. The use of K-solubilizing microbes to increase the concentration of available K ions in the soil may mitigate K deficiency. Thus, the potassium solubilisation ability of PGPR is one of the crucial characteristics that promote plant growth and development. The application of KSB as a biofertiliser could support sustainable crop production by improving agriculture development by reducing the use of inorganic chemical fertilisers or other agrochemicals.

**Phytohormone Production**

Plant cells typically communicate using chemical signals secreted from the sending cell and released to the neighbouring cells. The plant growth and development process is majorly impacted by the availability and communication of transporting mineral nutrients, hormones, and other secreting metabolites in the plant cells. In this case, PGPR has various characteristics and functions in influencing plant growth and development, including the production of plant growth regulators, also known as phytohormones, such as auxin, gibberellin, cytokinin, salicylic acid, and ethylene. Almost all communication in plant cells is brought by plant hormones produced by plant cells or by rhizobacteria (Maheshwari et al., 2015). The synergistic effect of hormone secretion is one of the main criteria of PGPR as the attraction to engage with the plant cells.

The most prevalent auxin phytohormone, indole acetic acid (IAA), is produced in the shoot apical meristem of plants and can be found across the body of the plant. IAA production was believed to be one of the bacterial colonisation strategies on plants other than phytostimulation of the basal plant defence mechanisms (Spaepan et al., 2009). IAA secretion by soil microorganisms was believed to be an important factor for plant growth and development. It encourages the growth of more and longer root hairs, increasing the surface area of the roots for better water and nutrient absorption (Vessey, 2003). Furthermore, optimal root growth boosts root vitality, safeguarding the plant, particularly from soil-borne pests and disease infections (Vessey, 2003). Many PGPRs, such as *Bacillus*, *Acetobacter*, and *Herbaspirillum*, are isolated from various rhizosphere crops that can produce IAA. It was also reported that IAA production by PGPR has significantly promoted rooting and growth in many crops such as rice, wheat, maise, kiwifruit, and oil palm (Biswas et al., 2000; Erturk et al., 2010; Om et al., 2009; Spaepan et al., 2009).

Plants that receive PGPR treatment frequently develop persistent, broad-spectrum systemic resistance to a variety of phytopathogenic bacteria and fungi. This situation develops through an induced resistance mechanism response via two forms, including induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Heil & Bostock, 2002). Certain PGPR that affect plant cells will produce salicylic acid as their exogenous metabolite that can induce the resistance mechanism response in plants (Pieterse et al., 2014). The induction of ISR and SAR is generally associated with salicylic acid signalling and the production of volatile organic compounds such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which decreases the plant ethylene levels, thus inhibiting the functioning of several phytopathogens (del Carmen Orozco-Mosqueda et al., 2023). The ACC deaminase will regulate the endogenous production of ethylene by PGPR, which is also helpful in sustaining plant growth and development under stress conditions (Shaharoona et al., 2011). Salicylic acid is the plant growth regulatory phenolic phytohormone that also serves as an intermediate precursor in pyochelin siderophores biosynthesis (Ankenbauer & Cox, 1988). According to Baldwin et al. (1997), salicylic acid application to plants has been found to inhibit the synthesis of jasmonic acid as an ISR response against pathogens' infection. In addition to the involvement of salicylic acid in SAR, this hormone is involved in the mitigation of various plant biotic and abiotic stresses, including both high and low temperatures, high levels of salt and toxic organic chemicals (del Carmen Orozco-Mosqueda et al., 2023).

Plant growth and performance are significantly influenced by the soil bacteria's synthesis of phytohormones. Different types of PGPR produce different levels of phytohormone, and one type may produce more than one type of phytohormone. PGPR, such as *Bacillus amyloliquefaciens*, was proven to produce gibberellins, auxin, and salicylic acids (Miljaković et al., 2020; Shahzad et al., 2016). Since then, it has also been noted in other bacterial species, including *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* (Bastián et al., 1998), and *Bacillus* spp. (Gutiérrez-Mañero et al., 2001). Gibberelic acid (GA) was initially described in *Rhizobium meliloti* (Atzorn et al., 1988). GA causes early flowering and budding, breaks seed dormancy, and delays plant senescence. Naturally occurring cytokinins, such as zeatin and adenine, have specific functions in cell division, leaf growth, and the induction of seed germination (Mok, 1994). Different bacteria from the genera *Proteus*, *Klebsiella*, *Bacillllus*, and *Pseudomonas* have been reported to have the ability to produce cytokinins.

## Siderophores Production

Iron (Fe) is an essential nutrient for soil microorganism's metabolism. It is contradictory to plant that needs only a trace amount of iron. The availability of iron in the soil is always limited because of the low

iron concentration and very low solubility of the ferric ion ($Fe^{3+}$) (Siddiqui, 2005). Iron in the soil builds up in typical mineral phases such as iron oxides and hydroxides, the minerals that give the soil its red and yellow hues, and these minerals cannot be readily used by organisms. Soil microorganisms release siderophores to scavenge iron from its solid phases, resulting in soluble iron ($Fe^{3+}$) that plants can absorb.

Under conditions of low iron stress, some bacteria and fungi produce siderophores, which are ferric-ion-specific chelating agents (Ngamau et al., 2014) with a molecular weight of below 1000 Da. Studies have shown that one crucial mechanism for biological control is the siderophore-mediated competition for iron between PGPR and soil-borne pathogens. Most plants can obtain iron from the soil via bacterial iron siderophore complexes. The implications of this condition have reduced phytopathogens' ability to compete for root colonisation (Ren et al., 2005).

*Pseudomonas aeruginosa* FP6 is the siderophores producer that was isolated from the rhizospheric soil and was found to significantly reduce the growth of *Rhizoctonia solani* and *Colletotrichum gloeosporiodes* that cause diseases in chilli (Sasirekha & Srividya, 2016). Siderophores production by *Chryseobacterium* C138 has significantly increased iron, chlorophyll content, and yield of the iron-starved tomato plants, indicating that siderophores are effective in providing iron to the plant (Radzki et al., 2013). A study found that the production of the siderophore by *Pantoea* sp. strain (EA106) has increased the ability of roots to absorb iron and promotes the development of a more oxidative environment in the rice rhizosphere (Lakshmanan et al., 2015). The inoculation with siderophores-producing microbes can change the levels of both arsenic and iron in rice, indicating that the bacterial strain may potentially improve rice quality by lowering the buildup of toxic arsenic species in the plant's aerial parts.

## NUTRITIONAL REQUIREMENTS FOR PGPR GROWTH

It is well known that environmental factors can impact how bacteria adapt, proliferate, and produce secondary metabolites. Two requirements for microbial growth are the nutritional and physical factors that vary greatly between species (Cappucino & Sherman, 2004). The formulation and production of biofertilisers, as well as the effective growth of microorganisms in the laboratory, depend on an understanding of these requirements. Moreover, bacterial fermentation must compete favourably with chemical synthesis in the biofertiliser market. It is essential since many potential microbiological uses that have been considered for developing biofertilisers depend on whether they can be generated economically. This is because the fermentation medium can reduce the cost of microbial fermentation by up to 30%, which is critical in the commercial industry (Hofvendahl & Hahn-Hagerdal, 2000). Complex media commonly employed for bacterial growth in the laboratory are unsuitable for commercial production and are not economically attractive due to their

high amount of expensive nutrients such as yeast extract, salts, and peptone (Batish et al., 1990).

All bacteria require certain basic nutrients for life sustenance, and the requirements vary greatly among species. Nutritional needs are supplied through a variety of media that have various essential nutrients for bacterial growth, such as carbon, nitrogen, metals and non-metals elements, vitamins, and water (Cappucino & Sherman, 2004). Many bacteria can be grown in laboratories in the nutrient medium, which are designed to provide all the essential nutrients needed by bacteria for their growth. It is one of the several non-selective media useful in the routine cultivation of microorganisms. Nutrient agar/broth is a general-purpose nutrient medium supporting the growth of a wide range of non-fastidious organisms. This medium contains many nutrients needed for bacterial growth and can grow various species of bacteria and fungi.

Before being used in the industry, microbes were typically cultivated in a nutritional medium of the necessary quantity. Bacterial cultivation uses a variety of carbon sources, including glucose, fructose, and lactose. However, using such pure or mixed media on an industrial scale would be quite expensive (Michailides et al., 2015). Industrial applications of microbes need to use a more economical carbon source. Thus, molasses is an important agro-industrial by-product containing high sugar (48-50%) (Quan et al., 2005). It can be utilised as a more affordable source of nutrients for microbial development compared to other biological or chemical mediums in the market. According to Curtin (1983), the chemical composition of molasses was almost similar and seemed to be a standard one. It was then proven by Sutigoolabud et al. (2004) that the composition of molasses produced in Thailand and Okinawa was almost similar, with high concentrations of total sugars and reduced sugars, as shown in Table 1.

Molasses is the basic raw material used for a lot of microbiological processes (Quan et al., 2005). The dark brown thick syrup remained as the residue of inverted sugar crystallisation. Molasses is one of the other organic materials used as carbon and nitrogen sources for bacterial growth. Due to its many benefits, molasses is preferred as a medium for microbial growth over chemical substances. It has higher biodegradability and is effective at extreme temperatures or pH values, and most importantly, it has low toxicity (Rodrigues et al., 2006). High values of caramelised and inverted sugar in high concentrations of molasses could usually cause cell toxicities (Baei et al., 2009). Usually, less than 10% of molasses is used in a bacterial fermentation medium, and the percentage depends on the purpose of the fermentation. Therefore, the precise amount of molasses to be utilised as a medium for bacterial growth must be measured accordingly to achieve optimum bacterial growth.

There are many types of molasses, but the one that has gained much attention is sugar cane molasses. This molasses has been reported to be used as the growth

medium in many fermentation processes of several bacteria and other microorganisms. The by-product of producing sucrose from sugarcane, which comprises more than 46% of inverted total sugar, is cane molasses (Curtin, 1983), which has a high concentration of total sugar (38.8%), which is made up of glucose (3.8%), fructose (7.9%), sucrose (27.7%), and reducing sugar (23.5%) (Aslan et al., 1997). Molasses has been shown to have other additional minerals other than the sources of carbon and nitrogen, including manganese, iron, calcium, potassium, magnesium, succinic acid, malic acid, citric acid, vitamin B6, and selenium (Aslan et al., 1997; El-Enshasy et al., 2008; Sutigoolabud et al., 2004).

Table 1
*The chemical and physical properties of molasses, as adopted from Sutigoolabud et al. (2004)*

| | Molasses | |
|---|---|---|
| Component | Produced in Thai | Produced in Okinawa |
| Brix (%) (1:100) | 5.1 ± 0.0 | 1.2 ± 0.0 |
| Moisture (%) | 24.6 ± 1.3 | 21.6 ± 1.3 |
| Ash (%) | 9.5 ± 1.3 | 16.8 ± 0.0 |
| Total sugar (%) | 38.8 ± 2.9 | 35.3 ± 1.6 |
| Reducing sugar (%) | 23.5 ± 1.9 | 24.9 ± 4.7 |
| Glucose (%) | 3.8 ± 2.2 | 4.1 ± 1.1 |
| Fructose (%) | 7.9 ± 1.9 | 10.9 ± 3.8 |
| Sucrose (%) | 27.7 ± 4.4 | 24.4 ± 4.6 |
| Citric acid (mg/kg) | 1 179 ± 81 | 1 002 ± 462 |
| Malic acid (mg/kg) | 410 ± 90 | 603 ± 32 |
| Succinic acid (mg/kg) | 2 134 ± 60 | 3 218 ± 179 |

*Note*. All the values are mean of triplicate analysis on a wet weight basis

## ENVIRONMENTAL FACTORS ON PGPR GROWTH

As biofertiliser, the microbial inoculants will be introduced to the soil, seeds, or plant itself. The introduced bacteria must adjust to the soil environment upon inoculation to achieve successful and effective colonisation and be vigorous enough to compete with local microorganisms. Bacterial growth and survival depend directly on several environmental factors, and the requirements differ among species (Figure 2). These specialised requirements show how bacteria have adapted to their surroundings. Environmental factors such as pH, temperature, available water, nutrient level, oxygen levels, and competition with other microbes and toxins could influence bacterial growth rate and activity. Bacteria have optimal growth conditions under which they flourish. However, the stress can result in reduced or stalled growth outside their required condition and environment. Some PGPR, such as *Bacillus* species,

may be dormant by the formation of spores to protect themselves. In more serious conditions, morphological changes could happen in the cell, or the emergence of resistance to the same stress factor or other types of stress factors or even death (Jones & Lennon, 2010; Święciło & Zych-Wężyk, 2013).

PGPR has various mechanisms, including the production of antibiotics, enzymes, metabolites, and scavenging of nutrients to protect themselves from biotic and abiotic stress. Other than protecting themselves, these mechanisms could also influence different physiological activities and induce systemic resistance, thus protecting plants from the biotic stress caused by other pathogenic infections and abiotic environmental stress factors (Shameer & Prasad, 2018). Studies on the effect of pH, temperature and salinity on bacterial growth and extracellular polymeric substances (EPS) and extracellular enzymes have been reported for various strains of microorganisms. A study found that the growth of *Rhizobium meliloti* was increased when the 10% molasses medium pH was increased from 6 to 8 at a constant temperature of 28°C (Singh et al., 2011). They added that at pH 7 in a 10% molasses medium, the growth of *R. meliloti* growth was higher in high temperatures (28–30°C) compared to lower temperatures (26–27°C), and the optimum temperature for the highest bacterial growth was at 28°C.

Other than the effects on bacterial growth, environmental conditions could also affect bacterial functions. The pH, temperature, nitrogen source, carbon source, organic acid, and iron concentration influence the production of siderophores by the *Bacillus* sp. strain VITVK5 and *Enterobacter* sp. strain VITVK6 isolated from the iron-enriched soil sample (Kumar et al., 2017). In other cases, the cultural conditions such as pH and temperature and media components, for example, carbon and nitrogen source as well as tryptophan concentration, have effects on IAA production of *Bacillus* and *Lactobacillus* species isolated from the rhizosphere soil of banana, cotton, and maise (Mohite, 2013).

Knowledge and understanding of the factors affecting microbial growth are very important in predictive microbiology approaches to recognise the level of bacterial response and its efficacy when used in different soil conditions. As in crop production, the use of chemical fertiliser will increase soil salinity. However, a combination of biofertiliser and chemical fertiliser has been reported to increase various crops' yield and quality. Thus, a comprehensive study needs to be done to determine the type of PGPR used as the inoculant in biofertiliser production. The ability of a microorganism to survive and react in other extreme environmental conditions, such as in too low or high salinity, high pH, or high temperature, is also an important characteristic to be emphasised.

## CONCLUSION AND FUTURE PERSPECTIVES

The demands for agricultural products and the supply of foods had caused robust

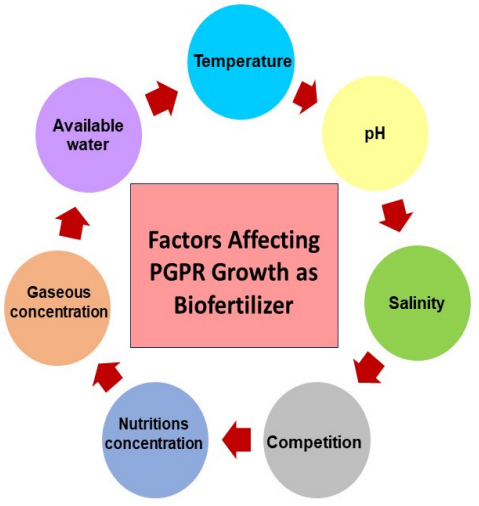


*Figure 2*. Environmental factors affecting plant growth-promoting rhizobacteria (PGPR) growth as biofertiliser

development in agriculture. This situation caused the use of many chemical fertilisers, which are expensive and harmful to the environment, soil health, and food safety. The use of biofertilisers is highly recommended and considered as an alternative to solve this chemical fertiliser issue. Biofertilizer is the organic fertiliser prepared by living microbial cells such as PGPR to activate the various natural processes and enhance plant growth and yield through various mechanisms. Biofertilisers are a promising tool for crop production and agricultural ecosystems as a supplementary, renewable, and eco-friendly source of plant nutrients. The application of biofertilisers was hoped to be a key element in maintaining crop productivity and soil fertility at a sufficiently high level and vital to achieving sustainable agricultural goals.

The changing approach to more sustainable agricultural practices makes biofertilisers a crucial part of crop production in this century. A number of rhizosphere microorganisms, especially PGPR, were identified to exert multifunction plant growth-promoting activities. The selection of the right PGPR with the desired characteristics and ability to adapt to the environment, as well as the ideal formulation of the biofertiliser, is the main criteria that should be emphasised and very important in determining the success of biofertiliser. Some PGPR is root-inhibiting, while others are free-living diazotrophs in soil and are believed to develop mechanisms for survival in the competitive soil environment. Survival PGPR are protected from competition with other soil microbes and adverse soil environmental conditions by producing secondary metabolites, scavenging nutrients, and becoming root endophytes.

Several research projects conducted worldwide have demonstrated the PGPR's contributions as a biofertiliser to improve agricultural productivity and quality and preserve soil and environmental health. Despite demonstrating their potential, biofertilisers are not widely used to replace chemical fertilisers. Therefore, there is an indispensable need to encourage individuals, farmers, and industry participants to explore the use of PGPR as biofertilisers to achieve the goal of higher agricultural sustainability. Farmers and the authorities need to jointly play a role in making this plan a success and should be educated and aware of the use and advantages of biofertilisers. Farmers should have easy access to biofertilisers, and government officials should start offering

rigorous training and capacity building for agricultural or industrial workers regarding biofertiliser use, production, maintenance, and quality control. In addition, it would be very significant if the authorities could provide subsidies on biofertilisers to farmers and encourage them to accelerate the use of biofertilisers for the time being.

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