Institute of Biodiversity and Environmental Conservation

Identification and Characterization of Plant Growth Promoting Rhizobacteria Indigenous to Rhizosphere of Sago Palm (*Metroxylon sagu*, Rottb.)

Flonia Binti Benet

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Identification and Characterization of Plant Growth Promoting Rhizobacteria Indigenous to Rhizosphere of Sago Palm (*Metroxylon sagu,* Rottb.)

Flonia Binti Benet

A thesis submitted

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

Name: Flonia Binti Benet

Matric No.: 18020053

Institute of Biodiversity and Environmental Conservation

Universiti Malaysia Sarawak

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ABSTRACT

Arising food consumption, climate impacts as well as dwindling arable land have put worries on the agriculture sector that the world's food cupboards may be bare in the coming decades. *Metroxylon sagu*, Rottb. is one of the underutilized crops which holds high potential to be cultivated for its high starch yield, deemed to strengthen food security especially in Sarawak, Malaysia. However, the quest for boosting *M. sagu* yields will potentially lead to the future abuse of synthetic agrochemicals and chemical fertilizers. Against this backdrop, biofertilizers have emerged as suitable alternatives to ameliorate sustainable agricultural ecosystems. They are derived from living or dormant microorganisms and one of the foremost candidates in this respect is Plant Growth Promoting Rhizobacteria (PGPR). Despite the fact that much research has been done on PGPR, there are still certain gaps to be addressed, particularly in those related to PGPR indigenous to underutilized crops such as *M. sagu*, which exposed to stressful environments. Thus, this study was conducted to identify the PGPR indigenous to *M. sagu*, Rottb. in selectedsites at Kuching and Dalat Division of Sarawak State, Malaysia. Given various Plant Growth Promoting (PGP) traits, the study has been approached with hierarchical strategy by screening one of essential nutrients required by plants, the nitrogen. The putative diazotrophic PGPR were initially tested for growth on Burks agar, a nitrogen-free medium. The isolates then were randomly picked and subsequently analyzed for their genetic differences, by Repetitive Extragenic Palindromic (rep-PCR), the $(GTG)_5$ PCR. The banding profiles obtained were analyzed by GelJ_v2.0 software to generate a dendrogram and the isolates were further identified by using 16S rDNA sequencing. These identified isolates then further screened for their PGP traits such as ammonia producer, phosphate solubilizer, IAA and siderophore producer. The superior of identified isolates for their PGP traits was determined by one way ANOVA and they were

further ranked based on bonitur scale method. About 54 isolates were isolated as diazotrophic bacteria and about 47 isolates were further subjected to $(GTG)_5$ PCR analysis. Based on the constructed dendrogram, about nine clusters were deduced and nine species were identified. The isolates were deduced to be in the Phyla of Proteobacteria and Firmicutes, consisting of 9 identified species, namely *Serratia marcescens, Bacillus* sp., *Bacillus cereus, Pseudomonas* sp., *Staphylococcus sciuri, Pseudomonas monteilii, Pseudomonas extremaustralis, Bacillus thuringiensis* and *Bacillus subtilis.* Isolates belonging to genus *Bacillus* made up 44.4% of the total number of PGPR identified, making it the most prominent genus. All test isolates turned positive for ammonia production. The rhizobacterial isolates of *Pseudomonas* being the highest phosphate solubilizer (23.08 ± 14.29 mg/L) with no statistically significant difference with other isolates. In IAA production, *Pseudomonas.* sp. was found to be a significant IAA producer with the presence of tryptophan (26.07 ± 8.53 µg/mL). Production of siderophore was detected in seven out of nine tested isolates. *S. sciuri* was the highest producer with solubilization index of 1.37 ± 0.06 cm with no statistically significant difference was observed in the amount of IAA produced. According to bonitur scale. *Pseudomonas.* sp. was determined to be at the top of the scale, followed by *S. sciuri, P. monteilii,P. extremaustralis, B. subtilis, B. thuringiensis, Bacillus* sp., *B. cereus* and *S. marcescens*. Thus,this study implied that the isolates indigenous to the rhizosphere of *Metroxtylon sagu,* Rottb. possessed PGPR traits. These identified isolates may potentially a good fit as consortium for biofertilizer as well as biocontrol agents which is beneficial for our agriculture industry.

Keywords: Plant growth promoting rhizobacteria (PGPR), phosphate solubilizer, IAA producer, siderophore producer, *Metroxylon sagu*, Rottb.

***Pengenalpastian dan Pencirian Rhizobakteria Penggalak Pertumbuhan Tumbuhan Asli kepada Rhizosfera Sagu* (Metroxylon sagu, *Rottb.*)**

ABSTRAK

Dek peningkatan *keperluan makanan, kesan daripada perubahan iklim serta tanah pertanian yang kian berkurangan, telah menimbulkan kebimbangan akan status bekalan makanan dalam tempoh mendatang.* Metroxylon sagu, Rottb. merupakan *salah satu tanaman yang kurang digunakan yang berpotensi tinggi untuk diusahakan kerana hasil kanjinya yang tinggi, disifatkan dapat mengukuhkan keselamatan makanan terutamanya di Sarawak, Malaysia. Bagaimanapun, usaha untuk meningkatkan hasil tanaman* M. sagu *ini berpotensi membawa kepada penyalahgunaan agrokimia sintetik dan baja kimia pada masa hadapan. Baja bio telah muncul sebagai alternatif yang sesuai untuk memperbaiki ekosistem pertanian yang mampan. Ia berasal daripada mikroorganisma hidup atau tidak aktif dan salah satu calon utama dalam hal ini ialah Rhizobakteria Penggalak Pertumbuhan Tumbuhan (PGPR). Meskipun beberapa penyelidikan telah dilakukan ke atas PGPR, masih terdapat jurang tertentu yang perlu ditangani, terutamanya mencari PGPR yang memberi kesan yang memberangsangkan terhadap tanaman yang terdedah kepada persekitaran alam yangtertekan. Oleh itu, kajian ini dilakukan bertujuan untuk mengenalpasti PGPR daripada M. sagu, Rottb. yang tumbuh di, sekitar Kuching dan Dalat,Sarawak, Malaysia. Lantaran wujudnya beberapa kriteria PGPR, kajian ini dimulai denganpenyaringan PGPR terhadap salah satu PGP terpenting, iaitu pengikatan nitrogen. Isolat bakteria ini telah diuji melalui pertumbuhannya di atas media tanpa nitrogen, media Burks.Pengasingan kemudiannya dipilih secara rawak dan seterusnya dianalisis untuk perbezaan genetiknya, oleh Repetitive Extragenic Palindromic (rep-PCR), PCR (GTG)5. Profil DNA yang diperolehi telah dianalisis oleh perisian GelJ_v2.0 untuk menghasilkan dendrogram dan filogeni isolat*

dikenal pasti dengan menggunakan penjujukan 16S rDNA. Isolat yang dikenal pasti ini kemudiannya disaring untuk ciri-ciri Penggalak Pertumbuhan Tumbuhan (PGP) mereka seperti pengeluar ammonia, pelarut fosfat, pengeluar IAA dan siderofor. Keunggulan bagi isolat yang dikenal pasti untuk ciri-ciri PGP mereka ditentukan oleh ANOVA sehala dan mereka selanjutnya disenaraikan berdasarkan kaedah skala bonitur. Kira-kira 54 isolat telah dikenalpasti sebagai bakteria diazotropik dan 49 isolat selanjutnya tertakluk kepada analisis PCR (GTG)5. Berdasarkan dendrogram yang dibina, sembilan spesies telah dikenal pasti, Serratia marcescens, Bacillus sp., *Bacillus cereus, Pseudomonas* sp., *Staphylococcus sciuri, Pseudomonas monteilii, Pseudomonas extremaustralis, Bacillus thuringiensis* dan *Bacillus subtilis. Semua isolat yang diuji menunjukkan tindakbalas positif untuk pengeluaran ammonia. Manakala, isolat Pseudomonas merupakan pelarut fosfat tertinggi (23.08 ± 14.29 mg/L) tanpa perbezaan yang signifikan secara statistik dengan isolat yang lain. Dalam pengeluaran IAA, Pseudomonas* sp. *didapati sebagai pengeluar IAA yang ketara dengan kehadiran triptofan (26.07 ± 8.53 µg/mL). Pengeluaran siderofor dikesan dalam tujuh daripada sembilan isolat yang diuji. S. sciuri adalah pengeluar tertinggi dengan indeks pelarutan 1.37 ± 0.06 cm tanpa perbezaan ketara secara statistik diperhatikan dalam jumlah IAA yang dihasilkan. Mengikut skala bonitur. Pseudomonas* sp. *telah ditentukan untuk berada di bahagian atas skala, diikuti oleh S. sciuri, P. monteilii, P. extremaustralis, B. subtilis, B. thuringiensis, Bacillus* sp., *B. cereus dan S. marcescens. Kesimpulannya,* isolat daripada rizosfera *M. sagu,* Rottb. *mempunyai ciri-ciri PGPR yang memberangsangkan. Isolat ini berpotensi dijadikan sebagai konsortium inokulan bagi pembangunan baja bio dan agen kawalan yang bermanfaat untuk industry pertanian kita.*

Kata kunci: *Plant growth promoting rhizobacteria (PGPR), pelarut fosfat, penghasilan IAA, penghasilan siderofor,* Metroxylon sagu, *Rottb.*

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LIST OF ABBREVIATIONS

ARB	Antibiotic resistance bacteria
ARG	Antibiotic resistance gene
ARGs	Antibiotic resistance genes
BLAST	Basic local alignment search tool
BNF	Biological nitrogen fixation
CAS	Chrome Azurol S
CFU	Colony forming unit
CGS	Centre for Graduate Studies
COVID-19	Corona virus disease 2019
DNA	Deoxyribonucleic acid
EtBr	Ethidium Bromide
FAO	Food and Agriculture Organization
HCN	Hydrogen cyanide
IAA	Indole acetic acid
ISR	Induced systemic resistance
MRB	Malaysian Rubber Board
NPR	Nodule promoting rhizobacteria
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
PGPRs	Plant growth promoting rhizobacterias

pH	Potential of hydrogen
PSI	Phosphate solubilization index
PVK	Pikovskayas
RAPD	Random amplification of polymorphic DNA
Rep-PCR	Repetitive Extragenic Palindromic
Sp.	Species
TBE	Tris Borate EFTA
TCP	Tri-Calcium Phosphate
UNIMAS	Universiti Malaysia Sarawak
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Growing population has driven up the food demand and this eventually exerted pressure on the global food security. According to the United Nations Food and Agriculture Organization (United Nations, 2019), worldwide food production would need to expand for at least 60% by year 2050 in order to fulfil rising global demands due to fast population growth and rising food consumption (United Nations, 2019). Under these circumstances, it is projected that present arable land will provide around 90% of increase in food production (Konuma, 2018). However, there are some challenges in which the capacity for agricultural land growth is getting limited.

Besides, climate change impact as well as others environmental stress has become another stress factor onto agricultural productivity. For instance, in consequence of soil salinity, about 20% cultivated land has turned into uncultivated region (Rasool et al., 2013). Also, the soil salinity brought major shift in plant development and plant metabolism as it affects crops morphology and physiology (Gupta & Huang, 2014). Likewise, flood is another abiotic stress affecting plant productivity. These abiotic pressures are becoming prevalent owing to global climate change. As a result, this wide spectrum of ecological instability has had an impact on crop output.

Vagsholm et al. (2020) stated that, in order to feed 10 billion people by 2050, there should be an effort to make greater use of food currently produced while striking the balance between agricultural sustainability, food security and food safety. The Green Revolution dramatically increased plant productivity and agricultural yields by introducing new high yielding seed types and expand the use of synthetic fertilisers, insecticides, and other

agrochemicals (Kesavan and Swaminathan, 2018). Since then, the global agricultural scene has evolved dramatically.

However, overuse of synthetic agrochemicals for crop productivity enhancement has posed detrimental impacts on sustainable agriculture effort. The extensive application of chemical fertilizers has damaged the biological and physicochemical health of arable soil, resulting in a global decline in agricultural output over the last several decades (Pingali, 2012; Yang and Fang, 2015). Slepetiene et al. (2020) found that prolonged exposure to strong chemicals has altered the soil acidity while Khatoon et al. (2020) stated that excess usage of pesticides has adversely affect the soil fertility, soil microbiota as well as the beneficial natural insect. As a result, the land resources as well as the biological wealth are being at risk while ensuring the effort to meet the growing demand being made.

Meanwhile, in light of COVID-19, the global food chain challenges have become apparent again. These challenges include: (i) the ongoing effects faced from climate change and the need for the food system to be resilient to a variety of extreme weather events; (ii) the necessity to secure long-term productivity development in order to feed a growing global population in a changing environment while lowering greenhouse gas emissions in the sector; and (iii) sustaining biodiversity against the backdrop of agricultural land use change, new variety management, and the introduction of new technologies (OECD, 2020). In this circumstance, Sridhar et al. (2022) stated one of the methods in developing resilient food system would be through sustainable agriculture approach. These can be adopted via various methods such as weed and pest management, urban agriculture, biodynamic farming, permaculture, crop rotation, and polyculture.

Due to environmental and regulatory pressure, it is essential in adopting environmentally friendly measures, such as sustainable use of efficient, safe and beneficial

bacteria. It is a beneficial approach as it provide the potential to fulfil not only the immediate demand but also assure healthy future (Santoyo et al., 2017). This situation has triggered an advancement to discover the purposeful use of Plant Growth Promoting Rhizobacteria (PGPR).

PGPR play a significant role in agriculture sector (Etesami & Maheshwari, 2018). These beneficial rhizobacteria is commonly found in rhizosphere region, a crucial ecologicalhabitat for plant-microbe interactions in the soil (Hayat et al., 2010). It capable in promotingthe plant growth through multiple mechanisms of actions. This included direct mechanism whereby the substances produced was expected to boost nutrient availability and stimulate plant growth. This is also achievable indirectly through the suppression of plant pathogens presence in the rhizosphere of the crop (Ribeiro & Cardoso, 2012). Nevertheless, the efficacy of PGPR varied on the soil ecology, species as well as age of crop (Mohanty et al., 2021).

Fan and Smith (2021) stated there are increasing interest received by PGPR in plant growth promotion and disease protection. Meanwhile, Pieterse et al. (2014) in their study has reported the existence of rhizobacterium-induced systemic resistance (ISR) has been discovered in many plant species such as rice, bean, cucumber, tobacco, tomato, and *Arabidopsis.* These showed there is emerging market for Plant Growth Promoting Rhizobacteria (PGPR) as bioinoculants on current major agricultural crops as well as other common species. Nevertheless, the research on PGPR related to our potential future tree of life, *M. sagu,* Rottb. has been scarce. This has sparked some research questions which being the motivation of this study:

(a) Does the rhizosphere indigenous to *M. sagu,* Rottb. possessed potential PGPR?

(b) Does the rhizobacteria inhabit the rhizosphere of *M. sagu,* Rottb. exhibit plant growth promoting traits?

(c) Does rhizobacteria isolates indigenous to *M. sagu,* Rottb. exhibit multiple PGP traits or at least one PGP traits?

Moreover, as has long been recognised, all soil microorganisms are less capable of surviving and coping with stress. However, those PGPRs indigenous to harsh environments possessed capabilities to thrive under stress and contribute positively towards plant development. This possibly due to well adaptation by those bacteria towards stress conditions. Hence, selecting efficient PGPRs is critical as this will determine the significant inoculation effects upon the plant growth (Rekha et al., 2007). As a result, this steered the current study objective in isolating PGPRs from crops grown in such environment.

Thus, to expand upon previous findings, the current study was conducted in purpose to identify the potential PGPRs indigenous to rhizosphere of sago palm (*M. sagu,* Rottb.) in selected locations of Sarawak State, Malaysia.

1.1 Problem Statement

Due to rising pressure on food production, the demand in food has been impacted in which it need to be increased by 60% by year 2050. However, there is little room to expand agricultural land while climate change is threatening the future agricultural production potential which eventually resulted in stagnation of productivity growth involving major starch crops. This has sparked research interest in identifying alternative food resources which able to be cultivated on underutilized lands while tolerant to stressful environment conditions and produce substantial amount of starch. Against this backdrop, *M. sagu,* Rottb. is one of the crops which fit in these criteria and deemed to help strengthen

the food security especially in Sarawak, Malaysia. Despite the current fertilizer application on *M, sagu* is manageable, potential future abuse of synthetic agrochemicals and chemical fertilizers to boost *M. sagu* yields might occur. Thus, finding alternative such as biofertilizer technology derived from PGPR is beneficial for the sustainable production of *M. sagu.* The presence of the helpful bacteria PGPR in the rhizosphere of sago palms may have helped them adapt to acidic peat soil and oxygen-deficient circumstances, claimed Nemenzo and Rivera (2018).

Moreover, such habitat suitable for the growth of microorganism which sago palms are mutually beneficial from (Gray and Smith, 2005). As a result, focusing on PGPR grown in environments where *M. sagu,* Rottb. had evolved may aid in extending knowledge of potential PGPR that could potentially affect their growth. This potential PGPR then could be harnessed as inoculum for biofertilizer. Nevertheless, there is little research has been done related to PGPR indigenous to rhizosphere of wild grown *M.sagu.* Thus, under these circumstances, the research on PGPR indigenous to rhizosphere of *M. sagu* as potential inoculum for biofertilizer has been placed in this research's objective limelight.

1.2 Objectives

The main objective of this study isto contribute knowledge on PGPR traits associated with underutilized crop of our country, the *M. sagu.* Rottb. Besides, the study has been set to serve these specific objectives:

(i) To isolate diazotrophic PGPR from selected rhizosphere of *M. sagu,* Rottb.

(ii) To identify the PGPR isolates indigenous to rhizosphere soil of *M. sagu,* Rottb.

(iii) To investigate the PGP traits possessed by the PGPR isolates.

(iv) To determine the rank of potential PGPR consortium according to bonitur scale method.

CHAPTER 2

LITERATURE REVIEW

2.1 The Outlook on The Status of Global Food Production

Over the last century, the world population has shown a vivid change in its growing rate. It took hundreds of thousands of years for the world population to reach one billion, and then it surged sevenfold in just another 200 years or so. The world population passed the 7 billion milestone in 2011, and it stands at over 7.6 billion (*World population trends*, 2010). Up to 2019, based on recent data estimated by United Nations (2019) there are 7.7 billion people, yielding approximately one billion inhabitants were added over the last twelve years. It is estimated the world's population will reach approximately 8.5 billion in 2030, increase further to 9.7 billion in 2050 and 11.1 billion by year 2100 (Table 2.1).

Table 2.1: Estimated world Population (Nations, n.d.)

Region	Population (millions)			
	2017	2030	2050	2100
World	7550	8551	9772	11184
Africa	1256	1704	2528	4468
Asia	4504	4947	5257	4780
Europe	742	739	716	653
Latin America and the Caribbean	646	718	780	712
Northern America	361	395	435	499
Oceania	41	48	57	72
Southeast Asia	648	668	727	794

Due to the rapid growing population, the United Nations estimates that by 2050, global food production would have to grow by at least 60% (Konuma, 2018). This scenario is indirectly driving up global food demand and eventually heightening fears that the world's cupboards may run bare in the coming few decades.

Besides, in order to increase global production, there is another concern whereby the world requires more room to expand agricultural land, facing climate change threat towards future agricultural production potential, greater use of chemicals including fertilizers, pesticides and herbicides. Despite the successful history brought by chemical fertilizer in meeting the current demand, it is putting risk on the environment health. This calls to practice a sustainable intensification of agriculture, which offering capability in balancing both

domains of socioeconomic-environmental sector. This was highlighted by FAO (2019) through establishment of some principles which focusing on improvement of food productivity through effective resource utilization and the importance on natural resource conservation.

2.2 Agriculture in Malaysia

The agriculture industry has played a significant part in Malaysia's economic growth. Three National Agricultural Policies have been in place since 1984 to aid in the development of the agricultural industry. The First National Agricultural Policy (1984–1991) emphasised export-oriented growth, whereas the Second National Agricultural Policy (1992–1997) emphasised boosting productivity, efficiency, and competition, as well as expanding acreage and growing the agro-based sector. Both strategies place a premium on the efficient use of local resources, with the goal of increasing agricultural revenue (Murad et al., 2008).

Later, from 1998 to 2010, the Third National Agricultural Policy was created to address the country's difficulties and demands, ensuring enough, safe, highly nutritious, and high-quality food production as well as long-term agricultural growth. The new strategy, in the Fourth National Agricultural policy (2011-2020) is aimed at maintaining national food security while also increasing agricultural earnings. Its goals are to lift farmers, livestock breeders, and fishers out of poverty by focusing on food security, rural development, and boosting domestic investments and international commerce (Dardak, 2015). Through these policies, Malaysia’s effort in aiming the balance of socioeconomic and environmental sector has been well recognized.

2.3 Agrochemicals Practice in Malaysia

As matter of fact, agriculture is a vital business in Malaysia since it promotes food security and boosts rural income. The industrial agriculture coupled with green revolution has resulted in a prominent achievement, but this also observed high trend in the utilization of agrochemicals. Agrochemicals are chemical compounds used in agriculture to improve crop yield and protect crops against pests, insects, weeds, fungus, and other organisms. These include fertilisers, plant-protection chemicals or insecticides, and plant-growth hormones (Mandal et al., 2020). In Malaysia, the agrochemicals market is divided into two categories: food-based crops such as grains, cereals, fruits, vegetables as well as oilseeds and cash crops such as oil palm, rubber or other crops.

The nature of the environment in tropical countries, with its evergreen splendour and warm, humid climate, encourages rapid of undergrowth development and hatching cycles of farm insects. Because many farmers operate on a subsistence and small scale, they cannot afford to conduct netting or organic farming thus alternative practice of synthetic fertilizers agriculture was preferred (Ali & Shaari, 2015). Even the Malaysia agrochemicals market is huge at which almost 75% of the total agricultural land was introduced with pesticides (Sabran & Abas, 2021). Herbicides, insecticides, fungicides, and fertilisers are among frequent agrochemicals used in tropical areas like Malaysia. All of these substances can be sprayed on the plant, sown in the soil, or administered in various ways (Ali & Shaari, 2015).

2.3.1 Deleterious Effects of Synthetic Fertilizers on Pollution

In spite being major player in crop productivity, excessive utilization of synthetic fertilizers led to several issues included water pollution. Plants requirement for N fertilizer usually takes only up to 50% and this resulted to high fractions of applied N potentially leached into groundwater. These was proven through the presence of nitrate in polluted

water. Nitrate is a form of dissolved nitrogen present in groundwater and high concentration of nitrate (50 mg NO_3-/L) present, may lead to gastric cancer, goiter, blue baby syndrome and birth defects (Ward et al., 2018). Figure 1.1 shows the possible side effects through consumption of polluted nitrate water.

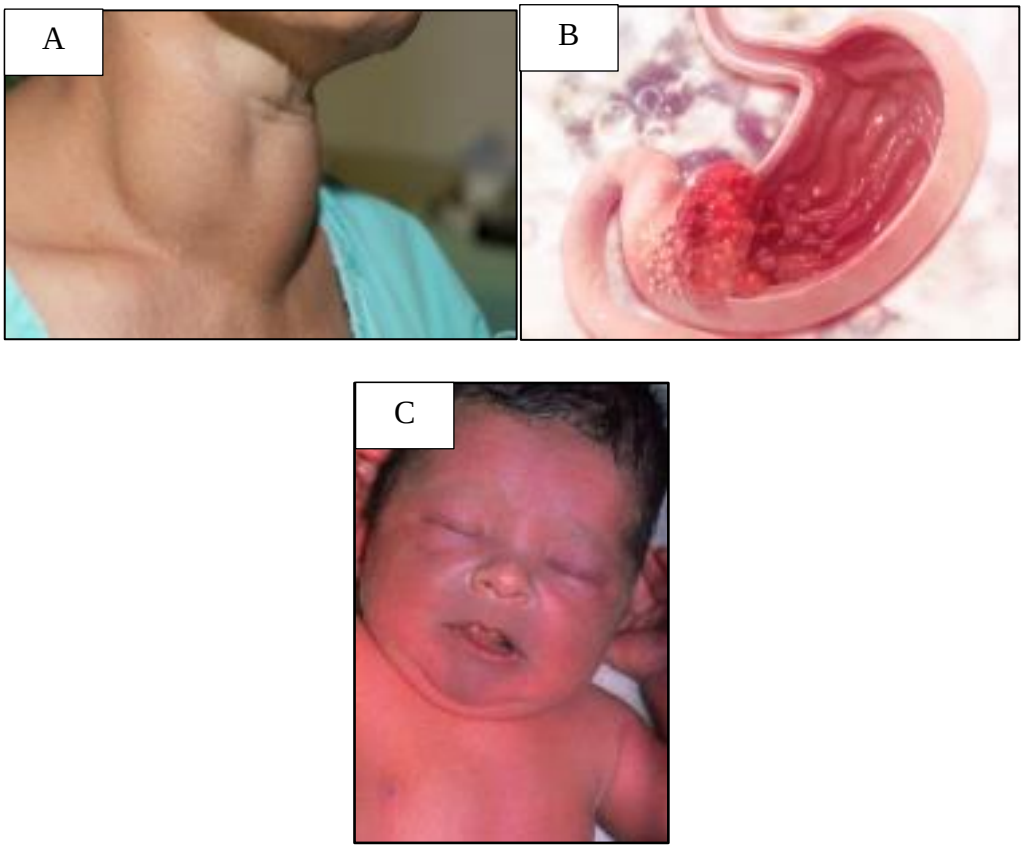


Figure 1.1: The side effects through consumption of polluted nitrate water (Ward et al., 2018), which is (A) gastric cancer, (B) goiter and (C) blue baby syndrome

Besides, excess nitrate poses deleterious effects on aquatic life by water eutrophication. The eutrophication occurred as the environment becomes enriched with nutrients, potentially led to proliferation of aquatic plants and algae. As a result, creating environment which is harmful to aquatic life due to reduction in oxygen supply (Chandini et al., 2019).

Besides, emission of deleterious greenhouse gases due to excessive utilization of chemical fertilizers are one of the main concerns in air pollution. For instance, the conversion of excess nitrogen fertilizer into nitrous oxide is responsible for air pollution. The emission of this gas consequently leads to formation of atmospheric holes which eventually resulted to excessive exposure of ultraviolet radiation towards human and animals (Rütting et al., 2018). Moreover, the global warming potential accounted by nitrous oxide is 310 times more alarming than other greenhouse gas such as carbon dioxide. Apart from that, excessive application of ammonium-based chemical fertilizers might cause acid rain from the chemical reactions. For instance, emitted ammonia eventually deposited and oxidized to nitric acid and sulfuric acid which cause the acid rain. This is damaging towards vegetation, as well as aquatic organisms inhabiting lakes and reservoirs (Sharma, 2017).

Meanwhile, application of chemical fertilizer beyond recommended usage also responsible for the detrimental effects on soil. The toxic metals which are some of the trace elements of fertilizers, get build up and eventually accumulated in harvest crops. These may lead to health problems of consumers coupled with deleterious effect on the soils itself. For instance, accumulation of cadmium and arsenic from Triple superphosphate fertilizers in the plant eventually reach consumers through the food chains. Moreover, this practice could cause soil acidification which reduce humus content responsible for storing nutrients, contributed to emission of greenhouse gases as well as formation of soil crust. This soil acidity will also result in stunted plant growth as the toxic ion concentration accumulated in the soil increase (Ilker et al., 2007).

Therefore, in effort of ensuring sustainable agriculture production while exerting beneficial effects on soil physical conditions, integrated application of nutrient supplicants such as biofertilizer with chemical fertilizer should be an option.

2.4 Application of Organic Fertilizer

Organic fertilizer is a fertilizer comprised of organic sources and have been organically used as fertilizer in agriculture. This including organic compost, poultry droppings, domestic sewage, and manures. Most of the manures' sources would come from the animal wastes. These could be the mixtures of animal faeces, as well as other materials associated with the animal production. For instance, the bedding materials, waste feed, soil and any physical or chemical amendment which commonly used along the manure handling and storage (Sims et al., 2005).

Despite of beneficial amendment brought by the manures, there was a concern on the long-term effects of application of manures. This is so because, prior studies have found that antibiotic resistance genes (ARGs) have been shown to enter the environment through the discharge of animal dung, contaminating soil, water, and crops (He et al., 2016).

Heuer et al. (2011) in their study stated that the application of manures markedly increased the ARG abundance which eventually align with the increased population of antibiotic resistance bacteria (ARB). This was due to the elevation of ARG levels exist or through the introduction of novel types of ARGs carried along by the manures (Udikovic-Kolic et al., 2014).

Besides, according to Xie et al. (2018), the horizontal gene transfer was able to promote the dissemination of ARGs among microbial community via the mobile genetic elements. Furthermore, through horizontal gene mechanisms, previous studies assumed that there is possibility of transfer environment ARGs into food chain, posing great risk to human health (Berendonk et al., 2015; Wang et al., 2015).

Manure application indeed has been proven to be an effective approach with respect to increase crop yield, maintaining soil organic carbon as well as the increase of carbon sequestration (Gai et al., 2018). Nonetheless, considering the manures as reservoir of ARGs and ARBs, there is still potential harm of the effect of manure fertilization of ARGs' spread in soils (Wang et al., 2018).

2.5 Application of Biofertilizer

Biofertilizer is a type of fertilizer which prepared or derived from living microorganisms, whereby it is colonizing rhizosphere or interior parts of plants as its being applied on the seeds or the crops itself. It is commonly offered as a growth-promoting inoculation liquid or as a biological insecticide, to improve plant development.

Biofertilizer production and utilization have increased dramatically during the last few decades. The worldwide biofertilizers industry has gained considerable attraction due to the health deterioration of cultivable land while the need to meet the growing demand for agricultural goods among the world's population must be fulfilled. The global market for biofertilizers is a fraction of the market for synthetic agrochemicals (Timmusk et al., 2017) which dominated by *Rhizobium* spp., *Azotobacter* spp., and *Azospirillum* spp. strain as the nitrogen fixers biofertilizers. Although these nitrogen-fixing biofertilizers are most used to produce pulses and other leguminous crops, they are also utilised to grow a variety of cereals and cash crops (Ferguson et al., 2019).

Biofertilizers are usually sold as growth promoting inoculation liquid or carrier-based fertilizers. The present biofertilizers available are nitrogen fixing, phosphate solubilizing and mobilizing as well as potassium solubilizing and mobilizing biofertilizers. Meanwhile, the modes of application are via seed treatment, foliar treatment, root dipping,

seedling root treatment as well as soil treatment. In China, the usage of biofertilizers has been used in 30 provinces for 55 products and producing minimum economic benefits 59 million dollar per annum (Haghighi et al., 2011). This eventually decreasing the usage of chemical fertilizers and indirectly making the detrimental effects tractable.

In Malaysia, the history of biofertilizer has initiated with industrial scale production of microbial inoculants, in late 1940s. The trend picking up in 1970s leaded by *Bradyrhizobium* inoculation on legumes crops in the inter rows of young rubber trees in large plantation by Malaysia Rubber Board (MRB). Meanwhile, in year 1980s, the research on the contribution of *Mycorrhiza* as well as nitrogen contribution by *Azospirillum* to oil palm seedling has been conducted (Bang et al., 2018).

Biofertilizers have historically been utilised in Malaysia by the pioneers such as SOField Agrobio Resources in Sarawak as well as BIO AG in Pahang. The application has been discovered to support the farmers in raising yields while cutting operational costs and carbon emissions. Biofertilizers are particularly useful for leguminous cover crops like peanuts. Rather than improving soil fertility or crop yields, they were used as defence against water losses, especially during the dry season as well as against erosion of soil (Wei, 2021).

According to Wei (2021), biofertilizers should be marketed in industrial scale as an alternative to the country's extensive use of chemical fertilisers. However, this are not intended for replacement means, yet as a preferred fertilizers' choice for the growth of the agricultural sector while supporting country's efforts in practicing green economy. It is possible that using biofertilizers all at once will cause the application of microbial killers and chemical fertilisers obsolete. Furthermore, Hii (2020) advocated for the use of biofertilizers as an effort to minimise emissions from Malaysian agricultural goods. Thus,

Wei (2021) proposed that biofertilizers should be incorporated into future National Agricultural Policy, specifically for the efforts to sustain the oil palm or agriculture in general. This will be coupled along with the goal of making it more climate-change resilient and as a contribution in the reduction of carbon emissions.

2.6 Potential Application of Plant Growth Promoting Rhizobacteria

Rhizosphere region is governed by the complex dynamic ecological interactions between plants and microbes (Verma at al., 2018). The rhizosphere is commonly described as soil area surrounding the plant roots and regarded as "hot spot" for microbial colonization and activity (Prashar et al., 2013). The root tissue area (endorhizosphere), root surface (rhizoplane) and rhizosphere soils which directly surrounding host roots (exorhizosphere) made up the rhizosphere region. This region harbours a multitude of microorganisms which interact directly and indirectly with the plants.

Though the plant-microbes interaction relationship can be either beneficial or detrimental (Nadarajah & Abdul Rahman, 2021), there are plenty interactions which beneficial towards the plants (Schirawski & Perlin, 2018). One of such beneficial microbes is plant growth promoting rhizobacteria (PGPR) (Singh, 2018).

PGPR is a naturally occurring soil bacterium that has the capacity to stimulate plant development (Kloepper, 1978). In fact, there is ample evidence by Smith and Read (1997) that the factor affecting the plant growth in nutrient poor environments is linked to the mutualistic associations with the PGPR.

Several PGPR strains found to be possessed capability in acting as biofertilizer and biopesticide which aided the plant growth. For instance, strains of *Burkholderia cepacia* as biofertilizer was integrated with the application of N and P fertilizer. The integrated

approach found to promote better maize growth as the root and shoot was found to increase in length (Sandanakirouchenane et al., 2017).

Agriculturists prefer PGPR over chemical fertilizer as root promoting hormone and biocontrol factors. This is due to the high potential of plant growth promoting bacteria in producing plant hormones while decreasing the damage brought by plant pathogen factor, yet, environmentally friendly.

Moreover, PGPR-based fertilizer possesses a characteristic required for increasing the rate or chances of success in rhizoremediation. Rhizoremediation is a process whereby the microorganisms degrading the soil contaminants present in the rhizosphere (Correa-García, 2018). These soil contaminants such as heavy metals have been demonstrated to have a major influence on soil microbial communities in a variety of ways, including a decrease in total microbial biomass, a reduction in the numbers of particular populations, and a shift in the microbial community structure (Ilyas & Bano, 2012). Through the utilization of PGPR, these rhizoremediation success rate is increase as it is aiding in solubilization of heavy metals.

According to Ahemad and Kibret, (2014) the PGPR could directly and indirectly influence the plant growth promotion. For instance, the PGPR directly influence the plant growth promotion via fixation of atmospheric nitrogen, minerals solubilizations, production of siderophores which solubilize and sequester iron as well as production of phytohormones that improve the plant development (Olanrewaju et al., 2017). This was aligned with Manoharachary and Mukerji (2006) study, whereby, PGPR able to increase the ability of plants in acquiring the nutrients from soil by either increasing the extent of root system or solubilizing macronutrients such as phosphorus or sulphur.

On the other hands, indirect growth promotion occurred as PGPR promotes the plant development in constrained settings. For instance, by producing antagonistic substances or through the induction of pathogen resistance (Kumari & Mallick, 2017). Thus, PGPRs are classified into three different forms (biofertilizer, phytostimulator, biopesticide) depending on their mechanism of action.

2.7 Plant Growth Promoting Rhizobacteria

Biofertilizer is one of the broadest categories of PGPRs. In this category, PGPRs aided in supplying necessary plant nutrients, either directly or indirectly. For instance, through nitrogen fixation, ammonia production as well as phosphate solubilization.

2.7.1 Nitrogen Fixation

Nitrogen and phosphorus are among of essential macronutrients needed in significant quantities by the plants. However, these nutrients availability is rather limited in soils, and it was found that the bacterial endophytes might aid the plants hosts in acquiring these nutrients. This was done so by converting nitrogen into useful form of ammonia by the nitrogen fixing microorganisms through biological nitrogen fixation process (Ahemad & Kibret, 2014).

Taurian et al. (2012) reviewed that diazotrophic symbionts often provide a large nitrogen input to their plant hosts, particularly in nitrogen-deficient soils. Biological N2 fixation by rhizobacteria has also been discovered to integrate significant quantities of nitrogen into various important agronomical plants such as rice, sugarcane as well as maize. These bacteria invaded root hairs and formed nodules, where they developed to fix atmospheric nitrogen into a usable form for plants. This is a symbiotic connection because the plant benefits from the nitrogen supply that is gained in return for plant carbon sources.

The biological nitrogen fixation comprised of two types, in which symbiotic nitrogen fixation and non-symbiotic nitrogen fixation. The symbiotic nitrogen fixation involved members under Family Rhizobiaceae (Dinnage et al., 2019), while various genera such as *Pseudomonas, Diazotrophicus, Arthrobacter, Acetobacter, Bacillus, Clostridium* as well as *Azotobacter* are commonly associated with non-symbiotic nitrogen fixation (Martins et al., 2019). These PGPR will eventually fix the large portion of the elemental nitrogen which entered the soil under normal settings (Ji et al., 2019). These beneficial plant-microbe interactions found to be a significant criterion in the biological nitrogen fixation which deemed to be beneficial for the development of organic fertilizers (Kuypers et al., 2018).

2.7.2 Ammonia Production

One of the fundamental properties associated to plant growth promotion is PGPR's ability to produce ammonia. In general, ammonia has been demonstrated to provide nitrogen to their host plants, promoting root and shoot elongation as well as biomass (Marques et al., 2010).

Besides, the ammonia emission has been suggested to be able to influence microbial diversity and plant-microbe interactions. In nature, high-protein resource decomposition (carcasses, whey and manure) is thought to be coupled with the emission of ammonia, which alters pH of the rhizosphere and therefore impacts organismal diversity and plant-microbe interactions. Hence, bacterial ammonia emission may be more important than previously assumed for plant colonisation and growth development (Weise et al., 2013).

There are several studies that have reported on ammonia producer by PGPR. Ammonia production was observed in 95% of isolates isolated from the rhizosphere of rice, mangroves, and soils affected by effluent (Samuel and Muthukkaruppan, 2011, Joseph et al., 2007). Besides, the ammonia-producing bacteria *B. subtilis* and *Pseudomonas fluorescens*

were shown to dramatically boost the biomass of medicinal and fragrant plants such as geranium (Mishra et al., 2019).

2.7.3 Phosphate Solubilization

Phosphorus is a crucial limiting nutrient for plants, and it may be found in both organic and inorganic forms (Khan et al., 2010). Commonly, most of the phosphates applied on agricultural soils undergone rapid immobilization. As a result, there is large reservoir of phosphate present. However, this phosphate precipitated into insoluble forms thus inaccessible to the plants. In acidic soils, most of the insoluble forms of phosphate exist as aluminium and iron phosphates, while it presents as calcium phosphates in alkaline soils (Rani & Goel, 2012).

PGPR, on the other hand, exerts phosphate solubilizing traits which is one of the common mechanisms of action to increase the phosphate availability of the plant (Taurian et al., 2010). These was achievable by lowering soil pH, chelation as well as mineralization. In order to lower the pH of the soils, PGPR secreting organic acids acted as good chelator agents. These acids chelating the cation which bound to phosphate with their hydroxyl and carboxyl groups, thus making the phosphate available to plants. Besides, these organic acids are also capable of forming soluble metal ion complexes, at which the metal ion was primarily co-complexed with insoluble phosphorus. As a result, the formation of this complexes allowing the phosphorus moiety to be released, improving the P availability for the plants. Some of these beneficial bacteria has been identified as members *of Kluyvera, Burkhoderia, Pseudomonas, Pantoea, Klebsiella, Streptomyces, Enterobacter* and *Bacillus* genera (Oliveira et al., 2021; Hariprasad & Niranjana, 2009).

Mineralization, which includes the breakdown of complex organic P molecules into utilisable forms taken up by plants, is another process responsible for P-solubilization

(Prasad et al., 2019). The presence of enzymes, especially phosphatases and phytases released by soil bacteria, catalyses the mineralization process (Wu et al., 2019). Phosphatases are extracellular enzymes that take organic forms of P as a substrate and convert them to inorganic forms (Ghosh et al., 2018). Phytases, another key class of enzymes, are involved in the release of phosphate from phytic acid, which is one of the principal components of organic phosphorus in the soil (Puppala et al., 2019). However, it was feared that the lack of consistency in the effects of these microbes in mobilising phosphate in field circumstances would be a barrier for their use. This is most likely due to competition with native rhizobacteria as well as environmental variables that affect the PGPR's activity. Phosphate can be easily taken up by plants once the organic and inorganic forms of phosphate have been converted into simpler forms. Given the importance of these phosphate solubilizing enzymes, it would be ideal to create bacterial inoculants that can manufacture these enzymes, since these enzymes might be a source of considerable practical benefit in sustainable agriculture (Rathinasabapathi et al., 2018).

2.8 Plant Growth Promoting Rhizobacteria as Phytostimulators

PGPR also has been found to act as phytostimulators, which referred to breakdown activity of organic contaminants in soil. The phytostimulation activity of PGPR is usually achieved by secreting growth hormones, which directly promote the plant growth. These phytohormones play a fundamental role in regulating plant growth and development as it is a signal molecule which acted as the chemical messengers in the plant.

2.8.1 Indole Acetic Acid (IAA) Production

Auxins are one of the phytohormones which play essential role in plant development. One of the most essential plant auxins secreted for significant growth of plant is IAA. IAA metabolite is derived from tryptophan by various pathway in plants and bacteria, either via

Tryptophan-dependent or Tryptophan-independent pathway. In both plants and microorganisms, L-tryptophan is an important precursor for IAA synthesis. In the microbial production of IAA, four tryptophan-dependent pathways play a major role; the indole-3-acetamide pathway, the indole-3-pyruvic acid pathway, the indole-3-acetonitrile pathway as well as the indole-3-tryptamine pathway (Imada et al., 2017).

In PGPR, the major route for IAA production is through the indole-3-pyruvic acid pathway. The conversion of tryptophan to indole-3-pyruvate, which is done by aminotransferases, is the first step in this process. In the next step, flavin-containing monooxygenases convert indole-3-pyruvate to IAA (Matthes et al., 2019). This major auxin biosynthesis pathway is the two-way conversion of tryptophan to IAA, which is involved in several plant developmental activities.

IAA found to be responsible in promoting division, enlargement, initiation of root growth as well as in increasing the number of root hairs responsible in nutrient uptake of the plants. Besides, auxins trigger signalling pathways for root epidermal hair cells, which leads to the growth of root hairs (Nascimento et al., 2020). Based on transcriptome sequencing data analysis, it has been found that auxins positively regulate 90% of genes associated to root development (Zhang et al., 2018).

It also has been found that auxin synthesis by plant growth-promoting rhizobacteria can also control and increase the antioxidant system in economically significant crops like wheat (Acuna et al., 2019). *Rhizobium, Pantoea, Agrobacterium, Pseudomonas*, and *Bacillus* are among the species that have been identified as PGPR synthesizing auxin (Tabassum et al., 2017).

2.9 Plant Growth Promoting Rhizobacteria as Biopesticides

By a variety of methods, beneficial PGPR also capable in inducing disease suppression in the plants. These includes through the production of siderophore.

2.9.1 Siderophore Production

PGPR produce siderophore in the means of inducing disease suppression of the plants. The siderophore-producing PGPR prevents harmful microorganism development by sequestering iron in the rhizosphere. The siderophore served as defence against root-invading parasites as it deprived the iron availability for the pathogens. This eventually resulted in the colonization of PGPR over pathogens at the plant roots, secreting array of antifungal metabolites which indirectly promote the plant growth (Haas & Défago, 2005). Thus, the acquisition of iron through siderophore synthesis is critical in determining the capability of bacteria when competing for iron with other microbes (Rani & Goel, 2012).

Besides, due to prolong co-evolvement between microbial strains and host plants, these PGPR are likely to benefiting the plants with more than one benefit such as by promoting plant growth along with pathogen control. Few studies have reported the successful deployment of PGPR as biocontrol. For instance, *Bacillus subtilis* were found to have significant suppression effect upon pathogen *Phytophtora capsica,* when the strains were applied on the host plants studied (Islam et al., 2016). This is corresponded with Punja et al. (2016) study in which the biocontrol effects by *B. subtilis* were also observed as the strains suppressed the *Penicilium* sp. and *Rhizopus stolonifera* effects on the fruits harvested.

Apart serving as disease suppressor, siderophore served as scavenging agents in supplying source of iron in plants. Iron is one of essential component which involves in biosynthetic pathways as well as in the formation of chlorophyll. However, it is relatively

insoluble in soils and exist as ferric (Fe3+) ion in common aerated soils, which is easily precipitated in iron-oxide forms. Meanwhile, the plants roots preferred to absorb iron from ferrous (Fe2+ ion), which is a reduced form of iron (Ems & Huecker, 2018).

In response of iron deficiency environments, PGPR, on the other hand, possessed a beneficial trait in chelating iron via siderophores. Siderophores which secreted by PGPR, scavenge the iron present in extracellular environment and form siderophore-iron complexes which will be transported into the cell via receptors found in the membrane (Neilands, 1981). As the iron solubility is limited at higher pH, these circumstances are mostly occurred in soils with neutral to alkaline pH.

2.10 Status of PGPR Research

Despite the fact that biofertilizer application has a history, it is not well established. As a result, there is a lot of room for innovation and improvisation of our country's abundant natural resources in producing a high-quality, long-lasting and effective biofertilizers. Devliegher et al. (1995) stated that if the environment in which PGPRs are injected, allows these bacteria to survive and develop, only then plant inoculation with these bacteria may be helpful. In other words, the capacity of the PGPRs to combat against well-adapted native microbes to the local environment are also crucial for their survival in the environment. Thus, choosing the most efficient root colonizing PGPRs strains is a necessity for achieving powerful PGPRs and reaping large inoculation effects (Rekha et al., 2007).

Besides, all soil microorganisms, as has long been known, are less capable to live and withstand stress. The key restriction of effective rhizobia-legume symbiotic interaction in Malaysia, according to Shamsudin et al. (1994), was soil acidity. Due to soil low pH, phosphorus and calcium concentrations while high in aluminium concentration, has affect the rhizobia growth as well as the legumes, hence affecting the symbiosis.

Nevertheless, Simons et al. (1996) found that the efficient colonisers isolated from stressed environments also possessed a higher capability to colonise plant roots. They found that a combination of bacteria that are injected onto the seedlings are resulted to plant growth in a gnotobiotic sand environment. Kuiper et al. (2001) in later years found that iterating this strategy boosts the bacteria ability to colonise root tips.

This could be due to the fact that these bacteria have previously been well acclimated to such stressful settings, hence only PGPRs isolated from stressful environments may live under stress and contribute to plant development (Shrivastava & Kumar, 2015). Salinity, for instance, has a detrimental impact on soil microbiological activity due to its high osmotic strength. Furthermore, in high salinity settings environment, salt induced toxicity can inhibit microbial development (Egamberdieva & Kucharova, 2009). Against this backdrop, salt tolerant-PGPRs can thrive in such extreme conditions. For instance, the PGPRs indigenous to wheat grown in salinized soil were found to promote plant development despite the salinized soil environment settings (Egamberdieva et al., 2008). As a result, there is high possibility that PGPRs isolated from stressful environments may enhance plant development in stress-affected conditions via direct or indirect routes, making them excellent as bio-inoculants.

This was supported by Glick (2010) and Grover et al. (2011) who mentioned that isolating native stress resistant PGPRs from stress impacted soils or plants cultivated in such soils, is one of beneficial techniques for obtaining effective strains that might be useful as bio-inoculants. In Malaysia, one of indigenous crop that hold these criteria would be *M. sagu,* Rottb.

2.11 *Metroxylon sagu,* Rottb.

The sustainable development agenda is jeopardised by an over-reliance on current agricultural commodities (FAO, 2019). According to Konuma (2018) the declining growth rate in major cereal crops such as wheat and rice has been observed and this could lead to stagnation productivity of growth. This might be owing to a reliance on a restricted number of crops, as well as a response to climate change which eventually act as stress factor.

This eventually piqued scientists' interest in developing novel food sources that can be grown in underutilised places with minimum or without competition from traditional food crops, are resistant to harsh environmental conditions, and deliver a considerable amount of food or starch. The United Nations (2019) acknowledged that the neglected and underused species possessed a role in the fight towards hunger, as well as being a major resource for smallholder farmers' agriculture and rural development. Furthermore, many overlooked on the underused species contribution to the preservation of cultural variety. They fill significant gaps in the landscape, preserving traditional landscapes while adapting to the unsafe and vulnerable situations that rural populations face.

In light of this, the sago palm (*M. sagu*, Rottb.) has been identified as one of the most promising yet underused traditional food crops (Konuma, 2018). It was found to adapt well with peat swamps and underutilized lands where other food crops cannot be grown economically, instead. As in contrast to other carbohydrate-producing crops, it is the most prolific (Bintoro et al., 2018). In which, it has been found to be capable in producing high starch yields as much as 150 kg to 300 kg of dry starch per plant harvested.

The sago industry in Malaysia, particularly in Sarawak, has grown to become a stable source of export earnings. Sarawak has been the world's single exporter of sago starch, despite not being the world's largest producer (Ming et al., 2018). After Indonesia and Papua

New Guinea, Malaysia is the world's third largest sago producer (Mohamad Naim et al., 2016). According to the most recent statistics from the Department of Agriculture Sarawak in Year 2012, all four districts in Mukah division, namely Mukah, Dalat, Matu, and Daro, farmed roughly 6,472 hectares, 28,196 hectares, 4,520 hectares, and 3,149 hectares of sago, respectively.

Meanwhile, the current fertilizer application for sago palm was usually done via foliar spray. At which a knapsack sprayer with a recommended capacity of 16 L was used to apply nitrogen, phosphorus, potassium (NPK) fertilizer. This application was done once every three months and the production of one sago sucker costs 1.1844E-03 L of NPK fertilizer (Sulaiman et al., 2021). Despite the application of fertilizer was manageable at current time, the needs to find alternative approach to prevent excessive application of chemical fertilizer in future is much needed. Hence, finding alternative such as biofertilizer technology which derived from PGPR, is beneficial for sustainable agricultural production.

M. sagu, Rottb. grows well in underutilized area yet slow in growth whereby it consumed 8 -9 years to reach maturity stage prior harvesting. It grows in marshy areas with standing water that is brown and somewhat acidic, yet the microorganisms that aid in the growth of sago palms thrive in such an environment. According to Nemenzo and Rivera (2018), the presence of the beneficial bacteria PGPR in the rhizosphere of sago palms might aided them in adapting to acidic peat soil and oxygen-deficient conditions. Hence, targeting PGPR grown in such environment where *M. sagu,* Rottb. had evolved might help in extending more information on potential PGPR which possibly influence their growth.

Moreover, the rhizosphere zone was found to be rich in nutrients due to the accumulation of variety plant exudates, such as amino acids and sugar (Gray & Smith, 2005).

This might contribute to the favourable condition for PGPR growth, of which *M. sagu,* Rottb. are mutually beneficial from. It was also found that the rhizobacteria inhabit the rhizosphere region of *M. sagu,* Rottb. are generally 10 to 100 times higher than other parts (Weller & Thomashow, 1994).

Previous research by Novero and Labrador (2014), has successfully identified beneficial microbial endophytes associated with sago palm cultivated in tissue culture. Besides, another study also identified the potential PGPR from acclimatized sago palm suckers, which cultivated in garden soil (Nemenzo & Rivera, 2018). Both of this research was working with PGPR associated with cultivated *M. sagu,* Rottb. instead of those grown in vivo, in stress-prone ecologically settings. Thus, in order to contribute additional knowledge beneficial for bioinoculant industry, as well as to contribute for narrowing the gap of PGPR study, the study on the PGPR indigenous to *M. sagu,* Rottb. rhizosphere soil was conducted.

2.12 $(GTG)_5$ PCR Fingerprinting

Repetitive element polymerase chain reaction (rep-PCR) is one of molecular tools at a great use in biodiversity study of phosphate solubilizing bacteria, at an intraspecific level (Rivas et al., 2006). Direct electrophoresis of amplified fragments, RAPD, BOX PCR as well as (GTG)5 are among the molecular techniques in rep-PCR. Among the techniques listed, (GTG)5 has been proven to possess the highest discriminating power (Mohapatra et al., 2007; Gevers et al., 2001). Further, it has effectively screened large number of bacterial strains, hence make it as an effective tool for identification and intraspecies identification of bacterial genomes.

Apart of giving a high discriminatory power in comparison to other techniques, (GTG)5 PCR is a reliable tool in classifying a broad range of Gram-negative and several

Gram-positive bacteria. Besides, it is a low-cost PCR based technique as it offers identification possibilities based on DNA fragment size instead of the DNA sequence, which eventually reduce the cost in acquiring sophisticated laboratory materials (Braem et al., 2011). Furthermore, this approach also has been proven to be suitable for high-throughput strains (Versalovic et al., 1991; Olive & Bean, 1999; Gevers et al., 2001).

2.13 16S rDNA for Identification of Rhizosphere Microorganisms

In the microbial ecology of bacteria and archaea, 16S rDNA gene sequencing has been widely employed as a phylogenetic marker and considered as a beneficial tool in population fingerprinting (Kim et al., 2014*)*. This is due to its reliability in identifying bacteria at genus as well as at its species level. Furthermore, even within closely related taxonomic groupings, 16S rDNA sequencing has revealed significant diversity (Wang et al., 2015).

The 16S rDNA approach relies on the 16S rDNA gene as the genetic marker to identify bacterial taxonomy and phylogeny. This was made feasible since the genetic marker utilised for bacterium identification retained hypervariable areas. The selection of these hypervariable regions as well as the amplicon primer design are crucial factors in 16S rDNA sequencing as it contributes to the results obtained.

Numerous of studies have employed 16S rDNA sequencing to identify the PGPR. For example, Ogut et al. (2010) identified their phosphate solubilizing bacteria as *Acinetobacter, Pseudomonas, Enterobacter, Enterococcus, Pantoea* and *Bacillus* genera, by analysing the 16S rDNA gene sequence data. Besides, Alsohim (2020) has also identified PGPR isolates using 16S rDNA and the most predominant genera of the bacterial isolates were *Pseudomonas* and *Bacillus.* Therefore, genotypic analysis is a reliable, useful approach in identifying bacteria species.

CHAPTER 3

METHODOLOGY

3.1 Sampling Sites

Sampling of rhizospheric soil samples of *Metroxylon sagu,* Rottb. were carried out from Southern and Central regions of Sarawak, as shown in Appendix 1 (page 117). The rhizosphere soil samples were isolated from cultivated sago palms in CRAUN Research, Kuching (1°33'11.1960"N, 110°20'42.1152"E) and Sago Research Plot Kuching (1°24'05.9"N, 111°20'16.7"E). Another rhizospheric soil samples were collected from wild grown sago palms in Dalat (2°25'39.7164"N 112°9'17.3844"E). At which it was specifically collected from Sungai Nunau (S), Sungai Ugui (SU), Intermediate of Sungai Taap and Sungai Petah (STSP) and Sungai Tabo (ST).

3.2 Soil Samples Collection

The soil sampling was done in April 2018 - September 2019 with three visits to the sampling site. The rhizospheric soil samples of *Metroxylon sagu,* Rottb. were collected from vicinity area of the plant, at the depth of 0 - 15cm. The samples were collected at least from four different points around single *Metroxylon sagu,* Rottb. tree. These samples were pooled together afterwards, which then considered as one composite sample. The samples were placed in zipped-lock plastic bags and kept at 4-8 ℃ subsequent arrived at Bacteriology Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS), before furthering next step.

3.3 Isolation of Plant Growth Promoting Rhizobacteria (PGPR)

Considering various activities by PGPR, the study was initiated by preliminary screening of PGP ability in nitrogen fixation activity. This was determined qualitatively by Wilson and Knight (1952) method and the potential growth of isolates was compared with the bacterial strain *Pseudomonas aeruginosa*, acted as the positive control while blank medium was used as negative control. Otherwise mentioned in the rest of the thesis, the experiments were conducted in the same condition as mentioned priorly. The study was done under culture-dependent manner, whereas the standard microbiological procedure of serial dilution method with modification (Kannan et al., 2018), was used for the isolation of rhizobacteria. About 10 g of rhizospheric soil samples were suspended in 90 mL of Phosphate Buffered Saline (PBS) solution (Amresco, USA). The suspension was shaken at 120 rotation per minutes (rpm) for 30 minutes, on an orbital shaker (New Brunswick Scientific, USA). After 30 minutes of sedimentation, about 1 mL of soil suspensions were mixed in another 9 mL PBS solution. In the same manner, these soil suspensions then were serially diluted, agitated at maximum speed by vortex mixer (Lab Depot, USA). About 100 µL aliquots of each dilution was evenly spread on Burks agar (HiMedia, India) and incubated at 27 ± 2 ºC for 24 – 48 hours. After incubation, the bacterial colonies were morphologically characterized, and colonies were randomly picked. Then, the code was assigned for each isolates depicted where the isolates were isolated from (Table 3.1).

Table 3.1: Code assigned for each isolates

Code	Source of soil samples
S	Sungai Nunau
SU	Sungai Ugui
STSP	Intermediate of Sungai Taap and Sungai Petah
ST	Sungai Tabo

The selection was also made based on their growth rate on the medium, at which the slow was discarded. The microbial count was performed and expressed as colony-forming unit (CFU) per gram of soil. An independent t-test was performed subsequently, to determine the difference in the mean of microbial population isolated.

3.4 Purification and Maintenance of Cultures

The bacterial colonies were purified onto nitrogen free medium Burks agar by streak plate method. The plates then were incubated for 7 days at 27 ± 2 ºC. These purified colonies were maintained according to Dexter (1955) method, with slight modification. The isolates were preserved in 15% (v/v) glycerol stock, at -20 ºC instead of -70 °C. This served as the stock cultures, for future use. Meanwhile, the working cultures were prepared by subculturing the purified colonies onto respective slant medium, kept at 4 ºC of refrigerator. The working cultures then were weekly sub-cultured onto new respective slant medium to maintain the cultures.

3.5 Characterization of Diazotrophic Bacteria

The morphology of the rhizobacterial colonies were characterized with a reference to Bergey's Manual of Systematic Bacteriology (1984). The distinct morphologies of the isolates based on its shape, size, opacity, elevation and margin was noted accordingly (Bergey et al., 1984). The codes then assigned for each isolate based on the location where its being isolated from. For instance, an isolate from Sungai Ugui was denoted as SUA. The SU indicated the location of isolates were isolated from, which is Sungai Ugui. While "A" represented the first distinct morphology was isolated from that very soil sample.

3.6 Taxonomic Identification of Plant Growth Promoting Rhizobacteria

3.6.1 DNA Extraction

Genomic DNA was extracted based on Soumet et al. (1994) with some modifications. About 2 mL of overnight rhizobacterial culture was centrifuged at 10,000 rpm for about 5 minutes. This step was repeated twice, and the supernatant was discarded. The pellet then was suspended with sterile distilled water before heated in dry bath heat block (Benchmark Scientific, USA) for 10 minutes at 100 ºC. Immediately, upon 5 minutes, the mixture was placed on ice and centrifuged for 10 minutes at 10,000 rpm. The supernatant was stored at -20 ºC for future use.

3.6.2 $(GTG)_5$ PCR

The genetic diversity among the bacterial isolates was performed by means of repetitive extragenic palindromic PCR (rep-PCR) analysis, to discriminate the same microbial strains profile (Ishii & Sadowsky, 2009). The rep-PCR was performed using (GTG)5 PCR analysis according to Kathleen et al. (2014), with slight modifications. The

PCR reaction was carried out in 25 µL of PCR mixture containing components as summarized in Table 3.2.

Table 3.2: Components of PCR mixture for (GTG)5 PCR analysis

Components	Concentration	Volume per reaction (µL)
Green Taq Buffer	5X	5
Magnesium chloride ($MgCl_2$)	25mM	3
Deoxyribonucleotide phosphate (dNTP)	10mM	2.5
$(GTG)_5$ primer (5'-GTGGTGGTGGTGGTG-3')	25mM	1
Sterile distilled water (dH_2O)	-	8
Go Taq Polymerase	5U	0.5
DNA Template	(20-30mg)	5

The $(GTG)_5$ primer used was purchased from Integrated DNA Technologies (USA). The amplification then was performed by using thermocycler (SensQuest LabCycler, Germany), following the programmed conditions as tabulated in Table 3.3.

Table 3.3: $(GTG)_5$ PCR conditions

Conditions	Temperature (°C)	Time(min)	Cycle
Pre-denaturation	95	7	1
Denaturation	95	1	30
Annealing	50	1	30
Elongation	72	1	30
Extension	72	5	1

The $(GTG)_5$ PCR amplification products were then subjected to gel electrophoresis to separate DNA fragments isolates, ranging in size.

3.6.3 Gel Electrophoresis

A 1.2% (w/v) agarose gel, which was pre stained with Ethidium Bromide (EtBr) (Promega, USA) was used to electrophorese 5 µL amplified PCR products. The gels were run in 1X Tris-Borate-EDTA (TBE) Buffer, at 80 V, 200 mA for 105 minutes. The gel then was visualized under UV transilluminator (Maestrogen, Taiwan). The size of amplicons was determined in which a comparison was made with the concurrently run DNA molecular 1 kb size marker (Thermo Fisher Scientific, USA). The comparative analysis of the fingerprints was performed by GelJ_v2.0 software and the dendrogram was constructed.

3.6.4 16S rDNA Sequencing

Based on the constructed dendrogram, the unique rhizobacterial isolates were chosen at its 60% of similarity. These selected isolates were further identified by using 16S rDNA sequencing identification method (Hutter et al., 2003). The universal primers, 27F forward

primer (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R reverse primer (5'-GWATTACCGCGGCKGCTG-3') was used in this study. A total of 25 µL of PCR reaction mixture was prepared accordingly as assorted in Table 3.4.

Table 3.4: PCR reaction mixture for 16S rDNA Sequencing

Components	Concentration	Volume per reaction (µL)
Green Taq Buffer	5X	6
Magnesium chloride ($MgCl_2$)	25mM	3
Deoxyribonucleotide phosphate (dNTP)	10mM	0.6
27F primer (5'-AGAGTTTGATCMTGGCTCAG-3')	25mM	1.5
519 R primer (5'-GWATTACCGCGGCKGCTG-3')	25mM	1.5
Sterile distilled water (dH_2O)	-	15.66
Go Taq Polymerase	5U	0.23
DNA Template	(20-30mg)	1.5

The PCR reaction mixture then were subjected to amplification. The amplifications were run by using thermocycler (SensoQuest, Germany), under conditions as tabulated in Table 3.5.

Table 3.5: 16S rDNA PCR amplification condition (Kathleen et al., 2014)

Conditions	Temperature (°C)	Time(min)	Cycle
Pre-denaturation	95	10	1
Denaturation	94	0.5	26
Annealing	55	1	26
Elongation	72	1.5	26
Extension	72	10	1

The PCR products then were electrophoresed in 1.5% (w/v) agarose gel, which was pre- stained with Ethidium Bromide (EtBr), beforehand. The agarose gel was electrophoresed in Tris-Borate-EDTA (TBE) Buffer at 90 V and 400 mA for 75 minutes. The size of amplicons then was determined by comparing each profiled band with concurrently run DNA molecular 1 kb size DNA marker (Thermo Fisher Scientific, USA).

3.6.5 DNA Purification

The PCR products were purified by using QIAQuick Gel Purification Kit (Qiagen, Germany). This was initiated by excising the DNA fragment (indicated by visible band from the agarose gel) into 2.0 ml sterile microcentrifuge tube, using a clean scalpel. The gel sliced was weighed, and Buffer QG was added. The buffer was added in three volumes to one gel ratio (100 mg gel ~ 100 µl). The tube was then incubated for 10 minutes at 50°C until the gel slice was fully dissolved. Following the full dissolution of the gel, 1 gel volume of isopropanol was added to the sample and was well mixed. The mixture was transferred into a new QIAquick spin column (which attached with collection tube) and was centrifuged at

10,000 rpm for 1 minute. The QIAquick column was reinserted into the collecting tube after the flow-through was discarded. About 500 µl of QG Buffer was added to the QIAquick column and centrifuged at 10,000 rpm for another minute. The flow-through was discarded and the washing procedure began. About 750 µl of Buffer PE was added to the QIAquick column and centrifuged for 1 minute at 10,000 rpm to complete the washing procedure. This operation was performed twice to eliminate any remaining wash buffer. Then, the QIAquick column was placed into a clean 1.5 ml microcentrifuge tube for elution of DNA. To elute DNA, about 50 µl of Buffer EB was added at the centre of the QIAquick membrane. The column then was centrifuged at 10,000 rpm for 1 minute. Another 30 µl of Buffer EB was added to the centre of QIAquick membrane. The column was let stand for 1 minute, and it was centrifuged at 10,000 rpm for another 1 minute. This last step was done to increase the DNA concentration. After centrifuge, the QIAquick column was removed and the flow-through (containing purified DNA products) was kept at -20 ºC. Prior sending these purified DNA products for DNA sequencing, about 5 µL of purified DNA products were electrophoresed at 90 V, 400 mA conditions, in a 0.8% (w/v) agarose gel for an hour. These purified DNA products were sent to Apical Scientific Sdn. Bhd. for DNA sequencing. The DNA sequences obtained were compared with GenBank Database by BLAST to find the regions of local similarity between biological sequences obtained.

3.7 In Vitro Screening of Plant Growth Promoting Activities

The selected rhizobacterial isolates were screened in vitro for the rest of its plant growth promoting properties. These PGP traits are solubilization of phosphate, production of indole acetic acid (IAA), siderophore production, and ammonia production.

3.7.1 Preparation of Identified Inoculum

All in vitro screening was performed by using a standardized inoculum. A bacterial cell suspension of 48 hours-old grown culture (OD 0.1 at 540 nm) was inoculated into nutrient broth. The turbidity of inoculated broth was determined by using UVmini-1240 UV-VIS Spectrophotometer (Shimadzu, Japan). Isolates with 0.5 McFarland turbidity, at which the absorbance reading of OD600 in the range of 0.08 to 0.1, was used. The turbidity of bacterial suspension then was adjusted to ~1.5 × 108 CFU/mL to standardize the microbial testing. Meanwhile, the un-inoculated broth served as the blank.

In order to ensure the validity of the experiments, all screening tests were carried out simultaneously with the control experiment, in natural settings. All tests were conducted in triplicates and the values were expressed as mean. A bacterial strain, *Pseudomonas aeruginosa* was chosen as positive control and uninoculated medium served as negative control. The selection was made based on the literature research and its availability at the lab. Hypothetically, the positive control (*Pseudomonas aeruginosa*) observed positive reactions in all screening tests. However, it is not being used as a standard benchmark in comparing the efficacy of PGPR screened.

3.8 Screening of Ammonia Production

For qualitative estimation of ammonia production, isolates were screened according to Cappuccino and Welsh (2017) method. The selected bacterial isolate was tested for its ammonia production in peptone water. The fresh inoculum was inoculated in 10 mL of peptone water, prior incubated on a rotary shaker for 96 hours at 28 º C ± 2. Following incubation, each inoculum was mixed with 0.5 mL of Nessler's reagent. The development of faint yellow indicates small production of ammonia while development of deep yellow to brownish colour indicate a weak, strong ammonia production, respectively.

3.9 Analysis of Phosphate Solubilization

The screening of phosphate solubilization was done qualitatively and quantitatively. Qualitative and quantitative determination of phosphate solubilization was performed by plate assay method according to Pikovskayas (1948) and Subba Rao (1982), respectively.

3.9.1 Qualitative Analysis of Phosphate Solubilization

Each rhizobacterial inoculum were streaked onto Pikovskayas agar (HiMedia, India). After 72 hours, the presence of halo zones was considered as positive result and the diameter was measured. The strain was classified based on its ability to produce halo zone diameter (Baig et al., 2010); low phosphate solubilizer (≤ 1 cm), medium phosphate solubilizer (1 – 2 cm), and high phosphate solubilizer (≥ 2 cm). The phosphate solubilization index (PSI) and the solubilization efficiency (SE) was calculated according to Equation 3.1 and Equation 3.2, respectively (Pande et al., 2017).

$$\mathrm{SI} = \frac{\text{Colony diameter} + \text{Solubilization diameter}}{\text{Colony diameter}} \qquad \text{Equation 3.1}$$

$$\mathrm{SE} = \frac{\text{Solubilization diameter}}{\text{Colony diameter}}\ x\ 100 \qquad \text{Equation 3.2}$$

3.9.2 Quantitative Analysis of Phosphate Solubilization

Each isolate was tested in Pikovskayas (PVK) medium (HiMedia, India) consisted insoluble tricalcium phosphate (Ca3PO4)2 as the sole phosphate source, and the pH variation was monitored. Meanwhile, sterile uninoculated medium was used as the negative control. About 0.1 mL of rhizobacterial inoculum were inoculated triplicately into Pikovskayas (PVK) medium (HiMedia India). The isolates were incubated at 27 ± 2 ºC on 120 rpm orbital shaker (New Brunswick Scientific, USA) for 9 days. The solubilized P were then estimated

spectrophotometrically using Vanadate Molybdate reagent against standard curve plotted at 400 nm. An aliquot of 5 mL was withdrawn on each 3 days and were centrifuged for 20 minutes at 10,000 rpm. About 2.5 mL of supernatant was collected, in which 1.5 mL of distilled water and 1 mL of Vanadate Molybdate Reagent (Merck, USA) was added. The mixture then incubated for 10 minutes at room temperature. After 10 minutes incubation, the absorbance reading of the mixture was obtained. The procedure was repeated for the aliquot withdrawn on day 6th and day 9th.

3.9.3 Standard Curve KH_2PO_4

The standard curve KH2PO4 was plotted using concentration of standard phosphate solution (Table 3.6) against absorbance reading at 400 nm.

Table 3.6: Dilution for standard potassium phosphate solution

Concentration (mg/L)	Solid KH_2PO_4(mg)	Standard Solution (mL)	Vanadate Molybdate Reagent (mL)	Distilled Water (mL)	Final Volume (mL)
0	0	0	1	4	5
2	0.01	0.2	1	3.8	5
5	0.025	0.5	1	3.5	5
10	0.05	1	1	3	5
15	0.075	1.5	1	2.5	5

The amount of solubilized P (mg/L) then were extrapolated from the standard curve plotted.

3.10 Screening of Indole Acetic Acid (IAA) Production

The Salkowski reagent was prepared by mixing 50 mL of 35% (v/v) perchloric acid, $HClO_4$ (Merck Milipore, USA) with 1 mL of 0.5 M iron (III) chloride, FeCl3 (Merck Milipore, USA).

The rhizobacterial inoculum were incubated for 72 hours in Nutrient Broth (Merck Milipore, USA) at 27 ± 2 ºC. After incubation, the bacterial cultures were centrifuged at 10, 000 rpm for 10 minutes. About 0.5 mL of supernatant was collected and mixed with 1 mL of Salkowski reagent. This reaction mixture then was incubated in dark for about 25 minutes and the light absorbance was measured immediately at 530 nm (Ahmad et al., 2008). The concentration of IAA produced by the rhizobacterial isolates was extrapolated from the standard curve of IAA.

3.10.1 Preparation of Standard Curve

Different concentration of IAA solution was prepared in Nutrient Broth (Merck Milipore, USA) medium (Table 3.7).

Table 3.7: Dilution of standard IAA stock solution

Concentration of IAA solution (µg/mL)	Dilution mixture
1000	10 mL acetone + 9 mL NB
100	1 mL of 1000 µg/mL + 9 mL NB
50	1 mL of 100 µg/mL + 5 mL NB
10	1 mL of 100 µg/mL + 9 mL NB
20	2 mL of 100 µg/mL + 8 mL NB
5	1 mL of 50 µg/mL + 9 mL NB
0	10 mL NB

A total of 10 mg of IAA (R&M Chemical, UK) was added into 10 mL of acetone (Merck Milipore, USA). This served as the standard IAA stock solution (1000 µg/mL). The standard IAA stock solution was diluted accordingly, with Nutrient Broth (Merck Milipore, USA) served as the solvent. The production of IAA was indicated by the development of pink colour of the medium. The colour intensity is directly proportional to the increase of IAA concentration produced (Appendix 3 – Page 119). The standard curve of IAA was plotted based on the absorbance reading value against the concentration of IAA (Appendix 4 – Page 120).

3.11 Evaluation of Siderophore Production

3.11.1 Preparation of Chrome Azurol S (CAS) Agar Plates

The test was initiated by preparing the CAS agar plates. There are three different stages involved in preparing the CAS assay. Firstly, two solutions: (A) Blue Dye Solution

and (B) Mixture Solution was prepared. These solutions then were mixed with Bacto agar (Merck Milipore, USA) to prepare the CAS agar. For this purpose, all the glassware used was soaked in 6M HCl (Merck Milipore, USA) and rinsed with distilled water, prior using. This step aimed to remove any trace elements prior preparing the CAS agar plates.

(A) Preparation of Blue Dye Solution

Solution I was prepared by dissolving 0.06 g of CAS (Fluka Chemical, USA) into 50 mL of distilled water. Then, about 0.0027g of iron (III) chloride hexahydrate, FeCl3-6H2O (Merck Milipore, USA) was mixed with 10 mL of 10 mM hydrochloric acid, HCl (Merck Milipore, USA), for solution II. Meanwhile, solution III was prepared by dissolving 0.073g of hexadecyltrimethylammonium bromide, HDTMA (Sigma-Aldrich, USA), in 40 mL of distilled water. Solution I and Solution III then was mixed with 9 mL of Solution II. The mixture was autoclaved and aseptically stored until further use.

(B) Preparation of Mixture Solution

The three stocks; (i) Minimal Media 9 (MM9) Salt Solution Stock, (ii) 20% (w/v) Glucose Stock and (iii) Casamino Acid Solution was prepared accordingly:

(i) Minimal Media 9 (MM9) Salt Solution Stock was prepared by dissolving 15 g of Potassium Dihydrogen Phosphate, KH2PO4 (Merck Milipore, USA), 25 g of Sodium Chloride, NaCl (Merck Milipore, USA) and 50 g of Ammonium Chloride, NH4Cl (Merck Milipore) in 500 mL of distilled water.

(ii) 20% (w/v) Glucose Stock was prepared by mixing 20 g of glucose (Merck Milipore, USA) in 100 mL of distilled water.

(iii) Casamino Acid Solution was prepared by mixing 3 g of Casamino Acid with 27 mL of distilled water. Then, it was extracted with 3% (w/v) of hydroxyquinoline

(Merck Milipore, USA) in chloroform (Merck Milipore, USA), to remove any trace iron. This liquid-liquid extraction was performed by using separatory funnel. This extracted product was filter sterilized by using 0.22 μm of membrane filter (Meltzer and Jornitz, 2003).

(C) Preparation of CAS agar

A total of 100 mL of MM9 Salt Solution was added into 750 mL of distilled water. Then, about 32.24 g of piperazine-N, N'-bis (2-ethanesulfonic acid) PIPES (Sigma- Aldrich, USA) was dissolved in this solution. PIPES (Sigma-Aldrich, USA) will not dissolve below pH of 5, hence the pH of the solution was monitored. Sodium hydroxide, NaOH (Merck Milipore, USA) was added gradually upon addition of PIPES (Sigma-Aldrich, USA) into MM9 Salt Solution to make sure the pH did not drop to pH of 5. On the same time, it was made sure that the pH of solution was not exceeding pH 6.8 as this will turn the solution into green colour. Upon dissolution of PIPES (Sigma-Aldrich, USA), about 15 g of Bacto agar (Merck Milipore, USA) was added into MM9/PIPES solution, prior autoclaving it. After autoclaved, about 30 mL of sterile casamino acid solution and 10 mL of sterile 20% (w/v) glucose solution was added. This followed by the addition of 100 mL of Blue Dye Solution before it was being aseptically poured into the petri dishes.

3.11.2 Screening of Siderophore Production by Selected Isolates

The screening of siderophore production were qualitatively tested by CAS plate assay (Schwyn & Neilands, 1987). About 30 μL of standardized bacterial inoculum were inoculated on the CAS agar plates. These plates were incubated for 24 - 48 hours at 30 ± 2 ^{o}C.

3.12 Statistical Analysis

The quantitative results were presented as means plus standard deviations (SE). Specifically for the microbial population study isolated, differences between means were assessed using an independent t-test with a significance threshold of 0.05.

3.13 Ranking Plant Growth Promoting Traits

The strains were graded based on the bonitur scale (Ambrosini and Passaglia, 2017) for their ability to stimulate plant development. The rankings considered all of the data acquired from the in vitro characterizations. The proportion of each PGP feature (nitrogen fixation and ammonia production) was turned into an arbitrary number ranging from zero to one; 0: no trait identified; 1: there is trait identified. Meanwhile for other PGP features (phosphate solubilization, IAA production, siderophore production), the arbitrary value ranging from zero to three was used based on the value of trait examined, accordingly.

CHAPTER 4

RESULTS

4.1 Sampling Sites

In our study, the sampling was conducted at Dalat and Kuching Division of Sarawak State, Malaysia. Dalat was chosen as sampling sites given plenty wild grown of *M. sagu,* in environmentally stress settings such as in standing water and acidic peat soil. Meanwhile, Kuching's sampling sites was chosen as the *M. sagu* was cultivated in garden soil which thought will brought variety of PGPR isolated from such environment, yet from the same host plant. The *M. sagu*. chosen (host plant of soil sample SN, SU and STSP) was wildly grown in peat soils by natural pollination occurrence. They were estimated to age within a range of 7-9 years old. Meanwhile, soil sample ST was collected from a clay-peat soils habitat. The sago palms were estimated by the land's owner as 1.5 years. Both of this location were located by the riverbank and fully exposed with sun and rain throughout the year (Appendix 5 – Page 121 and Appendix 6 – Page 122). Another sampling sites chosen for this study was located at Kuching. The host plant of rhizosphere samples was *Metroxylon sagu,* Rottb. seedlings cultivated in the greenhouse (Appendix 7 – Page 123).

4.2 Isolation and Enumeration of Microbial Population

A total of eight rhizospheric soil samples were collected and the coordinates for each sampling site can be found in Appendix 8 (Page 124). The enumeration of bacteria was performed by serial dilution and plating technique on Burks agar (HiMedia, India). The microbial count for all rhizospheric soil samples were tabulated in Table 4.1.

Table 4.1: Rhizobacterial population of soil samples

Location	Soil Samples	Microbial count (Log_{10} CFU/mL)
Sungai Nunau	SN	1.57×10^5
Sungai Ugui	SU	1.19×10^5
Intermediate of Sungai Taap and Sungai Petah	STSP	1.20×10^5
Sungai Tabo	ST	1.76×10^5
CRAUN Research Kuching	M0319	9.50×10^4
CRAUN Research Kuching	M063B	7.50×10^4
CRAUN Research Kuching	CR	9.90×10^4
Sago Research Plot Kuching	SRP	9.80×10^4

The population of microorganisms in rhizospheric soil was counted and reported as CFU/mL. The total viable count of the rhizobacterial varied from Log10 7.5 x 104 CFU/mL to Log10 1.19 x 105 CFU/mL There was an obvious trend between both locations, in which the microbial population indigenous to soil samples collected from Dalat Division was higher in comparison to those collected from Kuching Division.

4.3 Isolation and Characterization of Putative Diazotrophic Rhizobacteria

The preliminary screening was conducted by growing the isolates on N-free medium, Burks agar (HiMedia, USA). The presence of rhizobacterial growth on this agar indicated the

nitrogen fixation activity (Appendix 9 – Page 125). Isolates which were very slow to grow (more than 10 – 12 hours) were discarded. About 47 isolates were found to be putative diazotrophic PGPR (Table 4.2).

Table 4.2: Diazotrophic PGPR isolates

Locations	Soil Samples	Isolates	Number of isolates
Dalat	Sample Nunau	S1, S2, S3, S4, S5	5
	Sample Ugui	SUA, SUB, SUC, SUD, SUE, SUF, SUG, SUH, SUI, SUJ, SUK, SUL, SUM, SUN	14
	Sample TP	STSPA, STSPB, STSPC, STSPD, STSPDE, STSPF	6
	Sample Tabo	STA, STB, STC, STD, STE, STF, STG, STH	8
Kuching	Sample M0139	M0139A, M0139C, M0139D, M0139E, M0139F, M0139G	6
	Sample M063B	M063BA, M063BB, M063BC, M063BD, M063BE	5
	Sample CR	CR1, CR2, CR3, CR4	4

Most isolates were isolated from ST with a total of 8 isolates. While the lowest isolates were isolated from sample CR (n=4). Since not all isolates were able to produce band after Agarose Gel Electrophoresis (AGE), only 47 isolates were further subjected to $(GTG)_5$ PCR analysis. These isolates were also characterized for its morphology based on its appearance on the medium. The morphology of the viable 47 isolates was characterized with the reference of Bergey's Manual of Systematic Bacteriology (Bergey et al., 1984). These included the shape, size, elevation, margin, opacity, pigmentation, and the surface of the cell colonies (Table 4.3).

Table 4.3: Morphology of selected isolates

Isolates	Shape	Size	Elevation	Margin	Opacity	Pigmentation	Surface
S1	Circular	Medium	Flat	Lobate	Opaque	Yellow	Smooth
S2	Circular	Medium	Flat	Entire	Opaque	Non-pigment	Smooth
S3	Circular	Small	Flat	Entire	Opaque	Non-pigment	Smooth
S4	Circular	Small	Flat	Entire	Translucent	Non-pigment	Smooth
S5	Irregular	Small	Flat	Undulate	Opaque	Non-pigment	Rough
SUA	Circular	Large	Flat	Entire	Opaque	Non-pigment	Smooth
SUH	Irregular	Small	Flat	Lobate	Opaque	Cream	Smooth
SUI	Punctiform	Small	Flat	Entire	Opaque	Yellow	Rough
SUJ	Circular	Small	Flat	Undulate	Opaque	Yellow	Smooth

Table 4.3 continued

SUL	Circular	Medium	Flat	Undulate	Opaque	Yellow	Smooth
SUM	Circular	Small	Flat	Entire	Opaque	Light blue	Smooth
SUN	Circular	Small	Convex	Entire	Opaque	Red	Smooth
STSPA	Circular	Medium	Flat	Entire	Opaque	White	Rough
STSPB	Circular	Small	Flat	Entire	Translucent	Non-pigment	Smooth
STSPC	Punctiform	Small	Flat	Entire	Opaque	Non-pigment	Smooth
STSPD	Circular	Medium	Flat	Undulate	Opaque	Non-pigment	Smooth
STSPE	Circular	Medium	Flat	Entire	Opaque	Yellow	Smooth
STSPF	Circular	Medium	Flat	Undulate	Opaque	Non-pigment	Smooth
STA	Circular	Medium	Flat	Undulate	Opaque	Non-pigment	Rough

Table 4.3: continued

STB	Irregular	Large	Flat	Lobate	Translucent	Yellow	Smooth
STC	Circular	Small	Flat	Entire	Translucent	Non-pigment	Smooth
STD	Circular	Small	Flat	Entire	Opaque	Yellow	Smooth
STE	Circular	Large	Flat	Irregular	Opaque	Non-pigment	Smooth
STF	Circular	Small	Flat	Entire	Opaque	Non-pigment	Smooth
STG	Circular	Medium	Flat	Entire	Opaque	Non-pigment	Smooth
STH	Irregular	Medium	Flat	Undulate	Opaque	Non-pigment	Smooth
M0139A	Circular	Medium	Flat	Entire	Opaque	Non-pigment	Smooth
M0139C	Circular	Small	Flat	Entire	Opaque	Non-pigment	Smooth
M0139D	Circular	Medium	Flat	Entire	Opaque	Non-pigment	Rough
M0139E	Irregular	Medium	Flat	Undulate	Opaque	Non-pigment	Smooth

Table 4.3: continued

M0139F	Irregular	Medium	Raised	Undulate	Opaque	Non-pigment	Smooth
M0139G	Circular	Medium	Raised	Entire	Opaque	Non-pigment	Smooth
M063BA	Irregular	Large	Flat	Undulate	Opaque	Non-pigment	Smooth
M063BB	Irregular	Large	Flat	Entire	Translucent	Non-pigment	Smooth
M063BC	Irregular	Large	Flat	Entire	Translucent	Non-pigment	Smooth
M063BD	Circular	Medium	Flat	Entire	Translucent	Non-pigment	Rough
M063BE	Circular	Small	Flat	Entire	Translucent	Non-pigment	Smooth
CR1	Irregular	Medium	Flat	Undulate	Opaque	Non-pigment	Smooth
CR2	Circular	Medium	Raised	Entire	Opaque	Non-pigment	Smooth
CR3	Circular	Small	Flat	Entire	Opaque	Non-pigment	Smooth
CR4	Circular	Medium	Flat	Entire	Opaque	Non-pigment	Smooth

Table 4.3 continued

SR1	Irregular	Medium	Raised	Undulate	Opaque	Non-pigment	Smooth
SR2	Circular	Medium	Raised	Entire	Opaque	Non-pigment	Smooth
SR3	Irregular	Large	Flat	Entire	Translucent	Non-pigment	Smooth
SR4	Circular	Medium	Flat	Entire	Opaque	Cream	Smooth
SR5	Irregular	Large	Flat	Entire	Translucent	Non-pigment	Smooth
SR6	Circular	Medium	Flat	Entire	Translucent	Non-pigment	Rough

The morphological characterization of the 47 isolates showed that most isolates had circular colony configuration (n =32), which is medium in size (n =23), with smooth surface (n =40), grow flat (n=41) and entire margin (n = 31) on NA medium. These isolates also mostly grew as non-pigmented (n=36) and appeared opaque (n=36) on the Burks agar medium.

4.4 $(GTG)_5$ PCR Fingerprinting Analysis

The banding pattern of all isolates was performed to reduce genetic redundancy, by identifying the isolates genetic similarity. By analyzing the gel electrophoresis images (Figure 4.1) using Gelj_v2.0, the clustering scheme (Figure 4.2) was deduced.

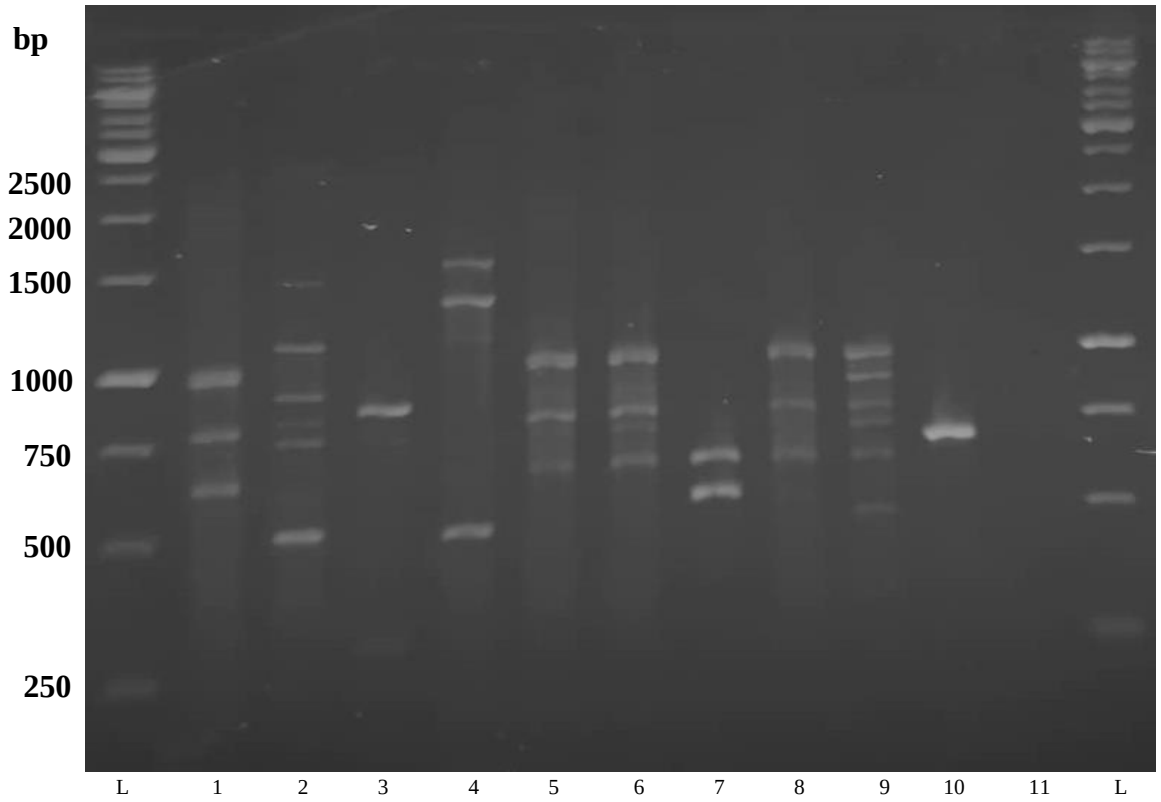


Figure 4.1: Example of banding profiles of $(GTG)_5$ PCR of isolates. Lane L: 1kb DNA Ladder (Thermo Fisher Scientific, UK), 1: STA, 2: STC, 3: STD, 4: STE, 5: STSPA, 6: STSPB, 7: STSPC, 9: STSPE, 10: STSPF, 11: Negative control.

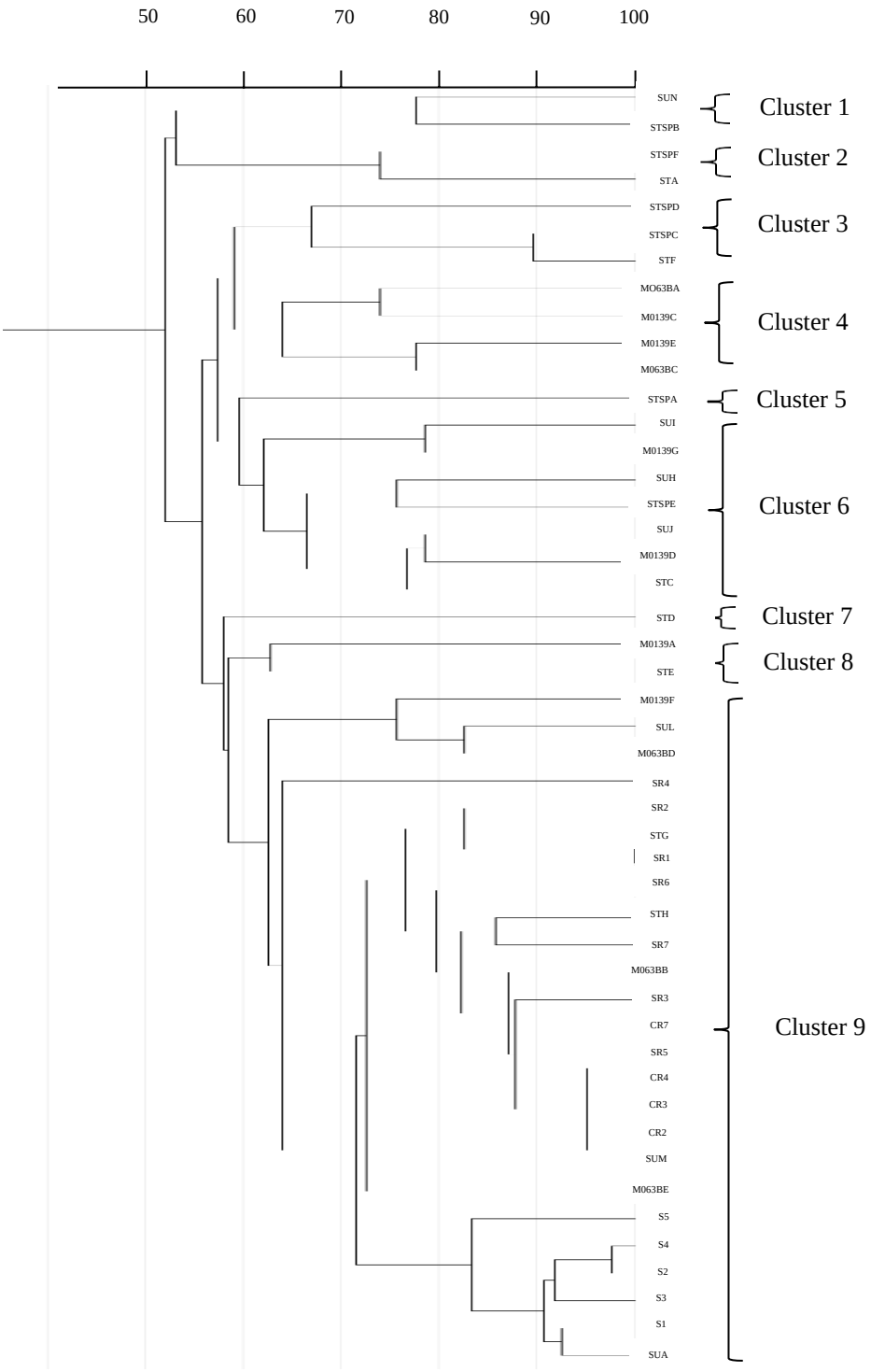


Figure 4.2: Dendrogram to determine the similarity between isolation

Since some isolates failed to produce DNA bands, not all isolates were managed to be included in the construction of dendrogram. The dendrogram was conducted using Dice similarity coefficient index and were automatically calculated with UPGMA. This method was recommended for genetic fingerprinting technique (Oueriaghli et al., 2018). Organisms with a genetic resemblance of at least 50% are regarded members of the same family, whereas organisms with a genetic similarity of 60% to 75% are considered members of the same species (Baron, 1996; Paradis et al., 2005). As depicted, all isolates were deduced as one family at 52% genetic similarity level. The isolates were clustered into 9 clusters at 60% similarity level (Table 4.4).

Table 4.4: Clusters of isolates depicted from the constructed dendrogram

Clusters	Related isolates (≥ 60% similarity)	Source of isolates	Isolates chosen for 16S rDNA sequencing
Cluster 1	SUN, STSPB	Ugui, STSP	SUN
Cluster 2	STSPF, STA	STSP, Tabo	STA
Cluster 3	STSPD, STSPC, STF	STSP, Ugui, Tabo	STSPC
Cluster 4	M063BA, M0139C, M0139E, M063BC	M063B, M0139, STSP	M063BA
Cluster 5	STSPA	STSP	STSPA
Cluster 6	SUI, M0139G, SUH, STSPE, SUJ, M0139D, STC	M0139	SUH

Table 4.4 continued

Cluster 7	STD	Tabo	STD
Cluster 8	M0139A, STE	M0139, Tabo	STE
Cluster 9	M0139F, SUL, M063BD, SR4, SR2, STG, SR1, SR6, STH, SR7, M063BB, SR3, CR7, SR5, CR4, CR3, CR2, SUM, M063BE, S5, S4, S2, S3, S1, SUA	Ugui, Tabo, M063B, CR	SUA

The major Cluster 9 comprised of 26 isolates with the smallest Cluster 5 and Cluster 7, each with one isolate. Meanwhile, Cluster 1, Cluster 2 and Cluster 3 each comprised two isolates. These followed by four isolates segregated into Cluster 4, two isolates into Cluster 8. The remaining seven isolates were clustered as Cluster 6. These isolates were considered belong to one family, at its lowest percentage of relatedness (52%).

4.5 Bacterial Identification by 16S rDNA Sequencing

From each cluster, one isolate was chosen and subjected to 16S rDNA sequencing. The isolates were identified by comparing the DNA sequences obtained with NCBI database (Table 4.5).

Table 4.5: Bacterial identities of selected isolates

Isolates	Bacterial identity	Identity Percentage (%)	Accession number
SUN	*Serratia marcescens*	99.20	EF035134.1
STA	*Pseudomonas extremaustralis*	99.19	MN826583.1
STSPC	*Bacillus* sp.	99.38	EU912475.1
M063BA	*Bacillus cereus*	99.8	MN691535.1
STSPA	*Bacillus subtilis*	99.8	HQ670439.1
SUH	*Bacillus thuringiensis*	99.8	KT895844.1
STD	*Staphylococcus sciuri*	98	KF876871.1
STE	*Pseudomonas* sp.	99.79	KY670738.1
SUA	*Pseudomonas monteilii*	99.79	OL889839.1

The BLASTn phylogenetic analyses revealed a clear clustering of these 47 isolates into two Phyla: Firmicutes (55.6 %) and Proteobacteria (44.4 %). At the genus level, these isolates were grouped within four genera: *Bacillus* (n=4), *Pseudomonas* (n=3), *Serratia* (n=1) and *Staphylococcus* (n=1).

4.6 Secondary *In Vitro* Screening of Plant Growth Promoting Traits in Isolates

4.6.1 Screening of Ammonia Production

Ammonia is the preferred nitrogen containing nutrient which potentially aiding the plant growth. This was done through the conversion of ammonia into nitrite and nitrate by PGPR. Hence, since the capability of the identified PGPR in nitrogen fixing has been tested priorly, the screening of ammonia production is mostly helping the study in determining the capability of PGPR in producing another source of nitrogen containing nutrient, which is ammonia. The selected bacterial isolates were screened as ammonia producer based on the development of brown and yellow colour of the medium (Table 4.6).

Table 4.6: Ammonia production of selected isolates

Isolates	Ammonia production	Color development
SUN	+	Yellow
STA	+	Yellow
STSPC	+	Yellow
M063BA	++	Light brown
STSPA	+++	Dark brown
SUH	++	Light brown
STD	+	Yellow
STE	+	Yellow
SUA	++	Light brown

- = no production, + = low production, ++ = moderate production, +++ = high production

All isolates were able to produce ammonia moderately, with isolate STSPA observed a high production activity. Isolates M063BA, SUH and SUA produced ammonia, fairly while isolate SUN, STA, STSPC, STD and STE were found to produce ammonia weakly.

4.6.2 Qualification of Phosphate Solubilization

Qualification of phosphate solubilization were observed based on their ability in producing clear zone in the minimal medium containing the insoluble phosphate, Pikovskayas agar. In vitro screening for phosphate solubilization traits was measured for all

identified isolates and the study observed there was phosphate solubilization activity by all 9 isolates (Table 4.7).

Table 4.7: The solubilization efficiency of isolates in plate assay

Isolates	Bacterial identity	Colony diameter (cm)	Solubilization zone (cm)	Phosphate solubilization index (PSI)	Phosphate solubilization efficiency, PSE (%)
SUN	*Serratia marcescens*	1.00 ± 0.10^{ab}	1.63 ± 0.06^{cd}	2.65 ± 0.23^{abc}	164.78 ± 22.11^{bcd}
STA	*Pseudomonas extremaustralis*	0.70 ± 0.10^{a}	2.33 ± 0.12^{e}	4.38 ± 0.63^{d}	339.30 ± 62.58^{a}
STSPC	*Bacillus* sp.	1.10 ± 0.10^{b}	1.50 ± 0.10^{bcd}	2.39 ± 0.18^{ab}	137.42 ± 19.58^{cde}
M063BA	*Bacillus cereus*	0.97 ± 0.12^{ab}	1.17 ± 0.06^{a}	2.21 ± 0.12^{ab}	120.87 ± 11.35^{de}
STSPA	*Bacillus subtilis*	0.90 ± 0.10^{ab}	1.23 ± 0.15^{ab}	2.39 ± 0.18^{ab}	137.69 ± 17.78^{de}
SUH	*Bacillus thuringiensis*	1.20 ± 0.20^{b}	1.30 ± 0.15^{ab}	2.13 ± 0.25^{a}	111.35 ± 27.27^{cde}
STD	*Staphylococcus sciuri*	2.53 ± 0.15^{c}	2.93 ± 0.10^{f}	2.15 ± 0.09^{a}	116.03 ± 7.78^{e}
STE	*Pseudomonas* sp.	0.67 ± 0.06^{a}	1.33 ± 0.12^{abc}	3.02 ± 0.17^{bc}	200.79 ± 15.50^{abc}

Table 4.7 continued

SUA	*Pseudomonas monteilii*	0.70 ± 0.10^{a}	1.70 ± 0.06^{d}	3.46 ± 0.41^{c}	246.82 ± 42.61^{ab}

The phosphate solubilizing index was varied to a great extent, with a range from 2.13 ± 0.25 to 4.38 ± 0.63. Based on the PSI and PSE value, it can be depicted that isolate STA showed the most statistically significant phosphate solubilizing activity on solid PVK, with 4.38 ± 0.63 cm and 339.30 ± 62.58 %, respectively. While isolate SUH being the lowest phosphate solubilizer based on the PSI and PSE value, 2.13 ± 0.25 and 111.35 ± 27.27 %, respectively.

4.6.3 Quantification of Phosphate Solubilization

Based on the solubilizing zone, all isolates were selected for secondary screening of phosphate solubilization. The phosphate solubilized as well as the changes in pH of Pikovskayas medium was recorded in Table 4.8 and Table 4.9.

Table 4.8: Phosphate solubilization of all isolates

Isolates	Bacterial identity	Phosphate solubilization (mg/L)		
		Day 3	Day 6	Day 9
SUN	*Serratia marcescens*	7.83 ± 12.65[a]	10.92 ± 15.51[a]	10.35 ± 12.53[a]
STA	*Pseudomonas extremaustralis*	7.55 ± 12.71[a]	13.79 ± 18.55[a]	7.19 ± 11.33[a]
STSPC	*Bacillus* sp.	4.02 ± 6.08[a]	5.24 ± 5.35[a]	2.76 ± 2.48[a]
M063BA	*Bacillus cereus*	9.17 ±12.59[a]	12.71 ± 17.40[a]	3.65 ± 5.21[a]
STSPA	*Bacillus subtilis*	7.12 ± 8.67[a]	7.41 ± 10.34[a]	6.49 ± 6.85[a]
SUH	*Bacillus thuringiensis*	11.34 ± 11.66[a]	7.53 ± 11.56[a]	6.20 ± 6.99[a]
STD	*Staphylococcus sciuri*	9.93 ± 14.61[a]	11.13 ± 16.63[a]	18.46 ± 22.76[a]
STE	*Pseudomonas* sp.	12.69 ± 17.73[a]	15.12 ± 16.84[a]	15.06 ± 17.01[a]
SUA	*Pseudomonas monteilii*	15.60 ± 13.45[a]	23.08 ± 14.29[a]	12.22 ±13.61[a]

Table 4.9 pH of the pikovskayas medium upon incubation time of isolates

Isolates	Bacterial identity	pH of Pikosvskayas medium		
		Day 3	Day 6	Day 9
SUN	*Serratia marcescens*	5.36 ± 0.11[a]	5.20 ± 0.16[a]	4.75 ± 0.50[a]
STA	*Pseudomonas extremaustralis*	5.55 ± 0.11[a]	5.11 ± 0.28[a]	4.65 ± 0.55[a]
STSPC	*Bacillus* sp.	5.24 ± 0.14[a]	4.95 ± 0.08[a]	4.56 ± 0.21[a]
M063BA	*Bacillus cereus*	5.25 ± 0.13[a]	4.81 ± 0.18[a]	4.37 ± 0.31[a]
STSPA	*Bacillus subtilis*	5.41 ± 0.29[a]	4.74 ± 0.24[a]	4.53 ± 0.07[a]
SUH	*Bacillus thuringiensis*	5.40 ± 0.12[a]	4.89 ± 0.19[a]	4.56 ± 0.45[a]
STD	*Staphylococcus sciuri*	5.44 ± 0.28[a]	5.06 ± 0.04[a]	4.46 ± 0.46[a]
STE	*Pseudomonas* sp.	5.39 ± 0.19[a]	5.00 ± 0.02[a]	4.63 ± 0.24[a]
SUA	*Pseudomonas monteilii*	5.23 ± 0.17[a]	5.03 ± 0.05[a]	4.56 ± 0.32[a]

The amount of phosphate solubilized was quantified by using the standard curve of KH2PO4 (Appendix 2 - page 118). There was no statistically significant variation in the results observed for all isolates, from day 3 to day 9. Most of isolates showed the same trend, in which the concentration of phosphate solubilized is increasing from day 3 to day 6. Then, the value descended upon day 9. However, isolate STD reacted oppositely, in

which the value consistently increasing till the ninth day. Nevertheless, isolate SUA showed highest phosphate solubilization activity (23.08 ± 14.29 mg/L) on day 6th while isolate STSPC solubilized lowest quantity of phosphate (5.24 ± 5.35 mg/L) on the same day.

Meanwhile, the pH of the medium was found to decline for all isolates, with increase in incubation time (Table 4.9). However, there is no statistically significant different was observed for each isolates.

4.6.4 Screening of Indole Acetic Acid (IAA) Production

IAA producer has been attributes of PGPR strains which could stimulate the plant growth via proliferation of lateral roots and root hairs. Thus, the significance of this study is the potential in identifying the identified PGPR capability in producing IAA, despite isolated from stressful environment, salinity condition. All 9 isolates were screened for the indole acetic acid (IAA) production activity. Thequantity of IAA was expressed in µg/mL (Table 4.9) extrapolated from the standard curve of IAA (Appendix 4 - page 121).

Table 4.9: IAA production by selected isolates

Isolates	Bacterial identity	Concentration (µg/mL)	
		With Tryptophan	Without Tryptophan
SUN	*Serratia marcescens*	0.00 ± 0.00[a]	0.00 ± 0.00[a]
STA	*Pseudomonas extremaustralis*	5.89 ± 5.46[a]	0.72 ± 0.38[a]
STSPC	*Bacillus* sp.	18.55 ±2.83[bcd]	0.21 ± 2.05[a]
M063BA	*Bacillus cereus*	9.14 ±2.72[abc]	0.46 ± 0.20[a]
STSPA	*Bacillus subtilis*	17.63 ±1.34[bcd]	0.83 ± 1.45[a]
SUH	*Bacillus thuringiensis*	15.08 ±1.84[bcd]	1.31 ± 0.91[a]
STD	*Staphylococcus sciuri*	19.02 ±4.12[cd]	1.85 ± 2.02[a]
STE	*Pseudomonas* sp.	26.07 ±8.53[d]	1.08 ± 1.62[a]
SUA	*Pseudomonas monteilii*	17.66 ±7.02[bcd]	0.23 ± 0.21[a]

Out of 9 isolates, 8 isolates were positive for indole acetic acid (IAA) production.

The value is varied to a great extend with higher value in medium supplemented by tryptophan and lower value in those without tryptophan. However, there is no statistically significant different between the amount of IAA produced by the isolates without the presence of tryptophan.

In the presence of tryptophan, the maximum amount of IAA was produced by isolate was SIE(26.07 ±8.53 µg/mL) while isolate STA (5.89 ± 5.46 µg/mL) produced the lowest amount of IAA. Isolate STD observed a higher concentration of IAA (1.85 ± 2.02 µg/mL) without the presence of tryptophan while isolate STSPC only produced about 0.21 ± 2.05 µg/mL. On the other hand, isolateSUN observed to be a non-IAA producer in both conditions.

4.6.5 Screening of Siderophore Production

All identified isolates were qualitative screened for their siderophore production traits, based on the formation of the halo zone (Appendix 10 – page 126). The diameter of zone for respective isolates are tabulated in Table 4.10.

Table 4.10: Siderophore production of selected isolates

Isolates	Bacterial identity	Solubilization zone (cm)	Solubilization index (SI)
SUN	*Serratia marcescens*	0.00 ± 0.00a	0.00 ± 0.00[a]
STA	*Pseudomonas extremaustralis*	0.30 ± 0.10[a]	0.40 ± 0.20[ab]
STSPC	*Bacillus* sp.	0.20 ± 0.00[a]	0.23 ± 0.06[ab]
M063BA	*Bacillus cereus*	0.00 ± 0.00[a]	0.00 ± 0.00[a]
STSPA	*Bacillus subtilis*	0.27 ± 0.21[a]	0.40 ± 0.26[ab]
SUH	*Bacillus thuringiensis*	0.27 ± 0.21[a]	0.40 ± 0.26[ab]
STD	*Staphylococcus sciuri*	1.37 ± 0.06[b]	1.97 ± 0.06[c]
STE	*Pseudomonas* sp.	1.30 ± 0.00 [b]	1.90 ± 0.00 [c]
SUA	*Pseudomonas monteilii*	0.33 ± 0.15[a]	0.47 ± 0.21[b]

Out of 9 isolates, only 7 isolates observed positive reactions toward qualitative screening of siderophore production. Isolate STD and STE produced much wider halo zone, with mean of 1.37 ± 0.06 and 1.30 ± 0.00, respectively.

4.7 Ranking of Plant Growth Promoting Traits

To aid in the selection of potential PGPR strains, the bacterial strains was graded

for their apparent plant growth-promoting ability. The assessment indicated nine strains with PGPR potential was tabulated in Table 4.11.

Table 4.11: Rank of PGPR according to bonitur scale

Isolates	Identity	[a]Nitrogen fixation	[b]Phosphate solubilization	[c]IAA production	[d]Siderophore production	[e]Ammonia production	Total value	Rank
STE	*Pseudomonas* sp.	1	3	2	2	1	9	1st
STD	*Staphylococcus sciuri*	1	3	1	2	1	8	2nd
SUA	*Pseudomonas monteilii*	1	3	1	1	1	7	3rd
STA	*Pseudomonas extremaustralis*	1	3	1	1	1	7	3rd
STSPA	*Bacillus subtilis*	1	2	1	1	1	6	4th

Table 4.11 continued

SUH	*Bacillus thuringiensis*	1	2	1	1	1	6	4th
STSPC	*Bacillus* sp.	1	2	1	1	1	6	5th
M06BA	*Bacillus cereus*	1	3	1	0	1	6	6th
SUN	*Serratia marcescens*	1	3	0	0	1	5	7th

Note: [a]Nitrogen fixation scores: 0: no trait is detected; 1: trait is detected

[b] Phosphate solubilization scores: 0: below the detection limit; 1: ≤ 4.1 mg/L; 2: ≥ 4.1 mg/L and ≤ 8.4 mg/L; 3: ≥ 8.4 mg/L.

[c]IAA scores (with tryptophan): 0, IAA below the detection limit; 1: ≤ 21.5 µg/mL IAA protein; 2: ≥ 21.5 µg/mL IAA protein and ≤ 44.7 µg/mL IAA protein; 3: ≥ 44.7 µg/mL IAA protein.

[d]Siderophore index (SI) scores (ratio of colored halozone: colony O): 0: no visible coloured halo in T-CAS media; 1: ≤ 1.1 SI; 2: ≥ 1.1 SI and ≤ 2.4 SI; 3: ≥ 2.4 SI.

[e]Ammonia production scores: 0: no trait is detected; 1: trait is detected

CHAPTER 5

DISCUSSION

The capability of PGPR as the emerging tools in supporting the sustainable agriculture effort has been well recognized. In Malaysia, extensive study involving PGPR was well researched especially related to major food crops such as rice. However, due to theimpact brought by climate change on the agriculture sector, this study recognized the gap of PGPR research in choosing effective PGPR which capable to thrive and pose a positive impact in stress prone environment settings. Thus, it is being thought that isolating and utilizing PGPR indigenous to crops grown in such settings is one of potential approach in addressing this challenge. Hence, in this study, exploiting the beneficial indigenous potentialfood crops of Sarawak, the isolation and identification of PGPR from *Metroxylon sagu,* Rottb. was done. To the author knowledge, little research was done with the association in Malaysia being published, with only sago palms in the Phillipines, Papua New Guinea and Indonesia.

5.1 Microbial Population in *Metroxylon sagu,* Rottb.

The microbial population indigenous to *Metroxylon sagu,* Rottb. varied to a great extent for both locations. An independent t-test was performed with the purpose to identify if the mean of the microbial populations isolated from the two separate sites, differed significantly. The results revealed that there was a significant difference in the microbial population of the rhizosphere isolated from Dalat (M = 5.15, SD = 0.09) and Kuching (M =4.96, SD = 0.06) conditions, t (6) = 3.69, p = 0.01.

In this study, there is a higher average of microbial population was isolated from Dalat soil samples, in which the host plant was grown in native in comparison to

the seedlings cultivated in greenhouse. The result of this study is in conformation with Lihan etal. (2021) who has also reported greatest microbial population was observed in wild grown sago palm sampling sites in comparison to new sago palm cultivation sites. The study suggested that these variances were due to different environment of which the *Metroxylon sagu,* Rottb. grown. These was also stated by Campbell (1985), at which, various attributessuch as location, sampling time, cultivar or variety type, age of crops, as well as physicochemical properties of the soil and the environment conditions of the selected locations could affect the microbial populations isolated.

Nevertheless, in this study, despite of similar cultivar type, there is slight difference in values of microbial population between each sample. Sample Tabo (Dalat) observed the highest microbial count of rhizobacteria (5.25 Log CFU/mL) in comparison to others. Thus, this study suggested the primary determinant of this research on bacterial communities isolated was the soil type. Sample Tabo was grown on clay soils which is known to give bestresults on *Metroxylon sagu,* Rottb. yield (Flach, 1997). This owes to the smaller particle sizefraction of clay soil which increase the surface area to the soil, hence increase the nutrient- holding capacity in respective environment. Sessitsch et al. (2001) also stated in their studythat the particle size fraction did significantly affect the microbial community structure to agreat extent than the kind of fertilizer applied.

However, it is worth mentioning that the above distinction was only applicable on the representative soil samples due to the limitation in cultivating fraction of vast microbial diversity of the soil. To be able to give conclusions and precise data on the microbial population of the chosen habitat, a metagenomic approach might be considered. Nevertheless, the study on microbial population of the sampling sites was not done since the main objective of the study was to identify the PGPR indigenous to Metroxylon *sagu*,

Rottb.

5.2 Preliminary Selection of PGPR Isolates

The isolation of putative PGPR usually resulted in large number of isolates (Guerrieriet al., 2020). Besides, considering various activities of PGPR, it has been decided to establisha strategy to sort out the culturable PGPR and restrict them to those which possess PGP traits. Hence, in this study, it was decided to perform preliminary screening of bacteria basedon one of the essential elements required in plant growth.

Nitrogen is a vital element in plant development. The biological nitrogen fixation (BNF) can be considered as one of major mechanisms that beneficial to the plants (Guerrieri et al., 2020). Thus, in this study the hierarchic approach was performed by starting to test the capability of rhizosphere isolates on their nitrogen fixation capability.

The samples were plated on Nitrogen free medium, Burks agar. The Burks agar contained inorganic salts along with the carbon source (sucrose) but lacks nitrogen source. Hence, the nitrogen fixing bacteria which able to fix atmospheric nitrogen will grow when cultured on this medium. This method is economical and rapid process; thus, it was chosen. Besides, Kifle and Laing (2016) also stated the reliability of this in vitro screening procedures (growth on N-free semi-solid medium, ARA, the ammonia production test) as itgave a quick and reliable finding of putative PGPR.

This approach was also conducted by Shipton et al. (2010) in order to perform a preliminary screening of putative nitrogen fixers from ground palm root samples. It was detected the nitrogen fixers isolated was highest in LG media supplemented with carbon source (2.4×10^5 CFU/mL), which is higher than those isolated in this study (1.76×10^5 CFU/mL). However, in general, the putative diazotrophic PGPR isolated in this study was higher (ranged from 1.76×10^5 to 7.5×10^4 CFU/mL) in comparison with those in their study

(ranged from 2.4 x 10^5 to 4.3 x 10^2 CFU/mL). It worth noting that the media used were different (Baz, LG and NFB media), supplemented with azelaic acid, malate, and sucrose ascarbon source. Hence, this study suggested the differences are possibly due to different media used as well as different incubation period which influence the efficacy of the microbial growth. Moreover, the slow grown microbes were discarded from this study thus possibly contribute to lower microbial count in comparison to previous study.

5.3 $(GTG)_5$ Fingerprinting Analysis and Identification of PGPR

In order to reduce the cost of sequencing of the unknown isolates, the putative PGPR were then subjected to $(GTG)_5$ PCR. It is known to be a powerful fingerprinting method used to form distinguishable patterns of DNA bands (Haghshenas et al., 2017). The organism wasregarded as members of the same family with at least 50% genetic similarity, while the organisms are regarded as same species when there is 60% - 75% genetic similarity (Baron, 1996; Paradis et al., 2005). The BLASTn phylogenetic analyses revealed a clear clustering of these PGPR isolates into nine clusters.

Based on the fingerprinting analysis, some of the bacteria from different samples were classified under the same cluster, despite of different sampling sites. This might suggestthat the distribution of PGPR isolates is almost similar in both locations, which might be dueto the same cultivar type used in the present study. This was in line with Jiang et al. (2017) study in which they stated that plant species and soil properties does playing a determinant role in the diversity and composition of the rhizosphere bacterial community.

Each cluster was assumed to represent one species and it was depicted that there were nine different species were isolated from rhizospheric sample indigenous to *Metroxylon sagu*, Rottb. Following the sequencing, these nine clusters were identified as two phyla: Firmicutes (55.6%) and Proteobacteria (44.4%). At the study knowledge, there was

no previous report described the same findings related to *Metroxylon sagu,* Rottb. as describedin this study. Nevertheless, considering the Palmae family, Kusai and Ayob (2019) reported the same findings, in which the PGPR isolated was dominated by Proteobacteria and Firmicutes in the peat soil ecosystem and oil palm plantation. However, the value was differed in both studies in which the current study reported lower percentage value. It is worth noting that this was due to different nature of study in which their study was focusing on identifying bacterial diversity at four different depths of the soil. Meanwhile the isolatedPGPR species in this study accounts to only small percentage of cultivable bacteria as to the actual bacterial PGPR community in the ecosystem. Hence, these might as well result to thedifferences of the results. Nevertheless, these findings corresponded with those reported byFlores-Núñez et al. (2018), at which in their study, the PGPR isolated was dominated by microbes under phylum Firmicutes followed by Proteobacteria. These were also similar to Albdaiwi et al. (2019) who reported the same bacterial communities isolated from durum wheat in saline areas.

Furthermore, the phylogenetic analysis revealed that these 47 rhizobacterial isolatesrepresent four different genera (Figure 4.2). Out of these 47 isolates, 4 isolates belong to *Bacillus,* 3 isolates belong to *Pseudomonas* and remaining isolates belong to *Serratia* and *Staphylococcus,* respectively. This result is corresponding with those reported in previous studies of different crops, at which *Bacillus* and *Pseudomonas* genera are the predominant genera of PGPR (Hashem et al., 2019). Despite of the harsh environment in this study, *Bacillus* genera was found to dominantly thrive in this condition. This might be due to its capability in producing a long lived and stress tolerant spores (Radhakrishnan et al., 2017), exhibit dominant isolation of respected genera in this study. Moreover, the *B. subtilis* which isolated in this study was well-known to hold beneficial PGP traits under

stress-prone environment settings (Etesami & Beattie, 2018).

5.4 In Vitro Screening of Identified PGPR

The screening of PGP traits of each identified isolates was done in vitro. This included their properties on ammonia production, phosphate solubilization, siderophore production and production of IAA.

5.4.1 Ammonia Production by Identified PGPR Isolates

In this study, all nine selected isolates were found to generate ammonia in peptone water. This screening test was crucial as it works as confirmation on the effectiveness of PGPR in supplying ammonia required by the crops, other than being solely a nitrogen fixer. Meanwhile, based on the development of colour in qualitative screening of ammonia production, it was found that genus *Bacillus* has produced ammonia prominently in comparison with another genus. These findings were in conformity with previous research by Abdelwahed et al. (2021) in which *Bacillus* genus responsible in producing highest amount of ammonia.

Ammonia is one of important PGP activity of PGPR strains (Singh et al., 2018). Marques et al. (2010) found that the isolates' synthesis of ammonia and hydrogen cyanide was linked to nitrogen accumulation, influencing the root elongation, as well as phosphorus accumulation, biomass production, and shoot elongation of the crops. Besides, the accumulation of ammonia in soil may act as a defence against plant pathogens as it created alkaline conditions which eventually suppress crops pathogen.

5.4.2 Phosphate Solubilization by Identified PGPR

In this study, all isolates were found to solubilize phosphate with *P. extremaustralis* being the highest phosphate solubilizer. This isolate produced largest halozone with 4.18

PSI in qualitative screening test of phosphate and 22.29 mg/L of phosphate was solubilized, quantitatively. Similar finding was reported previously byKudoyarova et al. (2017) in which, *Pseudomonas* genera were chosen as biofertilizer for their ability to mobilise phosphates and produce auxins in vitro. The effects of inoculating these bacteria on soil mobile phosphorus content, as well as phosphorus and hormone levelsin wheat plants, were investigated, and the results were linked to changes in plant development. However, at the best of current knowledge that the study being conducted, there is no study reported on *P. extremaustralis* being a PGPR related to*Metroxylon sagu*, Rottb. as found in this study.

Nevertheless, in regard of the same Palmae family, the current finding is contrary tothose reported. At which the phosphate solubilizer indigenous to oil palm rhizosphere was dominated by *Burkholderia* spp. (Fajar Irawan et al., 2020; Situmorang et al., 2015; Castanheira et al., 2014). This difference might be due to plant type factor which do affect the microbial community at the rhizosphere. Moreover, the culture-dependent method used in this study might have missed some potential PGPR present in the soil microbial community. This probability was reported by Austin (2017) in which the culture-dependentapproaches, which necessitate the growth of microorganisms, are incapable of reflecting the existing microbial diversity in the biosphere thoroughly.

Besides, microorganisms involved in phosphorous cycling through phosphate solubilization and fixing phosphorous present in soil, in a pH dependent manner (Bhattacharyya et al., 2020). In the present study, the similar finding was observed, whereby the phosphate solubilization by PGPR is accompanied with the declination of the pH medium. This study suggested that the production of acidic substances such as organic acids by PGPR facilitated the solubilization of insoluble phosphate. This is aligned with the

mechanisms used in solubilizing phosphate by PGPR, at which the organic acids might chelate the cations bound to phosphate, hence making phosphorus available for the plants. Besides, the organic acids also may form soluble complexes with metal ions associated with insoluble phosphate, thus releasing the soluble phosphate to the environment. Hence, the lower of pH observed in this study was corresponding to the solubilization of phosphate bythe isolates. This was supported by El-Azeem et al. (2007); Perez et al. (2007) study, at which all isolates that solubilize tri-calcium phosphate (TCP) in liquid and solid media may create organic acids, leading the medium's pH to become acidic.

5.4.3 IAA Production by Identified PGPR Isolates

The most prevalent auxin found in nature is IAA. Over 80% of rhizosphere bacteria may be capable of producing IAA, according to Spaepen & Vanderleyden (2010). In some situations, large levels of IAA synthesis by bacteria may improve total root biomass, allowing the plant to better absorb water and nutrients, which can help bacteria colonise theroot system (Spaepen & Vanderleyden, 2010).

Bacteria may produce IAA in two pathways, at which through tryphtophan-dependent and tryptophan-independent means. In this study, the screening has been designated in similar way in which the IAA production of each isolate was tested with and without the presence of tryptophan as precursor. As expected, all isolates produced higher IAA with the presence of tryptophan indicating these isolates are producing IAA through a tryptophan-dependent pathway. According to Zhang et al. (2019), these pathways could be indole-3-pyruvate (IPA), tryptamine (TPM), indole-3-acetonitrile (IAN) or indole-3-acetamide (IAM).

Genome studies reported that about 68.5% and 11.9% of Proteobacteria catalyse

tryptophan into IPA and TPM, respectively (Zhang et al., 2019). This was correlated with this study, in which *Pseudomonas* sp. produced highest amount of IAA (26.07 µg/mL) in the presence of the precursor L-tryptophan. Thus, there is possibility that respective PGPR are catalysing tryptophan into IPA and TPM in producing IAA.

Nevertheless, the IAA concentration produced in this study is much lower in comparison to previous research by Oliveira et al. (2021). In their study, *Pseudomonas* sp. were shown to be able to manufacture IAA as much as 90 µg/mL, in the presence of high levels of L-tryptophan. These differences could be due to the difference in amount of tryptophan used in both studies. These was reported by Sasirekha et al. (2012), in which the*Pseudomonas* bacteria observed an increase in IAA production from 80 to 123 µg/mL withan increase of tryptophan supplementation from 0.1 to 0.5 g/L. Besides, this difference suggests that despite the same genera of PGPR was isolated, the PGP effects of respective PGPR upon different crop is vary, due to the factor of host specificity by the PGPR.

5.4.4 Siderophore Production by Identified PGPR Isolates

On the other hand, the siderophores manufacturing ability may be categorized eitheras a direct or an indirect mechanism of plant growth stimulating rhizobacteria. They are low-molecular-mass organic compounds created by microbes that offer Fe nourishment to plantsto help them flourish in low-iron environments. At the same time, the PGPR's siderophore binds to iron, reducing Fe availability and effectively preventing the spread of fungal infections (Ahmed & Holmstrom, 2014).

In the present study, seven out of nine isolates found to produce siderophore via their screening on CAS agar. There is no siderophore production was observed by *S. marcescens* and *B. cereus.* This outcome was contrary to Devi et at. (2016) study, at which reported

that an endophytic bacterium identified as *Serratia* sp. AL2-16 was able to produce siderophore in iron-deficient conditions. These was also observed in Akhtar et al. (2021) study, in which the isolated *B. cereus* demonstrated the ability to solubilize phosphate and generate siderophore, phytohormones (indole acetic acid, cytokinin, and abscisic acid), and osmolyte (proline and sugar). However, it is worth noting that the literature comparison was involving different host plant species and different environmentalcondition, which might result in the differences of the current outcomes.

Moreover, despite the wide usage of CAS agar medium in screening of siderophore production bacteria, it may generate false negatives for some non-halo producing bacterial strains. Thus, there is a possibility that *S. marcescens* and *B. cereus* generated afalse negative result yet might react positively in CAS liquid solution.

5.5 Ranking of Identified PGPR Based on PGP Traits

In order to evaluate the prospective of PGPR isolates for commercialization as biofertilizers, the ranking of identified PGPR isolates has been done. The PGPR were rated based on its in vitro PGP traits.

In the current study, all nine bacterial isolates displayed at least one of the assessed PGP properties, hence considered as multi trait PGPR (Rana et al., 2011). Based on the ranking, about five isolates ranked at the top of the scale capable to exert all the traits evaluated. *Pseudomonas* sp., *S. sciuri, P. extremaustralis* ranked as first, second, and third, respectively, on the ranking scale (Table 4.8). However, it might be ranked at lower level than other isolates who may express only one specific PGP trait, yet with greater efficiency. It is worth noting that the ranking method is based on PGPR isolatespotential to express multiple PGPs properties. This was done in order to fit the objective of this study, in

determining the existence of multi trait PGPR indigenous to the sago palm. Asa result, the usage of this index should only be used to aid in the decision-making process, and its output should not be taken in strongest term.

Among these identified genera, the capability of *Bacillus* indigenous to *Metroxylon sagu,* Rottb. as PGPR has been proven by Nemenzo and Rivera (2018). They observed the potential of PGP properties isolated from *Metroxylon sagu,* Rottb. pose a positive impact toward the development of corn and tomato crops. Being the predominant of *Bacillus* as PGPR, it was expected to observe such results. Moreover, considering the bacterial culture to be used as bioinoculant such as liquid biofertilizers, *Bacillus* sp. is found to be suitable candidate as it is more tolerant towards high temperature. This owes to its capability in producing heat shock protein as well as the existence of dormant and resistant endospores.

Meanwhile, the rest of genera included those ranked at the top of the scale was not found to be corresponded in any research relate to *Metroxylon sagu,* Rottb., thus suggesting a novel PGPR findings related to this crop. However, the capability of *Pseudomonas* sp. and *Bacillus* sp. as a microbial consortia PGPR has been well recognized in other crops such asin *Cannabis sativa* (Comeau et al., 2021). Besides, the strains *S. sciuri* was also found to exhibit substantial potential as biofertilizer in maize. This was reported by Akram et al. (2016), at which the *S. sciuri* was found to be halotolerant PGPR capable in enhancing maize growth while alleviating the cellular oxidate damage of maize in salt stress settings. Thus, the strains isolated in this study might hold the same potential in being an effective PGPR and capable to pose a positive impact towards the plant grown in stress environment. Nevertheless, considering various abiotic and biotic factors, the observation isnot applicable to current study for there is a need to conduct the field trial by respected

bacteria.

Furthermore, Marques et al. (2010) in their study stated that the increase in the measured parameters in *Zea mays* is most likely due to a combination of the PGP traits of the employed species. The similar trend was observed in the current study, at which all of the identified PGPR isolates possessed multiple PGP traits. Besides, some of the studies found better results when the bacteria were mixed inoculated in comparison to single inoculation of these strains. Hence, there might be a possibility that this combination of PGPR as microbial consortia could exert growth on the plant, more effectively. Thus, further research in evaluating the PGPR behaviour in greenhouse and field trials should be considered.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In the current study, the PGPR was successfully isolated from the soil samples collected from Dalat and Kuching Division of Sarawak, Malaysia. These PGPR was identified as *S. marcescens, P. extremaustralis, Bacillus* sp., *B. cereus, B. subtilis, B. thuringiensis, S. sciuri, Pseudomonas* sp. and *P. monteilii.* These PGPR were also found to possess more than one PGP traits and was successfully ranked based on bonitur scale method.

6.2 Recommendations

The presence of several uncultivable microorganisms in the current study is well aware. For instance, some of the indigenous nitrogen fixing bacteria associated with the sago palm might not isolated in this investigation. Furthermore, no efforts were made in this work to identify slow-growing or anaerobic microbes, which might play a key role in identifying some beneficial microbial interactions. Thus, it is recommended further research by using culture independent method such as DGGE to be conducted for the purpose of improving cultivability.

Besides, the use of alternate solidifying agents and modifying the medium preparation (separate sterilisation of phosphate and gelling chemicals during medium production) increased the cultivability of bacterial populations associated with the wheat rhizosphere (Youseif et al., 2021). Thus, this study suggested that in culture dependent

approach, such different culture methodologies, should be used in tandem to cultivate more bacteria and identify novel PGPR candidates.

In order to assess the efficacy of chosen isolates' PGPR behavior, more study will be required, including greenhouse and field testing. This study also recommended for a genomic analysis prior to the in vivo experiment to validate the absence of pathogenic genes and the existence of putative genes implicated in PGPR activity.

REFERENCES

Abd-Aziz, S. (2002). Sago starch and its utilisation. *Journal of Bioscience and Bioengineering, 94*(6), 526-529.

Abdelwahed, S., Trabelsi, E., Saadouli, I., Kouidhi, S., Masmoudi, A. S., Cherif, A., Mnif, W., & Mosbah, A. (2021). A new pioneer colorimetric micro-plate method for the estimation of ammonia production by plant growth promoting rhizobacteria (PGPR). *Main Group Chemistry*, 1–14. https://doi.org/10.3233/mgc-210077.

Acuna, J. J., Campos, M., Luz Mora, M., Jaisi, D. P., & Jorquera, M. A. (2019). ACCD-producing rhizobacteria from an Andean Altiplano native plant (*Parastrephia quadrangularis*) and their potential to alleviate salt stress in wheat seedlings. *Applied Soil Ecology, 136,* 184–190. https://doi.org/10.1016/j.apsoil.2019.01.005.

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University Science*, *26*(1), 1–20. https://doi.org/https://doi.org/10.1016/j.jksus.2013.05.001.

Ahmad, F., Ahmad, I., & Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research, 163*(2), 173–181. https://doi.org/10.1016/j.micres.2006.04.001.

Ahmed, E., & Holmström, S. J. M. (2014). Siderophores in environmental research: roles and applications. *Microbial Biotechnology, 7*(3), 196–208. https://doi.org/10.1111/1751-7915.12117.

Akhtar, N., Ilyas, N., Yasmin, H., Sayyed, R. Z., Hasnain, Z., A Elsayed, E., & El Enshasy, H.A. (2021). Role of *Bacillus cereus* in improving the growth and phytoextractability of *Brassica nigra* (L.) K. Koch in chromium contaminated soil. *Molecules*, *26*(6), 1569. https://doi.org/10.3390/molecules26061569.

Akram, M. S., Shahid, M., Tariq, M., Azeem, M., Javed, M. T., Saleem, S., & Riaz, S. (2016). Deciphering Staphylococcus sciuri SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (Zea mays L.). *Frontiers in Microbiology*, *7*. 867. https://doi.org/10.3389/fmicb.2016.00867.

Albdaiwi, R. N., Khyami-Horani, H., Ayad, J. Y., Alananbeh, K. M., & Al-Sayaydeh, R. (2019). Isolation and characterization of halotolerant plant growth promoting rhizobacteria from durum wheat (Triticum turgidum subsp. durum) cultivated in saline areas of the dead sea region. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01639.

Ali, A., & Shaari, N. (2015). Mismanagement of chemical agriculture in malaysia from legal perspective. *Procedia Economics and Finance*, *31*, 640–650. https://doi.org/10.1016/S2212-5671(15)01152-1.

Ali, S., Khan, M. A., & Kim, W. C. (2018). Pseudomonas veronii KJ mitigates flood stress-associated damage in Sesamum indicum L. *Applied Biological Chemistry*, *61*(5), 575–585. https://doi.org/10.1007/s13765-018-0392-2.

Alsohim, A. S. (2020). Influence of Pseudomonas fluorescens mutants produced by transposon mutagenesis on in vitro and in vivo biocontrol and plant growth promotion. *Egyptian Journal of Biological Pest Control*, *30*(1). https://doi.org/10.1186/s41938-020-00220-5.

Ambrosini, A., & Passaglia, L. (2017). Plant Growth-Promoting Bacteria (PGPB): Isolation and screening of PGP activities. *Current Protocols in Plant Biology*, *2*(3), 190–209. https://doi.org/10.1002/pb.20054.

Austin, B. (2017). The value of cultures to modern microbiology. *Antonie van Leeuwenhoek*, *110*(10), 1247–1256. https://doi.org/10.1007/s10482-017-0840-8.

Azhar, A., Asano, K., Sugiura, D., Kano-Nakata, M., & Ehara, H. (2022). Waterlogged conditions influence the nitrogen, phosphorus, potassium, and sugar distribution in sago palm (Metroxylon sagu Rottb.) at seedling stages. *Plants*, *11*(5), 710. https://doi.org/10.3390/plants11050710.

Bang, W., Onyimba, I., & Egbere, O. (2018). Bio-fertilizers as key player in enhancing soil fertility and crop productivity: a review. *Direct Research Journal of Agriculture and Food Science, 6*(3), 73–83. https://doi.org/10.26765/DRJAFS.2018.4815.

Baig, K. S., Zahir, Z. A., & Arshad, M. (2010). Comparative efficacy of qualitative and quantitative methods for rock phosphate solubilization with phosphate solubilizing rhizobacteria. *Soil and Environment*, *29*(1), 82-86. https://www.researchgate.net/publication/282762307_Comparative_efficacy_of_qualitative_and_quantitative_methods_for_rock_phosphate_solubilization_with_phosphate_solubilizing_rhizobacteria.

Baron, E. J. (1996). *Medical Microbiology 4th Edition*. [e-book] Galveston, Texas: University of Texas Medical Branch at Texas. Available through: https://www.ncbi nlm.nih.gov/books/NBK8406/.

Beneduzi, A., Ambrosini, A., & Passaglia, L. M. P. (2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology, 35*(4), 1044–1051. https://doi.org/10.1590/s1415-47572012000600020.

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., & Walsh, F. (2015). Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology, 13*, 310–317. doi: 10.1038/nrmicro3439.

Bergey, D. H., Krieg, N. R., & Holt, J. G. (1984). *Bergey's manual of systematic bacteriology*. Baltimore, MD: Williams & Wilkins.

Bevivino, A., Sarrocco, S., Dalmastri, C., Tabacchioni, S., Cantale, C., & Chiarini, L. (1998). Characterization of a free living maize rhizosphere population of Burkholderia cepacia: effect of seed treatment on disease suppression and growth promotion of maize. *FEMS Microbiology Ecology*, *27*(3), 225–237. https://doi.org/10.1111/j.15746941.1 998.tb00539. x.

Bhattacharyya, C., Banerjee, S., Acharya, U., Mitra, A., Mallick, I., Haldar, A., Haldar, S., & Ghosh, A. (2020). Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-72439-z.

Bintoro, M. H., Iqbal N., Muhammad, P., Agief J., Ahmad, F., & Ayulia, L. (2018). Growing area of sago palm and its environment. *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods* (pp. 17–29). Springer Singapore. https://doi.org /10.1007/9789811052699_2.

Borneo Post Online. (2018) *Uggah: Government committed to develop sago industry.* https://www.theborneopost.com/2018/07/19/uggahgovernment-committed-to developsagoi ndustry/#:~:text=%E2%80%9CUnder%20the%20Sago%20Development%2Programme,re habilitation%20of%20sago%20small%20holdings.&text=The%20total%20ago%20area%2 0in,marg inal%20increase%20over%20the%20years. Braem, G., De Vliegher, S., Supré, K., Haesebrouck, F., Leroy, F., De Vuyst, L. (2011).

Braem, G., De Vliegher, S., Supré, K., Haesebrouck, F., Leroy, F., & De Vuyst, L. (2011). (GTG)5-PCR fingerprinting for the classification and identification of coagulase-negative Staphylococcus species from bovine milk and teat apices: a comparison of type strains and field isolates. *Veterinary Microbiology*, *147*(1-2), 67-74.https://d oi.org/10.1016/j.vetmic .2010.0 5.044.

Campbell, R. (1985). *Plant Microbiology* (p. 191). Baltimore: Edward Amold.

Cappuccino, J. G., & Welsh, C. T. (2017). *Microbiology: a laboratory manual*. Pearson Education.

Castanheira, É. G., Acevedo, H., & Freire, F. (2014). Greenhouse gas intensity of palm oil produced in Colombia addressing alternative land use change and fertilization scenarios. *Applied Energy, 114*, 958-967. https://doi.org/https://doi.org /10.1016/j.apenergy.2013.09.010.

Chandini, Kumar, R., Kumar, R., & Prakash, O. (2019). The impact of chemical fertilizers on our environment and ecosystem. In *Research Trends in Environmental Science*. AkiNik Publications.

Comeau, D., Balthazar, C., Novinscak, A., Bouhamdani, N., Joly, D. L., & Filion, M. (2021). Interactions between Bacillus Spp., Pseudomonas Spp. and Cannabis sativa promote plant growth. *Frontiers in Microbiology*, *12*.https://doi.org/10.3389/fmicb.2021.715758.

Correa-García, S., Pande, P., Séguin, A., St-Arnaud, M., & Yergeau, E. (2018). Rhizoremediation of petroleum hydrocarbons: a model system for plant microbiome manipulation. *Microbial Biotechnology*, *11*(5), 819–832. https://doi.org/10.1111/1751-7915.13303.

Dardak, R. (2015, February 04). *Transformation of Agricultural Sector in Malaysia Through Agricultural Policy*. https://ap.fftc.org.tw/article/818.

Department of Agriculture Malaysia, DOA. (2014). *Industrial Crop Statistics, Malaysia.* http://www.doa.gov.my/index/resources/aktiviti_sumber/sumber_awam/makluat_pertanian/perangkaan_tanaman/perangkaan_tnmn_industri_2018.pdf.

Devi, K. A., Pandey, P., & Sharma, G. D. (2016). Plant growth promoting endophyte Serratia marcescens AL216 enhances the growth of achyranthes aspera l., a medicinal plant. *HAYATI Journal of Biosciences*, *23*(4), 173–180. https://doi.org/https://doi.org/10.1016/j.hjb.2016.12.006.

Devliegher, W., Arif, M., & Verstraete, W. (1995). Survival and plant growth promotion of detergent-adapted Pseudomonas fluorescens ANP15 and Pseudomonas aeruginosa 7NSK2. *Applied and Environmental Microbiology*, *61*, 3865–3871.

Dexter, H., & Howard. (1955). The preservation of bacteria by freezing in glycerol broth. *Department of Infectious Diseases*, 625. https://doi.org/10.1128/jb.71.5.625- 625.1956.

Dinesh, R., Anandaraj, M., Kumar, A., Srinivasan, V., Bini, Y. K., Subila, K. P., Aravind, R., & Hamza, S. (2013). Effects of plant growth promoting rhizobacteria and NPK fertilizers on biochemical and microbial properties of soils under ginger (Zingiber officinale) cultivation. *Agricultural Research*, *2*(4), 346–353. https://doi.org/10.100 7/s4000 30130080 8.

Dinnage, R., Simonsen, A. K., Barrett, L. G., Cardillo, M., Raisbeck-Brown, N., Thrall, P. H., & Prober, S. M. (2019). Larger plants promote a greater diversity of symbiotic nitrogen-fixing soil bacteria associated with an Australian endemic legume. *Journal of Ecology, 107*(2), 977–991. https://doi.org/10.1111/1365-2745.13083.

Egamberdieva, D., Kamilova, F., Validov, S., Gafurova, L., Kucharova, Z., & Lugtenberg, B. (2008). High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. *Environmental Microbiology, 10*, 1–9.

Egamberdieva, D., & Kucharova, Z. (2009). Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biology and Fertility of Soils, 45*, 563–571.

El-Azeem, S., Mehana, T., & Shabayek, A. (2007). Some plant growth promoting traits of rhizobacteria isolated from Suez Canal region. In *8th African Crop Science Conference Proceedings*, 1517-1525.

Ems, T., & Huecker, M. R. (2018, October 27). *Biochemistry, Iron Absorption*. https://www.ncbi.nlm.nih.gov/books/NBK448204/.

Etesami, H., & Beattie, G. A. (2018). Mining Halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.00148.

Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and prospects. *Ecotoxicology and Environmental Safety*, *156*, 225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013.

Fajar Irawan, A., Baskara, G., Wandri, R., & Asmono, D. (2020). Isolation and solubilisation of inorganic phosphate by *burkholderia* spp. from the rhizosphere of oil palm. *Pakistan Journal of Biological Sciences*, *23*(5), 667–673. https://doi.org/10.3923/pjbs.2020.667.673.

Fan, D., & Smith, D. L. (2021). Characterization of selected plant growth-promoting rhizobacteria and their non-host growth promotion effects. *Microbiology Spectrum*, *9*(1). https://doi.org/10.1128/Spectrum.00279-21.

FAO. (2019, May 15). FAO - News Article: Excessive reliance on agricultural commodities puts Sustainable Development Agenda at risk. *Www.fao.org*. https://www.fao.org/director-general/former-dg/da-silva/newsroom/news/detail/en/c/1195597/.

Ferguson, B. J., Mens, C., Hastwell, A. H., Zhang, M., Su, H., Jones, C. H., Chu, X., & Gresshoff, P. M. (2019). Legume nodulation: The host controls the party. *Plant, Cell and Environment*, *42*(1), 41–51. https://doi.org/10.1111/pce.13348.

Flach M. (1997). Sago palm (*Metroxylon sagu* Rottb.). Promoting the conservation and use of underutilized and neglected crops. *No. 13. Institute of Plant Genetics and Crop Plant Research*, Gatersleben.

Flores-Núñez, V. M., Amora-Lazcano, E., Rodríguez-Dorantes, A., Cruz-Maya, J. A., & Jan-Roblero, J. (2018). Comparison of plant growth-promoting rhizobacteria in a pine forest soil and an agricultural soil. *Soil Research*, *56*(4), 346. https://doi.org/10.1071/sr17227.

Gai, X., Liu, H., Liu, J., Zhai, L., Yang, B., & Wu, S. (2018). Long-term benefits of combining chemical fertilizer and manure applications on crop yields and soil carbon and nitrogen stocks in North China Plain. *Agricultural Water Management*. *208*, 384– 392.

Gevers, D., Huys, G., & Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of Lactobacillus species. *FEMS Microbiology Letters, 205,* 31-36.

Ghosh, P. K., Maiti, T. K., Pramanik, K., Ghosh, S. K., Mitra, S., & De, T. K. (2018). The role of arsenic resistant *Bacillus aryabhattai* MCC3374 in promotion of rice seedlings growth and alleviation of arsenic phytotoxicity. *Chemosphere, 211*, 407–419. https://doi. org/10.1016/j.chemosphere.2018.07.148.

Glick, B. R. (2010). Using soil bacteria to facilitate phytoremediation. *Biotechnology Advances*, *28*(3), 367–374. 10.1016/j.biotechadv.2010.02.001.

Gray, E. J., & Smith, D. L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biology and Biochemistry*, *37*(3), 395–412. https://doi.org/https://doi.org/10.1016/j.soilbio.2004.08.030.

Grover, M., Ali, S. Z., Sandhya, V., Rasul, A., & Venkateswarlu, B. (2011). Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology and Biotechnology, 27*, 1231–1240.

Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics*. 2014:701596. doi: 10.1155/2014/701596.

Guerrieri, M. C., Fanfoni, E., Fiorini, A., Trevisan, M., & Puglisi, E. (2020). Isolation and screening of extracellular pgpr from the rhizosphere of tomato plants after long-term reduced tillage and cover crops. *Plants*, *9*(5), 668. https://doi.org/10.3390/plants9050668.

Haghighi, B. J., Alizadeh, O., & Firoozabadi, A. H. (2011). The role of Plant Growth Promoting Rhizobacteria (PGPR) in sustainable agriculture effect of different gamma radiation on quantitative and qualitative characteristics of sesame (Sesamum indicum). *Advances in Environmental Biology*, *5*(10), 3079–3083.

Haghshenas, B., Nami, Y., Almasi, A., & Abdullah, N. (2017). Isolation and characterization of probiotics from dairies evaluation of sophorolipid produced by Starmerella bombicola using palm-based olein as carbon source. *Article in Iranian Journal of Microbiology*, *26*, 539.

Hariprasad, P., & Niranjana, S. R. (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant Soil*, *316*, 13–24. doi: 10.1007/s11104-008-9754-6.

Hashem, A., Tabassum, B., & Fathi Abd Allah, E. (2019). Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, *26*(6), 1291–1297. https://doi.org/10.1016/j.sjbs.2019.05.004.

Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, *3*(4), 307–319. https://doi.org/10.1 038/nrmic ro1129.

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*, *60*(4), 579– 598. https://doi.org/10.1007/s1321301001171.

He, L., Ying, G., Liu, Y., Su, H., Chen, J., Liu, S., & Zhao, J. (2016). Discharge of swine wastes risks water quality and food safety: Antibiotics and antibiotic resistance genes from swine sources to the receiving environments. *Environment International*, *92- 93*, 210–219. https://doi.org/https://doi.org/10.1016/j.envint.2016.03.023.

Heuer, H., Schmitt, H., & Smalla, K. (2011). Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology*, *14*, 236– 243. doi: 10.1016/j.mib.2011.04.009.

Hii, R. (2020, December 13). *Agriculture in Malaysia must be included in national policies on climate change*. https://www.malaysiakini.com/letters/554987.

Hutter, G., Schlagenhauf, U., Valenza, G., Horn, M., Burgemeister, S., Claus, H., & Vogel, U. (2003). Molecular analysis of bacteria in periodontitis: evaluation of clone libraries, novel phylotypes and putative pathogens. *Microbiology, 149*, 67-75.

Ilker, S., Sahriye, S., & Mustafa, K. (2007). An investigation of seasonal changes in nitrate contents of soils and irrigation waters in greenhouses located in Antalya-Demre region. *Asian Journal of Chemistry*, *19*(7), 5639–5646.

Ilyas N., & Bano A. (2012) Potential Use of Soil Microbial Community in Agriculture. In: Maheshwari D. (eds), *Bacteria in Agrobiology: Plant Probiotics.* Springer. https://doi.org/10.1007/978-3-642-27515-9_3.

Imada, E. L., Oliveira, A. L. M., Hungria, M., & Rodrigues, E. P. (2017). Indole-3-acetic acid production via the indole-3-pyruvate pathway by plant growth promoter *Rhizobium tropici* CIAT 899 is strongly inhibited by ammonium. *Research in Microbiology, 168*(3), 283–292. https://doi.org/10.1016/j.resmic.2016.10.010.

Ishii, S., & Sadowsky, M. J. (2009). Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution. *Environmental microbiology, 11*(4), 733–740. https://doi.org/10.1111/j.1462-2920.2008.01856.x.

Islam, S., Akanda, A. M., Prova, A., Islam, Md. T., & Hossain, Md. M. (2016). Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.01360.

Ji, S. H., Kim, J. S., Lee, C. H., Seo, H. S., Chun, S. C., Oh, J., & Park, G. (2019). Enhancement of vitality and activity of a plant growth-promoting bacteria (PGPB) by atmospheric pressure non-thermal plasma. *Scientific Reports*, *9(*1), 1–16. https://doi.org/10.1038/ s41598-018-38026-z.

Jiang, Y., Li, S., Li, R., Zhang, J., Liu, Y., Lv, L., Zhu, H., Wu, W., & Li, W. (2017). Plantcultivars imprint the rhizosphere bacterial community composition and associationnetworks. *Soil Biology and Biochemistry, 109*, 145–155. https://doi.org/1 0.10 16 / j.soilbio.2017.02.010.

Joseph, B., Ranjan, P. R., & Lawrence, R. (2007). Characterization of plant growth promoting rhizobacteria associated with chickpea. *International Journal of Plant Production, 1*, 141-151.

Kathleen, M., Samuel, L., Felecia, C., & Lesley, N. (2014). (GTG) 5 -PCR analysis and 16S rDNA sequencing of bacteria from Sarawak aquaculture environment. *International Food Research Journal, 21*(3), 915–920.

Kesavan, P. C., & Swaminathan, M. S. (2018). Modern technologies for sustainable food and nutrition security. *Current Science, 115*(10), 1876–1883. https://www.jstor.org/stable/26978518.

Khan, A., Singh, J., Upadhayay, V.K., Singh, A.V., & Shah, S. (2019). Microbial biofortification: a green technology through plant growth promoting microorganisms. *Sustainable Green Technologies for Environmental Management* (pp. 255–269). Springer. https://doi.org/10.1007/978-981- 13-2772-8_13.

Khatoon, Z., Huang, S., Rafique, M., Fakhar, A., Kamran, M. A., & Santoyo, G. (2020). Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. *Journal of Environmental Management*, 273, 111–118. doi: 10.1016/j.jenvman.2020.111118.

Kifle, M. H., & Laing, M. D. (2016). Isolation and screening of bacteria for their diazotrophic potential and their Influence on growth promotion of maize seedlings in greenhouses. *Frontiers in Plant Science, 6*. https://doi.org/10.3389/fpls.2015.01225.

Kim, M., Chun, J., Goodfellow, M., Sutcliffe, I., & Chun, J. (2014). Chapter 4: 16S rDNA genebased identification of bacteria and archaea using the eztaxon server. *New Approaches to Prokaryotic Systematics* (pp. 61–74). Academic Press. https://doi.org/ht tps://doi.org/10.1 016/bs.mim.2014.08.001.

Kloepper, J. W. (1978). Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the 4th international conference on plant pathogenic bacteria. *Station de Pathologie Vegetale et Phytobacteriologie* (pp 879–882). Anger (FRA).

Koch, R. (1883). New Research Methods for Detection of Microcosms in Soil, Air and Water.

Konuma, H. (2018). Status and outlook of global food security and the role of underutilized food Resources: sago palm. *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods* (pp. 3–16). Springer Singapore. https://do i.org/10.1007/978981105 2699_1.

Kudoyarova, G. R., Vysotskaya, L. B., Arkhipova, T. N., Kuzmina, L. Y., Galimsyanova, N. F., Sidorova, L. V., Gabbasova, I. M., Melentiev, A. I., & Veselov, S. Y. (2017). Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. *Acta Physiologiae Plantarum*, *39*(11), 253. https://doi.org/10.1007/s1173801725569.

Kuiper, I., Bloemberg, G. V., & Lugtenberg, B. J. (2001). Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. *Molecular Plant-Microbe Interactions, 14*, 1197–1205.

Kumari, S., Kiran, S., Kumar, P., & Singh, A. (2021). Optimization of siderophore production by bacillus subtilis dr2 and its effect on growth of coriandrum sativum. *Research Square*. https://doi.org/10.21203/rs.3.rs-567897/v1.

Kusai, N. A., & Ayob, Z. (2020). Bacterial diversity in peat soils of forest ecosystems and oil palm plantation. *Eurasian Soil Science, 53*(4), 485–493. https://doi.org/1 0.1134/s106422 9320040080.

Kuypers, M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Reviews Microbiology*. *16*(5), 263. https://doi.org/10.1038/nrmicro.2018.9.

Lihan, S., Benet, F., Awang Husaini, A. A. S., Apun, K., Roslan, H. A., & Hassan, H. (2021). Isolation and identification of plant growth promoting rhizobacteria from sago palm (*Metroxylon sagu,* Rottb.), *Tropical Life Sciences Research, 32*(3), 39–51. https://doi.org/10.21315/tlsr2021.32.3.3.

Lim, W. K. L., & Chung, H. H. (2020). Salt tolerance research in sago palm (metroxylon sagu rottb.): past, present and future perspectives. *Pertanika Journal of Tropical Agricultural Science, 43*(2), 91–105.

Lugtenberg, B. J., Chin-A-Woeng, T. F., & Bloemberg, G. V. (2002) Microbe–plant interactions: principles and mechanisms. *Antonie Van Leeeuwnhoek, 81*(1–4), 373– 383.

Mandal, A., Sarkar, B., Mandal, S., Vithanage, M., Patra, A. K., & Manna, M. C. (2020). Chapter 7: Impact of agrochemicals on soil health. *Agrochemicals Detection, Treatment and Remediation* (pp. 161–187). Butterworth Heinemann. https://doi.org/https:// doi.org/10.1016/B9780081030172.000076.

Manoharachary, C., & Mukerji, K. G. (2006). Rhizosphere biology an overview. *Soil Biology, 7*, 1–15.

Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S., & Castro, P. M. L. (2010). Assessment of the plant growth promotion abilities of six bacterial isolates using Zea mays as indicator plant. *Soil Biology and Biochemistry, 42,* 1229–1235. https://doi.org /10.1016/j.soilbio.2010.04.014.

Martins, A. O., Omena-Garcia, R. P., Oliveira, F. S., Silva, W. A., Hajirezaei, M. R., Vallarino, J. G., & Araújo, W. L. (2019). Differential root and shoot responses in the metabolism of tomato plants exhibiting reduced levels of gibberellin. *Environmental and Experimental Botanuy, 157,* 331–343. https://doi.org/10.1016/j.envexpbot.2018.10.036.

Matthes, M. S., Best, N. B., Robil, J. M., Malcomber, S., Gallavotti, A., & McSteen, P. (2019). Auxin EvoDevo: conservation and diversification of genes regulating auxin biosynthesis, transport, and signaling. *Molecular Plant, 12*(3), 298–320. https://doi.org/ 10.1016/j.molp.2018.12.012.

Mishra, P., Singh, P. P., Singh, S. K., & Verma, H. (2019). Sustainable agriculture and benefits of organic farming to special emphasis on PGPR. *In Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology, 75-87.* https://doi.org/10.1 016/B978-0-12-817004-5.00005-1.

Mohamad Naim, H., Yaakub, A. N., & Awang Hamdan, D. A. (2016). Commercialization of sago through estate plantation scheme in Sarawak: The way forward. *International Journal of Agronomy, 2016,* 1–6. https://doi.org/10.1155/2016/8319542.

Mohanty, P., Singh, P. K., Chakraborty, D., Mishra, S., & Pattnaik, R. (2021). Insight into the role of PGPR in sustainable agriculture and environment. *Frontiers in Sustainable Food Systems, 5*. https://doi.org/10.3389/fsufs.2021.667150.

Mohapatra, B. R., Broersma, K., & Mazumder, A. (2007). Comparison of five rep-PCR genomic fingerprinting methods for differentiation of fecal Escherichia coli from humans, poultry and wild birds. *FEMS Microbiology Letters, 277*(1), 98–106. https://doi.org/10.1111/j.1574-6968.2007.00948.x/.

Murad, M. W., Mustapha, N. H. N., & Siwar, C. (2008). Review of malaysian agricultural policies with regards to sustainability. *American Journal of Environmental Sciences*, *4*(6), 608–614. https://doi.org/10.3844/ajessp.2008.608.614.

Nadarajah, K., & Rahman, A. (2021). Plant–microbe interaction: aboveground to belowground, from the good to the bad. *International Journal of Molecular Sciences, 22*(19). https://doi.org/10.3390/ijms221910388.

Nascimento, F. X., Hernandez, A. G., Glick, B. R., & Rossi, M. J. (2020). Plant growth-promoting activities and genomic analysis of the stress-resistant *Bacillus megaterium* STB1, a bacterium of agricultural and biotechnological interest. *Biotechnology Reports*, *25*. https://doi.org/10.1016/j.btre.2019.e00406.

Neilands, J. B. (1981). Iron absorption and transport in microorganisms. *Annual Review of Nutrition, 1*, 27–46. https://doi.org/10.1146/annurev.nu.01.070181.000331.

Nemenzo, P., & Rivera, W. (2018). Characterization of potential plant growth-promoting rhizobacterial isolates from sago (*Metroxylon sagu*, Rottb.) palms. *The Philippine Agricultural Scientist, 95*(1), 99-105.

Novero, A., & Labrador, K. (2014). Isolation and characterization of bacterial endophytes associated with sago palm (*Metroxylon sagu*, Rottb.). *Asian Journal of Microbial Biotechnology and Environmental Sciences, 16*(4), 877-855.

OECD. (2020, April 29). *COVID-19 and the Food and Agriculture sector: Issues and Policy Responses*. https://www.oecd.org/coronavirus/policy-responses/covid-19-and-the- food-and-agriculture-sector-issues-and-policy-responses-a23f764b/.

Ogut, M., Er, F., & Kandemir, N. (2010). Phosphate solubilization potentials of soil Acinetobacter strains. *Biology and Fertility of Soils*, *46*(7), 707–715. https://doi.org/10. 1007/s00374-010-0475-7.

Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology*, *33*(11), 197. https://doi.org/10.1007/s11274-017-2364-9.

Olive, D. M., & Bean, P. (1999). Principles and applications of methods for DNA based typing of microbial organisms. *Journal of Clinical Microbiology, 37*, 1661-1669.

Oliveira, D. A., Ferreira, S. D. C., Carrera, D. L. R., Serrão, C. P., Callegari, D. M., Barros, N. L. F., Coelho, F. M., & Souza, C. R. B. (2021). Characterization of Pseudomonas bacteria of Piper tuberculatum regarding the production of potentially bio- stimulating compounds for plant growth. *Acta Amazonica*, *51*(1), 10–19. https://doi.org/10.1590/1809-4392202002311.

Oueriaghli, N., Castro, D. J., Llamas, I., Béjar, V., & Martínez-Checa, F. (2018). Study of bacterial community composition and correlation of environmental variables in Rambla Salada, a hypersaline environment in South-Eastern Spain. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01377.

Pande, A., Pandey, P., Mehra, S., Singh, M., & Kaushik, S. (2017). Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology, 15*(2), 379–391. https://doi.org/10.1016/j.jgeb.2017.06.005.

Paradis, S., Boissinot, M., Paquette, N., Belanger, S. D., Martel, E. A., Boudreau, D. K., Picard, F. J., Ouellette, M., Roy, P. H., & Bergeron, M. G. (2005). Phylogeny of the*Enterobacteriaceae* based on genes encoding elongation factor Tu and F-ATPase β-subunit. *International Journal of Systematic and Evolutionary Microbiology, 55*, 2013-2025.

Perez, E., Sulbarán, M., Ball, M. M., & Yarzábal, L. A. (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology and Biochemistry 39*, 2905–2914.

Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C., & Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology, 52*, 347–375. https://doi.org/10.1146/annurev-phyto-082712- 102340.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology*, 17, 362-370.

Pingali, P. L. (2012). Green revolution: impacts, limits, and the path ahead. *Proceedings of The National Academy of Sciences of the United States of America*, *109*(31), 12302– 12308. https://doi.org/10.1073/pnas.0912953109.

Prasad, M., Srinivasan, R., Chaudhary, M., Choudhary, M., & Jat, L. K. (2019). Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. *PGPR Amelioration in Sustainable Agriculture* (pp. 129-157). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-815879-1.00007-0.

Prashar, P., Kapoor, N., & Sachdeva, S. (2013). Rhizosphere: its structure, bacterial diversity and significance. *Reviews in Environmental Science and Bio/Technology, 13*(1), 63– 77. https://doi.org/10.1007/s11157-013-9317-z.

Punja, Z. K., Rodriguez, G., & Tirajoh, A. (2016). Effects of Bacillus subtilis strain QST 713 and storage temperatures on post-harvest disease development on greenhouse tomatoes. *Crop Protection*, *84*, 98–104. https://doi.org/10.1016/j.cropro.2016.02.011.

Puppala, K. R., Bhavsar, K., Sonalkar, V., Khire, J. M., & Dharne, M. S. (2019). Characterization of novel acidic and thermostable phytase secreting *Streptomyces* sp. (NCIM 5533) for plant growth promoting characteristics. *Biocatalysis and Agricultural Biotechnology, 18.* https://doi.org/10.1016/j.bcab.2019.101020.

Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*. https://doi.org/10.3389/fphys.2017.00667.

Rani, A., Souche, Y., & Goel, R. (2012). Comparative in situ remediation potential of Pseudomonas putida 710A and Commamonas aquatica 710B using plant (Vigna radiata (L.) wilczek) assay. *Annals of Microbiology*, *63*(3), 923–928. https://doi.org/10.1007/s13213-012-0545-1.

Rasool, S., Hameed, A., Azooz, M. M., Siddiqi, T. O., & Ahmad, P. (2013). Salt stress: causes, types and responses of plants. *In: Ecophysiology and Responses of Plants Under Salt Stress*. (New York, NY: Springer), 1–24. doi: 10.1007/978-1-4614-4747- 4_1.

Rathinasabapathi, B., Liu, X., Cao, Y., & Ma, L. Q. (2018). Phosphate-solubilizing *Pseudomonads* for improving crop plant nutrition and agricultural productivity. *In: Crop Improvement Through Microbial Biotechnology* (pp. 363–372). Elsevier. https:// doi.org/10.1016/B978-0-444-63987-5.00018-9.

Rekha, P. D., Lai, W. A., Arun, A. B., & Young, C. C. (2007). Effect of free and encapsulated Pseudomonas putida CC-FR2-4 and Bacillus subtilis CC-pg104 on plant growth under gnotobiotic conditions. *Bioresource Technology, 98*(2), 447–451.

Ribeiro, C. M., & Cardoso, E. J. (2012). Isolation, selection and characterization of root associated growth promoting bacteria in Brazil Pine (Araucaria angustifolia). *Microbiological Research, 167*(2), 69-78. https://doi.org/1 0.1016/j.micres.2011.03.003.

Rivas, R., Peix, A., Mateos, P. F., Trujillo, M. E., Martínez-Molina, E., & Velázquez, E. (2006). Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. *Plant and Soil*, *287*(1), 23–33. https://doi.org/10.1007/ s1110400 690 62y.

Rütting, T., Aronsson, H., & Delin, S. (2018). Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems*, *110*(1), 1–5. https://doi.org/10.1007/s10705- 017-9900-8.

Sabran, S. H., & Abas, A. (2021). Knowledge and awareness on the risks of pesticide use among farmers at Pulau Pinang, Malaysia. *SAGE Open*, *11*(4), 215824402110648. https://doi.org/10.1177/21582440211064894.

Samuel, S., & Muthukkaruppan, S. M. (2011). Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil. *Current Botany*, *2*, 22–25.

Sandanakirouchenane, A., Haque, E., & Geetha, T. (2017). Recent Studies on N2 Fixing Burkholderia Isolates as a Biofertilizer for the Sustainable Agriculture. *International Journal of Current Microbiology and Applied Sciences*, *6*(11), 2780–2796. https://doi .org/10.20546/ijcmas.2017.611.329.

Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., & Hernández-León, R. (2017). The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. *Spanish Journal of Agricultural Research*, *15*(1), e03R01. https://doi .org/10.5424/sjar/2017151-9990.

Sasirekha, B., & Shivakumar, S. (2012). Statistical optimization for improved indole-3-acetic acid (iaa) production by Pseudomonas aeruginosa and demonstration of enhanced plant growth promotion. *Journal of Soil Science and Plant Nutrition*. https://doi.org/10.4067/s0718-95162012005000038.

Schirawski, J., & Perlin, M. H. (2018). Plant microbe interaction 2017 - the good, the bad and the diverse. *International Journal of Molecular Sciences*, *19*(5), 1374. https://doi.org/10.3390/ijms19051374.

Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, *160*(1), 47–56. https://doi.or g/https://doi.org/10.1016/00032697(87)906129.

Sessitsch, A., Weilharter, A., Gerzabek, M. H., Kirchmann, H., & Kandeler, E. (2001). Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Applied and Environmental Microbiology*, *67*(9), 4215–4224. https://doi .org/10.1128/AEM.67.9.4215-4224.2001.

Shahid, M., Ahmed, T., Noman, M., Javed, M. T., Javed, M. R., Tahir, M., & Shah, S. M. (2019). Non-pathogenic Staphylococcus strains augmented the maize growth through oxidative stress management and nutrient supply under induced salt stress. *Annals of Microbiology*, *69*(7), 727–739. https://doi.org/10.1007/s13213-019-01464-9.

Shamsuddin, Z. H. (1994). Microbial inoculants for increased crop production. [Research UPM].

Sharma, A. (2017). A review on the effect of organic and chemical fertilizers on plants. *International Journal for Research in Applied Science and Engineering Technology*, *V*(II), 677–680. https://doi.org/10.22214/ijraset.2017.2103.

Shipton, W. A., Baker, A., Blaney, B. J., Horwood, P. F., Warner, J. M., Pelowa, D., & Greenhill, A. R. (2010). Nitrogen fixation associated with sago (*Metroxylon sagu*) and some implications. *Letters in Applied Microbiology*, *52*(1), 56–61. https://doi.org/10.1111/j.1472-765x.2010.02967.x.

Shrestha, A., Toyota, K., Okazaki, M., Suga, Y., Quevedo, M. A., Loreto, A. B., & Mariscal, A. A. (2007). Enhancement of nitrogen-fixing activity of Enterobacteriaceae strains isolated from sago palm (*Metroxylon sagu*) by microbial interaction with non- nitrogen fixers. *Microbes and Environments*, *22*(1), 59–70. https://doi.org/10.1264/jsme2.22.59.

Shrivastava, P., & Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences, 22,* 123–131.

Simons, M., Van der Bij, A. J., Brand, I., Weger, L. A., Wijffelman, C. A., & Lugtenberg, B. J. (1996). Gnotobiotic system for studying rhizosphere colonization by plant growth promoting Pseudomonas bacteria. *Molecular Plant-Microbe Interactions, 9,* 600– 607.

Sims, J. T., Maguire, R. O., & Hillel, D. (2005). Manure management. *Encyclopedia of Soils in The Environment* (pp. 402–410). Elsevier. https://doi.org/https://doi.org/10.1016/B01 234 85304/005154.

Singh, I. (2018). Plant Growth Promoting Rhizobacteria (PGPR) and their various mechanisms for plant growth enhancement in stressful conditions: a review. *European Journal of Biological Research*, *8*(4), 191–213. https://doi.org/10.5281/zenodo.1455995.

Singh, R. P., & Jha, P. N. (2016). The multifarious PGPR Serratia marcescens CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (Triticum aestivum L.). *PLOS ONE*, *11*(6). https://doi.org/10.1371/journal.pone.0155026.

Singh, V., Dincer, I., & Rosen, M. A. (2018). Life cycle assessment of ammonia production methods. *Exergetic, Energetic and Environmental Dimensions*, 935–959. https://doi.org/10.1016/b978-0-12-813734-5.00053-6.

Situmorang, E. C., Prameswara, A., Sinthya, H. C., Toruan-Mathius, N., & Liwang, T. (2015). Indigenous phosphate solubilizing bacteria from peat soil for an eco-friendly biofertilizer in oil palm plantation. *KnE Energy*, *1*(1), 65. https://doi.org/10.18502/ken.v1i1.324.

Slepetiene, A., Volungevicius, J., Jurgutis, L., Liaudanskiene, I., Amaleviciute-Volunge, K., & Slepetys, J. (2020). The potential of digestate as a biofertilizer in eroded soils of Lithuania. *Waste Management. 102*, 441–451. doi: 10.1016/j.wasman.2019.11.008.

Smith, S. E., & Read, D. J. (1997). *Mycorrhizal Symbiosis*. Academic Press.

Somers, E., Vanderleyden, J., & Srinivasan, M. (2004). Rhizosphere bacterial signalling: a love parade beneath our feet. *Critical Reviews in Microbiology*, *30*(4), 205–240.

Soumet, C., Ermel, G., Fach, P., & Colin, P. (1994). Evaluation of different DNA extraction procedures for the detection of Salmonella from chicken products by polymerase chain reaction. *Letters in Applied Microbology*, *19*, 294 – 298.

Spaepen, S., & Vanderleyden, J. (2010). Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology*, *3*(4). https://doi.org/10.1101/cshperspect.a001438.

Subba Rao, N. S. (1982). Advances in agricultural microbiology. In: Subba Rao NS, editor. *Studies in the Agricultura and Food Sciences* (pp. 295-303). Butterworth Scientific.

Tabassum, B., Khan, A., Tariq, M., Ramzan, M., Khan, M. S. I., Shahid, N., & Aaliya, K. (2017). Bottlenecks in commercialisation and prospects of PGPR. *Applied Soil Ecology, 121*, 102–117. https://doi.org/10.1016/j.apsoil.2017.09.030.

Taurian, T., Anzuay, M. S., Angelini, J. G., Tonelli, M. L., Ludueña, L., & Pena, D. (2010). Phosphate-solubilizing peanut associated bacteria: screening for plant growth- promoting activities. *Plant Soil, 329,* 421–431. doi: 10.1007/s11104-009-0168-x.

Taurian, T., Ibáñez, F., Angelini, J., Tonelli, M. L., & Fabra, A. (2012). Endophytic Bacteria and their role in legumes growth promotion. In *Bacteria in Agrobiology: Plant Probiotics* (pp. 141–168). https://doi.org/10.1007/978-3-642-27515-9_8.

Timmusk, S., Behers, L., Muthoni, J., Muraya, A., & Aronsson, A. C. (2017). Perspectives and challenges of microbial application for crop improvement. *Frontiers in Plant Science, 8,* 49. https://doi.org/10.3389/fpls.2017.00049.

Udikovic-Kolic, N., Wichmann, F., Broderick, N. A., & Handelsman, J. (2014). Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *The Proceedings of The National Academy of Sciences, 111*(42), 15202–15207. doi: 10.1073/pnas.1409836111.

United Nations. (2019). *World population prospects 2019*. https://population.un.org/wpp/ Publications/Files/WPP2019_Highlights.pdf.

Vagsholm, I., Arzoomand, N. S., & Boqvist, S. (2020). Food security, safety, and sustainability-getting the trade-offs right. *Frontiers in Sustainable Food Systems*, *4*, 1-14. https://doi.org/10. 3389/fsufs.2020.00016.

Vejan, P., Abdullah, R., Ismail, S., & Boyce, A. N. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability-a review. *Molecules*, *21*(5), 573-590. https://doi.org/10.3390/molecules21050573.

Verma, A., Kumar, S., Hemansi, K. G., Saini, J. K., Agrawal, R., Satlewal, A., & Ansari, M. W. (2018). Rhizosphere metabolite profiling: an opportunity to understand plant- microbe interactions for crop improvement. *Microbial Biotechnology*, 343–361. https://doi.org/10.1016/b978-0-444-63987-5.00017-7.

Versalovic, J., Koeuth, T., & Lupski, J. R. (1991). Distribution of repetitive DNA-sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research, 19,* 6823–6831.

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil, 255*(2), 571–586.

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., & Ali, S. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research, 184*(2016), 13–24.

Wang, F., Chen, S., Wang, Y., Zhang, Y., Hui, C., & Liu, B. (2018). Long term nitrogen fertilization elevates the activity and abundance of nitrifying and denitrifying microbial communities in an upland soil: implications for nitrogen loss from intensive agricultural systems. *Frontiers in Microbiology, 9*, 1-12. doi:10.3389/fmicb.2018.02424.

Wang, F. H., Qiao, M., Chen, Z., Su, J. Q., & Zhu, Y. G. (2015a). Antibiotic resistance genes in manure-amended soil and vegetables at harvest. *Journal of Hazardous Materials, 299*, 215–221. doi: 10.1016/j.jhazmat.2015.05.028.

Wang, X., King, J. I., Mayer, L. W., Tang, Y., Sussman, M., Liu, D., Poxton, I., & Schwartzman, J. (2015b). Chapter 29: A phylogenetic perspective on molecular epidemiology. *Molecular Medical Microbiology (Second Edition)* (pp. 517–536). Academic Press. https://doi.org/https://doi.org/10.1016/B9780123971692.000299.

Ward, M., Jones, R., Brender, J., De Kok, T., Weyer, P., Nolan, B., Villanueva, C., & Van Breda, S. (2018). Drinking water nitrate and human health: an updated review. *International Journal of Environmental Research and Public Health*, *15*(7), 1557. https://doi. org/10.3390 /ijerph15071557.

Wei, J. L. S. (2021, January 10). *Promote biofertilisers as alternative to chemical ones.* https://www.dailyexpress.com.my/read/4166/promote-biofertilisers-as-alternative-to-chem ical-ones/.

Weise, T., Kai, M., & Piechulla, B. (2013). Bacterial ammonia causes significant plant growth inhibition. *PLOS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0063538.

Weller, D. M., & Thomashow, L. S. (1994). Current challenges in introducing beneficial microorganisms into the rhizosphere. In O'Gara F, Dowling DN and Boesten B (Eds.), *Molecular Ecology of Rhizosphere Microorganisms, Biotechnology and The Release of GMOs (*pp. 1-18). VCH Verlagsgesellschaft.

Wilson, P. W., & Knight, S.C. (1952). *Experiments in Bacterial Physiology* (pp.49). Burguess.

World Population Trends. (2010). Unfpa.org. https://www.unfpa.org/world-population-trends.

World Weather Online. (n.d.). *Dalat climate weather averages* [PNG]. https://www.wo rldweatheronline.com/dalat-weather-averages/sarawak/my.aspx.

Wu, J., Kamal, N., Hao, H., Qian, C., Liu, Z., Shao, Y., & Xu, B. (2019). Endophytic *Bacillus megaterium* BM18-2 mutated for cadmium accumulation and improving plant growth in hybrid Pennisetum. *Biotechnology Reports, 24.* https://doi.org/10.1016/j. btre. 2019.e00374.

Xie, W., Shen, Q., & Zhao, F. (2018). Antibiotics and antibiotic resistance from animal manures to soil: a review. *European Journal of Soil Science*, *69*(1), 181–195. https://doi.org/10.1111/ejss.12494.

Yang, X., & Fang, S. (2015). Practices, perceptions, and implications of fertilizer use in East-Central China. *Ambio*, *44*(7), 647–652. https://doi.org/10.1007/s13280-015-0639-7.

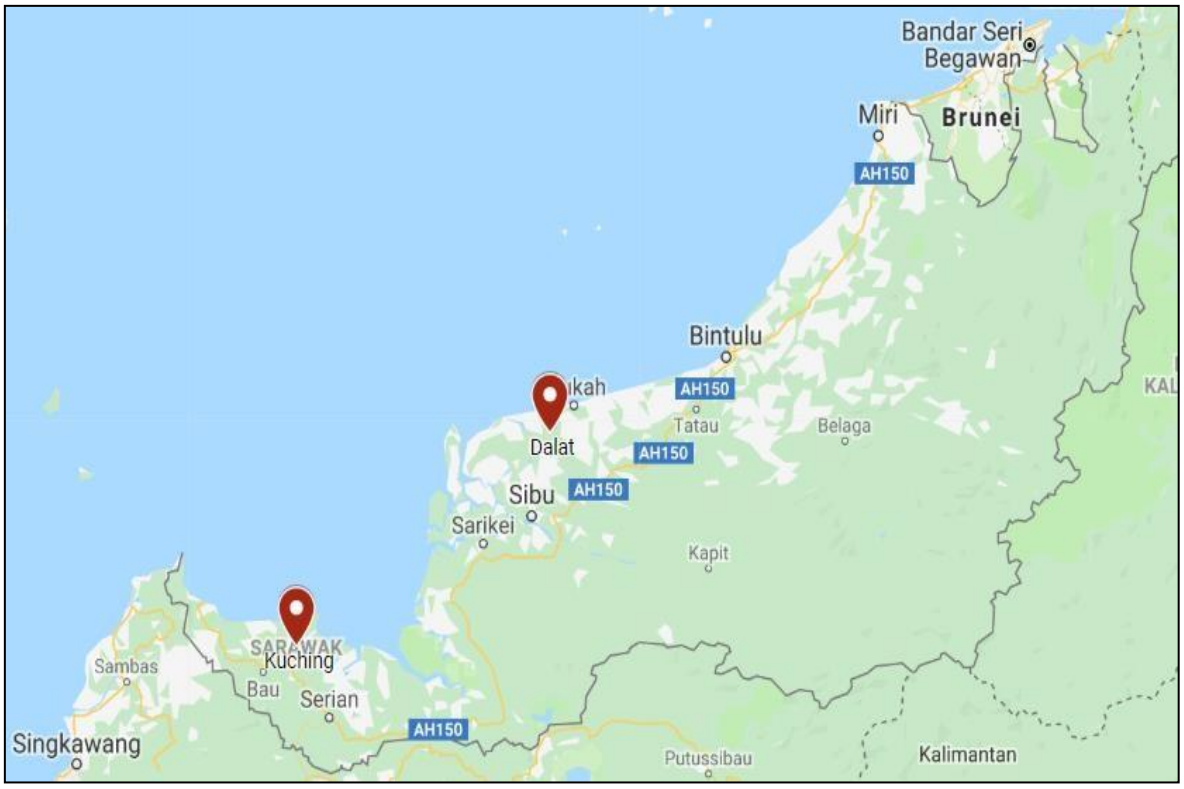
Youseif, S. H., Abd El-Megeed, F. H., Humm, E. A., Maymon, M., Mohamed, A. H., Saleh, S. A., & Hirsch, A. M. (2021). Comparative analysis of the cultured and total bacterial community in the wheat rhizosphere microbiome using culture-dependent and culture-independent approaches. *Microbiology Spectrum*, *9*(2). https://doi.org/ 10.1128/spectrum.00678-21.

Zhang, D. J., Yang, Y. J., Liu, C. Y., Zhang, F., Hu, W., Gong, S. B., & Wu, Q. S. (2018). Auxin modulates root-hair growth through its signaling pathway in citrus. *Scientia Horticulturae*, *236,* 73–78. https://doi.org/10.1016/j.scienta.2018.03.038.

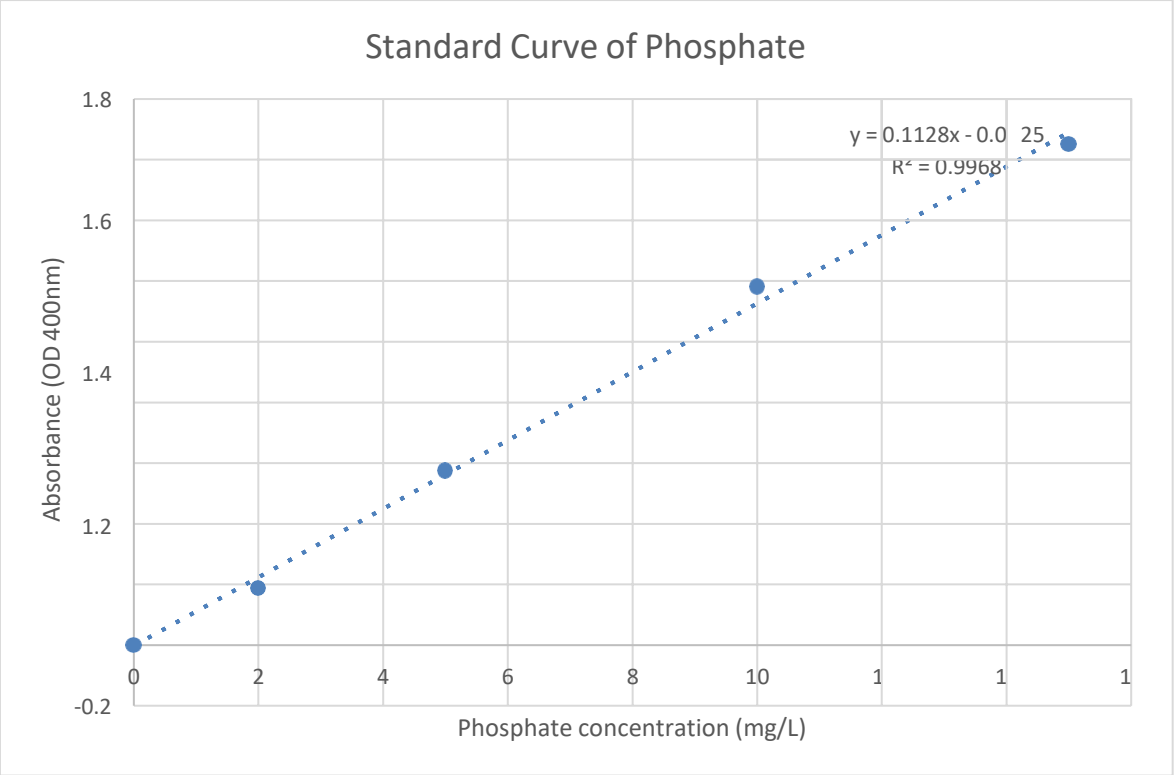
Zhang, P., Jin, T., Kumar, S. S., Xu, J., Shi, Q., Liu, H., & Wang, Y. (2019). The distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large-scale genomic analysis. *Molecules*, *24*(7), 1411. https://doi.org/10.3390/molecules 24 071411.

APPENDICES

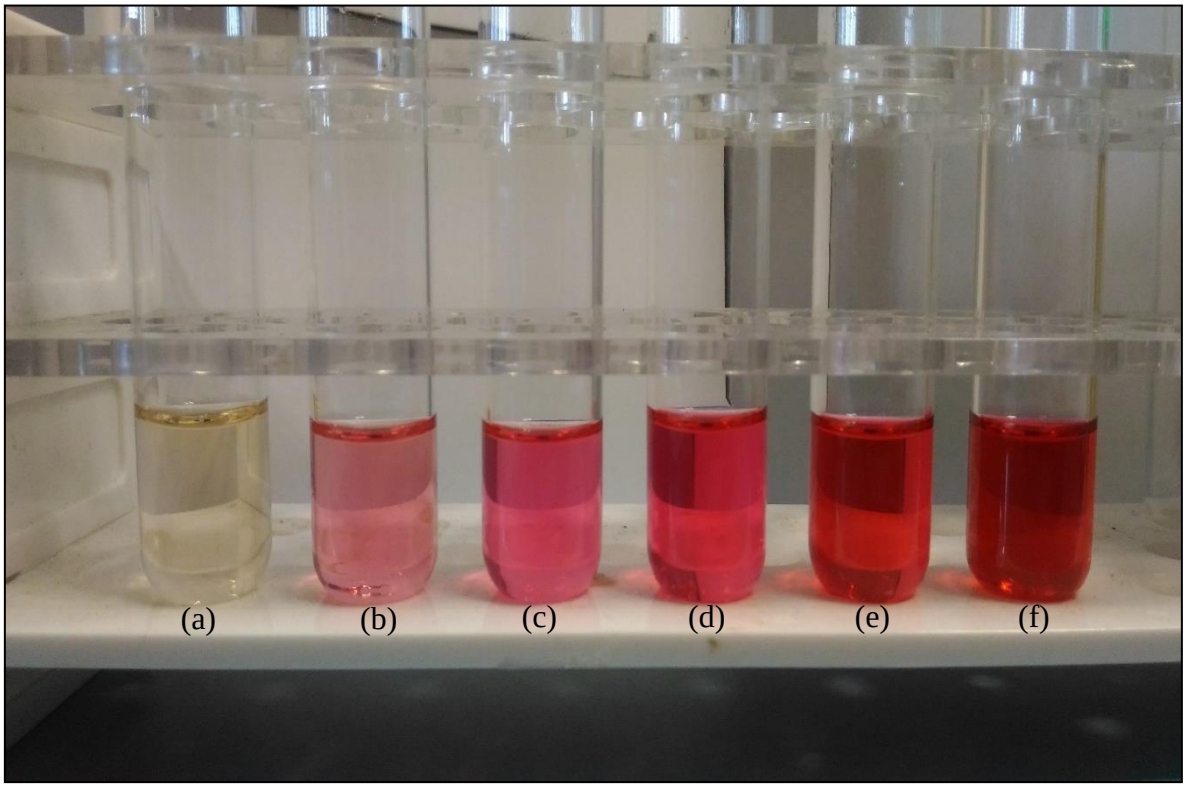
Appendix 1: The red marks indicated the sampling site's location chosen for this study, in Sarawak, Malaysia



Appendix 2: Standard curve of KH_2PO_4

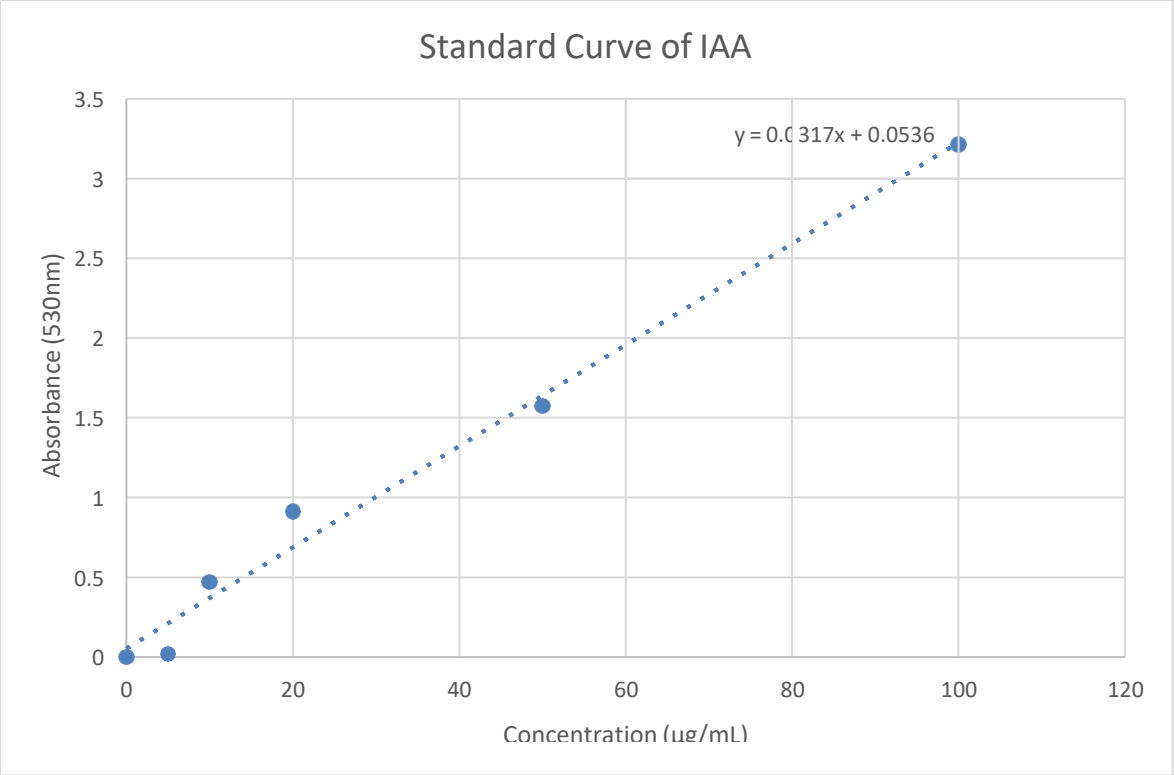


Appendix 3: Development of pink colour in different IAA concentrations

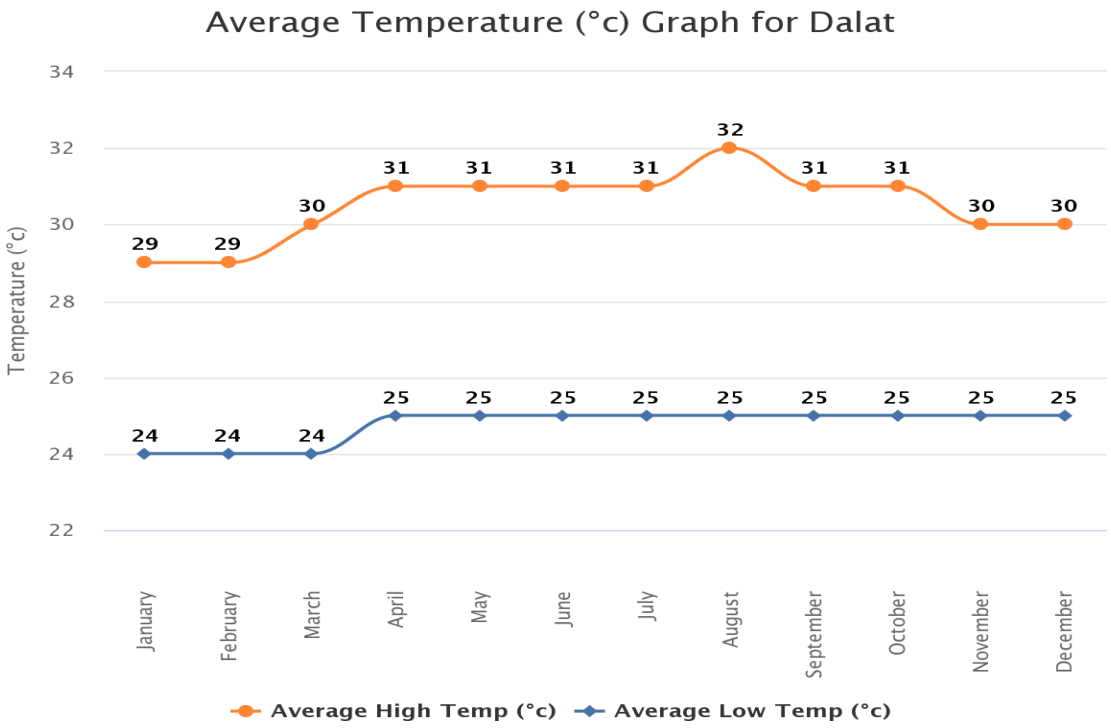


Note: (a) 0 µg/mL; (b) 5 µg/mL; (c) 10 µg/mL; (d) 20 µg/mL; (e) 50 µg/mL; (f)100 µg/mL

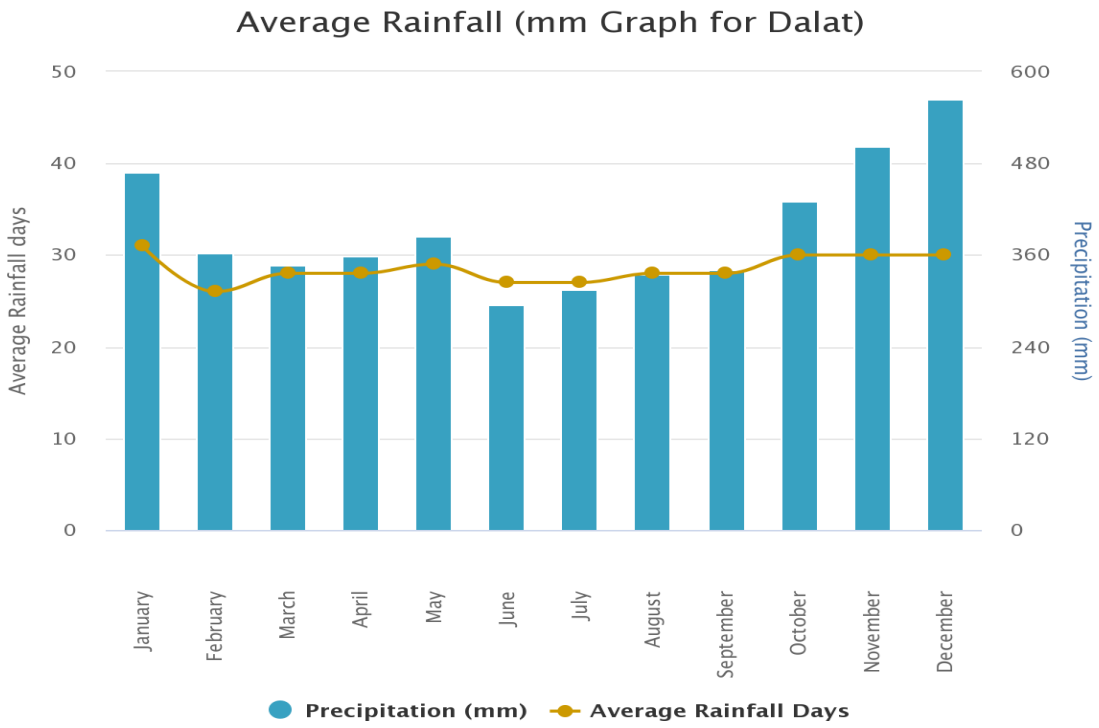
Appendix 4: Standard curve of IAA



Appendix 5: Dalat average temperature



Appendix 6: Dalat average rainfall



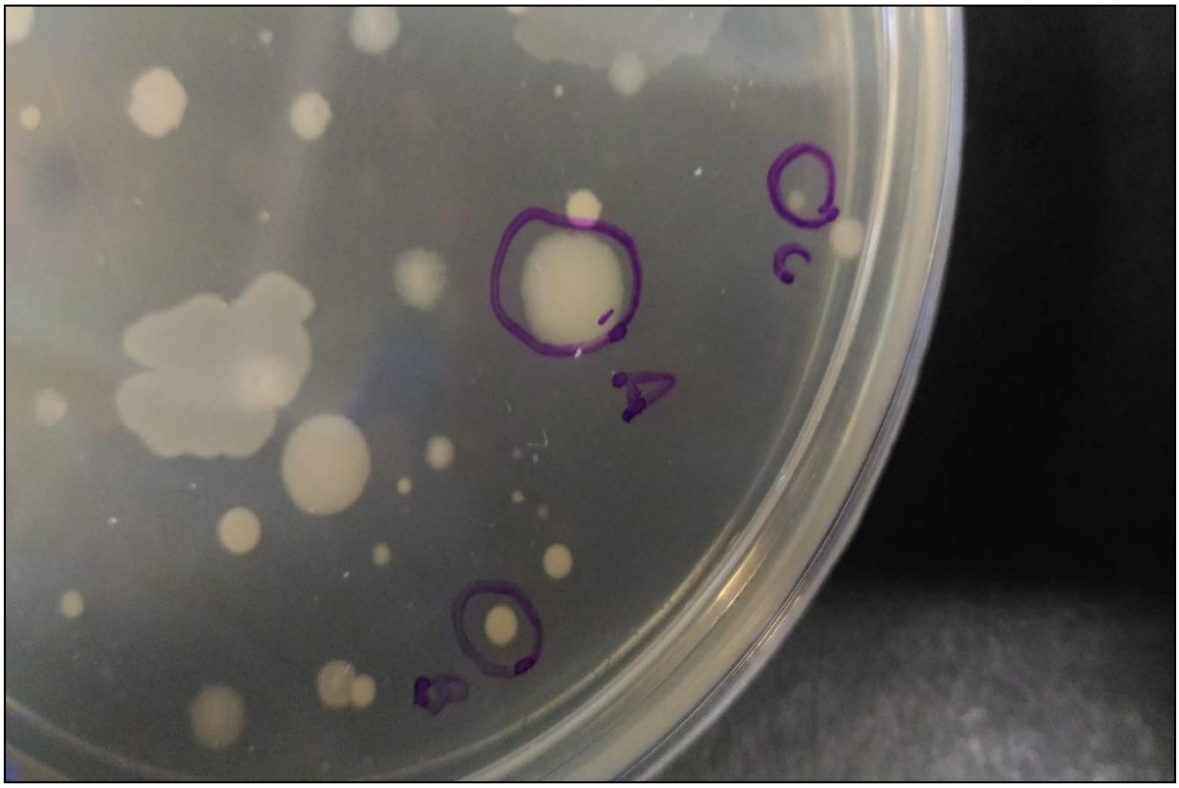
Appendix 7: Sago seedlings at sampling sites Kuching

Appendix 8: GPS coordinates for each sampling sites

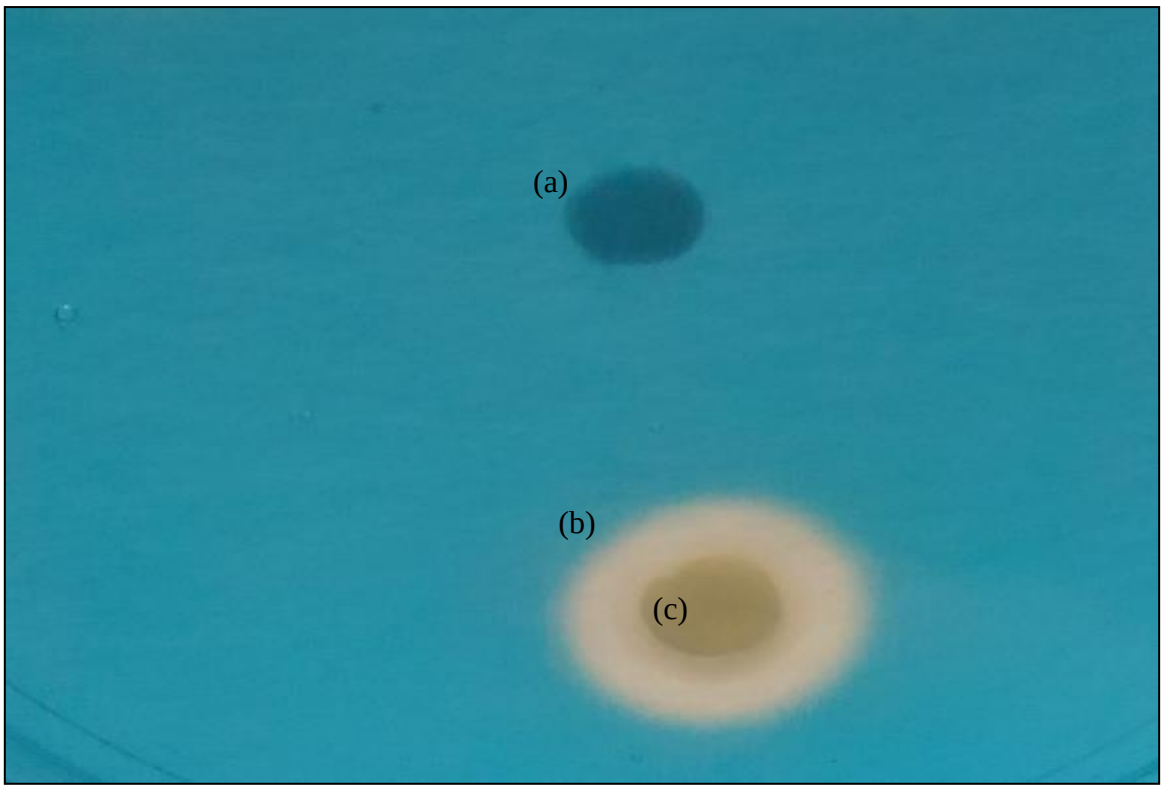
Locations	SoilSamples	GPSCoordinates
Dalat	Sample Nunau	2°45’14.32526’’N 111°56’48.61795’’E
	Sample Ugui	2°46’45.69676’’N,111°56’8.0025’’E
	Sample TP	°45'54.56592"N,111°56'15.34063"E
	Sample Tabo	2°42’15.79403’’N,111°55’22.59239’’E
Kuching	Sample M0139	1º37’37.2” N, 110º20’42.36” E
	Sample M063B	1º37’37.2” N, 110º20’42.36” E
	Sample CR	1º37’37.2” N, 110º20’42.36” E
	Sample SRP	1°24’05.9’’N 111°20’16.7’’E

Note: The soil samples were coded based on its location

Appendix 9: Examples of isolates with different morphology grown on Burks agar



Appendix 10: Example of solubilization zone formed in screening of siderophore production



Note: (a) Uninoculated control; (b) Halozone formed; (c) Colony growth