Isolation and Characterization of Effective Microorganism from Oil Palm Rhizospheric Soil and Evaluation of Their Potential as Biofertiliser

M N M Nor

1Faculty of Plantation and Agrotechnology, UiTM Cawangan Melaka Kampus Jasin, 77300, Merlimau, Melaka, Malaysia

Abstract. Plant nutrients are vital components of sustainable agriculture. Soil microorganisms play a significant role in regulating dynamics of organic matter decomposition and availability of plant nutrients such as nitrogen (N), phosphate (P) and sulphur (S), as well as stimulating plant growth. Soil microbial inoculants associated with rhizosphere region of plants work as biofertilisers. In this study, beneficial bacteria were isolated from rhizospheric soils collected from an oil palm plantation in Temerloh, Pahang, Malaysia. Rhizospheric soils (n = 6) were sampled from oil palm plants with heavy production of fresh fruit bunches, due to supplementation of biofertiliser. Isolation of bacteria was carried out on soil extract agar using a dilution plate method. Total bacterial count from isolation plates was in the range of $6.0 \times 10^7$ to $7.3 \times 10^7$ cfu per gram of soil. All bacterial isolates (n = 150) were purified and characterised for production of plant beneficial enzymes and hormones such as indole-3-acetic acid (IAA), siderophore, nitrogenase and phosphatase. Two bacterial isolates that were positive in phosphate solubilising assay, nitrogen fixers and IAA producers assay were identified as *Brevibacillus* sp., and *Burkholderia* sp. respectively, based on 16S rRNA gene sequences.

Keywords: Effective Microorganisms, Microbial Inoculants, Rhizospheric Soils, Plant Growth-promoting Rhizobacteria

1. Introduction
Oil palm (*Elaeis guineensis* Jacq.) is the most important industrial crops in Malaysia. Oil palm is a monocotyledon tree, belonging to the family Palmae and the genus *Elaeis*. Oil palm was commercially exploited as an oil crop when the first oil palm planting estate in Malaysia was established at Tennamaran Estate, Selangor in 1917 [1]. Approximately 90% of palm oil which is commercially fractionated into olein and stearin is used in food products. The remaining 10% is used as oleochemicals [2]. Recent investigations suggest the potential use of palm oil fatty acid esters as a biodiesel for engines combustion, similar to diesel fuel [3]. Various usages of palm oil has lead to a rapid growth of the oil palm industry. Latest statistics show that the planted area of oil palm in Malaysia as of the end of 2012 stood at 5.0 million hectares [4].

Recently, it has been reported that endophytic diazotroph bacteria such as *Acetobacter diazotrophicus*, *Herbaspirillum* spp., *Azoarcus* spp., *Pseudomonas* spp., *Proteus mirabilis* and *Azospirillum* spp., which colonised the root cortex of sugarcane, rice, maize and palm trees had enhanced plant growth. This has led to a considerable interest in exploiting these plant – microbe
interactions [5]. These bacteria have the potential to fix nitrogen, are able to produce IAA (indole-3-acetic acid), and showed some P-solubilising activity [6]. Shen et al., 2016 have reported that, introduction of diazotrophic rhizobacteria to the plant tissues during in vitro propagation process would maintain the beneficial organism within tissues of the host plant. Therefore, application of plant growth enhancer or microbial fertilizer can be considered as one of the potential alternative to mineral fertilizer[7].

Plant growth promoting rhizobacteria have been used as biofertilizer for over a century in the agricultural systems. Research has successfully shown that these bacteria has affected growth of the host plant significantly. Results from different experiments showed that up to 50 – 70 % yield increase were reported due to inoculation of plant growth promoting rhizobacteria [6]. Other reports on influence of plant growth promoting rhizobacteria for enhancing crops yields were also recorded for sugar beet, sugarcane, wheat, oil palm, maize, pineapple, kallar grass and rice [8].

The recommended rate of fertilizer suggested for mature oil palm on peat, is an annual application of 8.0 kg BA (bunch ash) palm\(^{-1}\) year\(^{-1}\), 5.0 kg Muriate of potash (MOP) palm\(^{-1}\) year\(^{-1}\) [9], 1.0 kg urea and rock phosphate [10] each to meet the nutrient demands of the oil palm. This high nutrient demand and high cost of mineral fertilizers for oil palm industry has encourage the growers to find cheaper alternatives that may contribute to efficient nutrient and better profit [11]. In addition, the high input of mineral fertilizers could also affect the environment, in terms of air, soil and water pollutions and contribute to health hazards and environmental pollution [12].

2. Methods

2.1 Rhizospheric soil sampling
Oil palm rhizospheric soil sample from locations where biofertilizers had previously been applied was collected on September 2013 (3° 32’ 11.9904” N, 102° 26’ 39.0516” E). 1 g rhizospheric soil from each oil palm rhizosphere was collected by scrapping the soil attached to the root of oil. The pH of soil samples were determined by suspending the samples in 9 mL of Ringer’s solution followed by measurement with pH meter.

2.2 Isolation of presumptive plant growth enhancing bacteria
Soil samples underwent serial dilution up to 10\(^{-5}\) in Ringer’s solution and spread plating were done onto soil extract agar. Soil extract agar was prepared as described by Hamaki et al., 2005 [13]. 1000g of soil was mixed in 2000 ml of 50mM NaOH and incubated overnight at room temperature. The mixture was filtered and the filtrate was centrifuged for 60 minutes at 18000 rpm. Then, the supernatant was sterilized by passing through a 0.2 µm membrane filter. 15 g of agar was dissolved with 500 ml of distilled water and autoclaved at 121°C for 15 mins. The medium was then left to cool prior to the addition of 500 ml of filtered supernatant and 50 mg/l cyclohexamide. The warm medium was then poured into petri dishes and left to be solidified. All the isolation plates were incubated at 28°C up to two weeks. Numbers of colonies obtained on all these agar plates were counted, and their colony characteristics were recorded.

2.3 In vitro assessment of Plant Growth Promoting traits of isolated Rhizospheric microflora
2.3.1 Qualitative screening of bacteria producing indole-3-acetic-acid (IAA) production
The production of IAA was carried out as described by Suttinan & Akira, 2009 [13]. Isolates were inoculated on yeast malt extract agar (YM) and incubated for 5 days at 28 °C. At the end of incubation, agar plugs (8 mm in diameter) were inoculated into 5ml YM broth containing 0.2% L-tryptophan (Sigma) using sterile cork borer. Broth cultures were incubated at 28°C with shaking at 125 rev/min for 7 days. After 7 days, broth cultures were centrifuged at 11 000 rev/min for 15 minutes. One milliliter of the supernatant was mixed with 2ml of Salkowski reagent. Formation of pink color indicated IAA production. Positive control used in the test was pure IAA (Sigma) while uninoculated broth was used as the negative control.
2.3.2 Qualitative screening of phosphate solubilisation
Production of phosphate was carried out by using National Botanical Research Institute’s phosphate growth medium (NBRIP) as described by Mehta and Nautiyal, 2000 [14]. Four strains were stabbed in triplicate on a single NBRIP plate by using sterile toothpicks. Both diameter of the halo zones and colony size were measured to the nearest millimeter after 14 d of incubation of the plates at 28 °C (Islam et al., 2006). Ability of bacteria to solubilize phosphate was indicated by the solubilization index which refer to the ratio of the total diameter of both colony and halo zone to the colony diameter (Edi Premono et al., 1996). Positive control used in phosphate solubilizing assay test was Pseudomonas aeruginosa while Escherichia coli was used as the negative control.

2.3.3 Qualitative screening of bacteria producing siderophore
Screening for siderophore producing bacteria was performed according to Meyer & Abdallah, 2008 [15]. The isolate were grown in the Succinate medium broth at 28°C for 2 days. A total of 4 wells were made by using a 5 mm cork borer on a Chrome Azurol Sulphonate (CAS) agar plate. Then, 100 µl of broth was transferred into each well. CAS plates were incubated at 28 °C for 4 to 8 hours and color changes of the medium were observed. Positive result was indicated by formation of yellow halo zone. Size of yellow halo zone surrounding each well was measured to the nearest millimeter. The positive control used in this assay was Deferoxamine mesylate salt (Sigma) SSM media whereas without inoculation was used as the negative control.

2.3.4 Qualitative screening of nitrogen fixing bacteria
To evaluate nitrogen fixing ability of rhizobacterial isolates plates of, pH 6.3 – 7.3 ±0.2 were prepared and streaked with rhizobacterial isolates. The evaluation for nitrogen fixing ability of rhizobacterial was carried out by using free Malate Medium as described by Joshi and Bohra (2011) [16]. The plates were incubated for 4 days at 28 °C. Isolates that able to fix nitrogen exhibited growth on the medium, and change the colour from purple to yellow.

2.4 Identification of potential plant growth promoting bacteria (PGPB)
16S rRNA gene sequencing of potent PGPB was performed to idenitify the organisms, 16S rRNA gene from genomic DNA of screened isolate was amplified using universal Forward Primer (27f) : 5’ TACGGYTACCTTGTTACGACTT 3’ and Reverse Primer 1492r 5’ AGAGTTTGATCMTGGCTCAG 3’ by means of polymerase chain reaction. The BLASTn search program (http://www.ezbiocloud.net/eztaxon) was used to look for nucleotide sequence homology. The sequence obtained was then aligned by ClustalW using MEGA 4.0 software [17] and a neighbor-joining (NJ) tree with bootstrap value 500 was generated using the software.

2.5 Test for Phytotoxicity of Selected Isolates
2.5.1 Surface sterilisation of maize seeds
The maize seeds used for this experiment were obtained from a Persatuan Peladang Malaysia. The maize seeds were soaked for overnight in distilled water. The distilled water was then drained out and the seeds were then soaked in 2 % sodium hypochlorite for five minutes. The seeds were then rinsed five times with sterile distilled water prior to use [18].

2.5.2 Seed viability test
Prior of the phytotoxicity test, seed viability test were first conducted to test the quality of the seeds. Seed viability test was conducted using the paper towel method [19]. One hundred surface sterilised seeds were placed on a damp paper towel in a petri dish and grown in at room condition (28 °C ± 2 °C). After seven days, the number of seeds the germinated was counted using formula:

\[
\left(\frac{\text{germinated seed} - \text{non germinated seed}}{\text{100}}\right) \times 100
\]
For the phytotoxicity analysis, 30 ml of ISP2 with 2% (w/v) agar was dispensed into plant tissue culture glass jars and autoclaved at 121°C for 15 minutes. Five ml of the bacteria suspension prepared earlier was then added into the cooled molten agar medium. The content was mixed well by gentle shaking and was left to solidify. Five surface sterilized maize seeds were placed onto the solidified agar surface and left to germinate under light condition for 24 hours per day of fluorescent lighting for 10 days. Agar medium without addition of spore suspension served as the control in this experiment. The root length, plant height, number of leaves and number of secondary roots were recorded. After 10 days of incubation, the plant height, main root length, number of leaves and number of secondary root branches were recorded. The mean and standard deviation of triplicate for selected isolates from each set were calculated. Data were analyzed using SAS software (windows). Mean separation was accomplished using Duncan’s Multiple Range Test. The statistical significance was determined at P ≤ 0.05.

2.6 Greenhouse experiment

Out of 150 isolates, two isolates were recognized as the potential isolates and were selected for greenhouse experiment. These two isolates were positive for 3 out of 4 enzyme and hormone assay. The greenhouse study was conducted at University Teknologi Mara (UiTM Jasin). One and half months old oil palm seedling clone D x P YANGAMBI obtained from Federal Land Development Agency (FELDA), Pusat Penyelidikan Pertanian Tun Razak, Jerantut, Pahang was used in this experiment as experimental plant. The seedlings were transferred into plastic pot containing sterilized soil (6 kg per pot). 38 cm x 45 cm black polybags was used for planting purposes. Roots of the seedling were washed clean from the nursery soil before planting them. A plastic sheet was placed below each polybag to avoid penetration of roots from the polybags into the nursery soils.

In this experiment, there were three types of treatments. The first treatment was the control treatment where the oil palm seedling is only fertilised using chemical fertiliser recommended by MPOB (Detail of chemical fertiliser as in Table 1). The second and the third treatment is the seedling that inoculated with SN 40 and SA 25 (1×10^8 CFU/ml). Once every two months, about 20 ml of each isolates (1×10^8 CFU/ml) was inoculated around the rhizosphere using sterile pipette. Seedling that was fertilized with chemical fertiliser as recommended by MPOB was used as control (Table 1). Oil palm seedlings were irrigated with same volume of water to each pot daily.

Destructive sampling was done after 120 days, 240 days and 360 days of growth. Days zero is counted from the first day of the plant seedling treated with the chemical fertiliser, SN 40 and SA 25. Parameters such as root dry weight, root volume, primary root numbers, top dry weight, total leaf area, plant height, stem diameter and leaf number were recorded. Data were analysed using SAS for Windows. Mean separation was accomplished using Duncan’s Multiple Range Test. The statistical significance was determined at P ≤ 0.05.

| Oil Palm age | Type of fertiliser      | Amount of fertiliser |
|--------------|-------------------------|----------------------|
| 0 to 150 days| NPK Yellow (15:15:6:4)   | 7 g / palm           |
| 150 to 270 days| NPK Yellow (15:15:6:4) | 14 g / palm          |
| 270 to 360 days| NPK Blue (12:12:17:2)  | 21 g / palm          |

Note: Supplied only to the control treatment
3. Results and Discussion

3.1 Colony counts of culturable microorganisms on soil extract agar

Table 2. Colony counts of culturable microorganisms on soil extract agar

| Sampling Point | pH     | Colony count (CFU/g) |
|----------------|--------|----------------------|
| 1              | 5.81   | \(6.1 \times 10^7\)  |
| 2              | 5.90   | \(6.6 \times 10^7\)  |
| 3              | 5.80   | \(7.1 \times 10^7\)  |
| 4              | 5.70   | \(7.3 \times 10^7\)  |
| 5              | 5.80   | \(6.0 \times 10^7\)  |
| 6              | 5.90   | \(6.9 \times 10^7\)  |

Note: cfu/g = colony forming unit per gram of soil

3.2 Screening Test

3.2.1 Screening for bacteria producing Indole acetic acid (IAA)

The IAA production assay was conducted to screen for the isolate that capable to produce IAA. Pink colour form was visible after addition of Salkowski reagent indicates the positive production of IAA. Figure 1. 22 bacterial isolates (14.67%) were successfully isolated as IAA producer from oil palm rhizosphic soil.

3.2.2 Screening for phosphate solubilising bacteria

In this experiment, a total of 150 of bacterial strains was isolated and purified from the rhizosphere of oil palm. All isolates were tested for their phosphate solubilizing activity using NBRIP medium supplemented with 1.5 % (w/v) Bacto-agar. The phosphate solubilisation indexes of the isolates were varied from 0.9 to 6.7 (Table 2). Solubilisation index is calculated by total diameter (colony + halo zone)/colony diameter [20]. The isolate SN 40 showed the highest phosphate solubilisation with index (6.7). Total of 22 isolates out of 150 (14.67 %) are able to solubilise phosphate.

Formation of halo zone around the colony indicates positive result, while no changes in the medium indicate negative result (Figure 2). Phosphate solubilisation ability of all oil palm rhizosphere isolates was further evaluated in NBRIP liquid broth medium. All isolates showed consistent results in solubilizing phosphate in both method; either liquid broth or agar. Consistent results of phosphate solubilisation were also reported by Nautiyal (1999).
Figure 2. Screening for phosphate solubilising bacteria using free NBRIP medium. Halo zone development (left) on the media showed positive result of phosphate solubilising test. No halo zone development (right) showed negative result of phosphate solubilising activity.

Table 3. Phosphate solubilization index by bacterial isolates in agar using National Botanical Research Institute’s phosphate (NBRIP) growth medium.

| Bacterial isolate | Phosphate solubilisation index |
|-------------------|--------------------------------|
| SN40              | 6.7                            |
| SA25              | 5.6                            |
| SN14              | 5.6                            |
| SN16              | 6.4                            |
| SN18              | 4.5                            |
| SN20              | 4.6                            |
| SN26              | 4.4                            |
| SN32              | 6.6                            |
| SS5               | 5.2                            |
| SS8               | 3.6                            |
| SS16              | 1.2                            |
| SS20              | 5.3                            |
| SA24              | 4.2                            |
| SA26              | 3.3                            |
| S12               | 1.9                            |
| S16               | 0.9                            |
| S31               | 4.4                            |

3.2.3 Screening for siderophore producing bacteria

The CAS assay was conducted to screen for the isolate that capable to produce siderophore. Production of the orange halo zones after four to eight hours of incubation indicates the positive result (Figure 3). These inhibition zones were categorised according to their respective strengths recorded by visual observation (qualitative). Sizes of the orange halo zone formed indicate the concentration of siderophore that able to be synthesised by the isolate.

Strong siderophore production was indicated when orange halo zone formed on the CAS agar is big (≥ 2.0 cm). Moderate siderophore production was indicated when the yellow zone diameter is range from
1.5 cm to 1.9 cm. Weak siderophore production was indicated when the yellow zone diameter is range from 0.7 cm to 1.4 cm. Out of the 150 isolates 30 isolates (20 %) are capable to produce siderophore.

![Figure 3. Positive results of siderophore test are labelled with D1, D2, D3, D4, D5 and S1. Negative results of siderophore test are labelled with B1 and B2.](image)

### 3.2.4 Screening for Nitrogen fixing bacteria (N-fixers)
Total of 22 isolates, (14.67 %) isolates out of 150 rhizobacteria were able to fix atmospheric nitrogen in vitro. Free Malate medium plates were incubated for 4 days at 28 °C as described by Joshi and Bohra (2011) [16]. Isolates that able to fix nitrogen exhibited growth on the medium, and change the colour from purple to yellow (Figure 4).

![Figure 4. Positive (+) and negative (-).](image)

### 3.3 Grouping of the isolates based on their results for hormones and enzymes test
From all the screening results in the experiment, all the isolates that screened were divided into 13 groups based on the pre-screening tests; phosphate solubilisation test, Indole-3-acetic acid (IAA), nitrogen fixing ability, and siderophore assay. Table 3 below shows the detail about how the isolates are grouped into each group. Isolates from Group 1(2 isolates) are capable of producing phosphatase, nitrogenase, and indole-30 acetic acid (IAA) hormone.
Table 4. Classification of bacteria based on enzymes and hormones production assays. A number of 150 bacteria were classified into 13 groups as listed. Production of enzyme and hormone were indicated as ‘positive (+)’ and otherwise, as ‘negative (-)’

| Pre-screening test | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 | GROUP 6 | GROUP 7 | GROUP 8 | GROUP 9 | GROUP 10 | GROUP 11 | GROUP 12 | GROUP 13 |
|--------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Phosphate          | +       | +       | +       | -       | -       | -       | +       | -       | -       | -       | -       | -       |         |
| IAA                | +       | -       | +       | -       | +       | -       | +       | -       | -       | -       | -       | -       |         |
| Nitrogen fixers    | +       | +       | -       | -       | +       | -       | -       | -       | +       | -       | -       | -       |         |
| Siderophore        | -       | -       | -       | -       | -       | +       | +       | -       | -       | -       | -       | -       |         |
| Number of strains  | 2       | 4       | 4       | 5       | 5       | 4       | 11      | 6       | 6       | 2       | 14      | 8       | 79      |

3.4 Identification of potential isolates

From the screening result, isolates from Group 1 were chosen for 16S rRNA nucleotide sequence analysis. The 16S rRNA nucleotide sequence of two selected strains for each strain; SA25 and SN40 is showed in Table 4. The nucleotide sequence were identified by the calculation of pairwise sequence similarity using global alignment algorithm, which was implemented at the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim, et al. (2012) [21]. All two isolates contained different nucleotide sequences for the 16S rRNA gene, indicating that they were different strains.

Strain SN 40 was closely related to *Burkholderia cenocepacia* LMG16656T (99.86 %), *Burkholderia contaminans* LMG23361T (99.85 %), *Burkholderia territiorii* LMG28158T (99.79%), and *Burkholderia cepacia* ATCC25416T (99.72 %) based on pairwise sequence similarity. Strain SA 25 was closely related to *Brevibacillus brevis* NBRC15304T (99.10 %), *Brevibacillus choshinensis* DSM85527T (98.96 %), *Brevibacillus formosus* DSM988T (98.81 %) and *Brevibacillus nitrificans* DA2T (98.81 %) in its neighbour.

Table 5. Blast results of the 16S rRNA gene sequences for all strains showing the closest relatives (type strains) based on nucleotide similarity.

| Strain | Sequence Length (bp) | Closest match                | Pairwise similarity (%) | Accession   |
|--------|----------------------|-----------------------------|-------------------------|-------------|
| SN40   | 1401                 | *Burkholderia cenocepacia*  | 99.8                    | JTDP01000003|
| SA25   | 1340                 | *Brevibacillus brevis*      | 99.1                    | AB271756    |

3.5 Phytotoxicity evaluation of the selected isolates

Before the potential strains were tested in a greenhouse trial, they were screened for phytotoxic activity. This assay was conducted using two selected isolates (SN 40 and SA 25) which is from Group 1 (Group that has most positive results from the assay). Maize seeds were preferred in this study compared to oil palm seedling because it have a faster germination rate (3 days) compared to oil palm seedling (14 to 21 days). The batch of maize seeds were chosen for this assay since it could germinate consistently at average germination rate of 84.12%.

The average plant height obtained from the assay was from 16.35 cm to 24.14 cm for each strain tested. The average of main root length was from 32.36 cm to 39.74 cm for each strain tested. The mean for plant height and main root length for the control seedlings were 16.35 cm and 32.36 cm respectively.

Generally, the treatment with strains SN 40 and SA 25 increase the height of the maize seedlings compared to the control seedlings. For the treatment with low dosage ($1 \times 10^6$ CFU/ml) of strains SN 40 and SA 25, the height was increased by 42.95 % and 23.37 % respectively. As for the high dosage ($1 \times 10^8$ CFU/ml) treatment, the plant height was increased by 47.66 % and 30.97 % respectively for
strains SN 40 and SA 25. The plant height was increased for the low dosage treatment and high dosage treatment.

The treatment with strains SN 40 and SA 25 also increase the main root length of the seedlings compared to control seedlings. Low dosage treatment with strains SN 40 and SA 25 enhance the main root length of the seedlings by 19.24 % and 15.08 % respectively, and it further increase in high dosage treatment to 22.79 % and 17.29 % respectively.

In the number of leaves produced by the maize seedlings, treatments with strains SN 40 and SA 25 also show improvement of the leaves number. Treatments with strain SN 40 promoted the number of leaves by 30.77 % in the low dosage treatment and by 38.46 % in the high dosage treatment. For strain SA 25, the number of leaves increased by 23.07 % and 38.46 % respectively in the low and high dosage treatments.

Treatments with strains SN 40 and SA 25 also improved the number of secondary roots. For strain SN 40, the number of secondary roots for the seedlings increased by 18.51 % and 37.03 % respectively in the low and high dosage treatments. Similarly, the number of secondary roots increased by 37.03 % and 33.33 % in the low and high dosage treatments with strain SA 25.

3.6 Greenhouse experiment

Results from the pot experiment (glasshouse experiment that harvested after D 120, 240 and 360 days of planting (D120, D240 and D360) demonstrated the potential of SA 25, (99.1 % of similarity to Breviballus brevis) and SN 40, (99.4 % of similarity to Burkholderia cenopacia) as a biofertiliser for oil palm seedlings through enhancing the development of roots (root dry weight, volume, and primary root numbers) and tops (total dry matter, top dry weight, leaf area, plant height and stem diameter) of the host plants as shown in Table 5.

Generally, inoculation of biofertiliser at two monthly intervals were more promising to the oil palm seedling growth (D 120-D 360) with similar or better response compared to the control with chemical fertiliser especially for all root growth observations, top dry weight and total dry matter of the host plants.

The above findings provided evidence that SN 40 and SA 25 are potentially effective biofertilisers and bioenhancers for sustainable oil palm seedling production

Table 6. Effects of rhizobacterial inoculation on plant height (cm plant⁻¹), stem diameter (mm plant⁻¹) and leaf numbers of oil palm seedlings at day 120, 240 and 360 days after planting in greenhouse conditions.

| Days | Treatments | Observations |
|------|------------|--------------|
|      |            | Height (cm plant⁻¹) | Stem Diameter (mm plant⁻¹) | Leaf number |
| 120  | Control    | 35.66 a        | 8.81 a                  | 5.25 a     |
|      | SN 40      | 39.62 b        | 9.048 b                 | 6.24 b     |
|      | SA 25      | 37.87 c        | 9.048 b                 | 6.24 b     |
| 240  | Control    | 68.31 a        | 17.03 a                 | 8.36 a     |
|      | SN 40      | 75.83 b        | 18.94 b                 | 10.14 b    |
|      | SA 25      | 76.45 b        | 18.80 b                 | 9.80 c     |
| 360  | Control    | 77.62 a        | 27.35 a                 | 10.12 a    |
|      | SN 40      | 81.60 b        | 32.10 b                 | 12.20 b    |
|      | SA 25      | 80.98 b        | 31.90 b                 | 11.99 b    |

Note: Means with the same letters are not statistically significant at 5% level for each respective harvest (D120, D240 and D360)
4. Conclusion

There is diverse soil microorganism presence in soil. Some of the microbes have multi-function to solubilise phosphate, fixing nitrogen and produce IAA hormone. Based on the preliminary screening 10.53% of total isolates able to solubilise phosphate, 9.02% isolates were produced IAA, 22.56% isolates were Nitrogen fixers and 17.29% isolates able to produce siderophore. 2.25% of the isolates capable to solubilise phosphate, fixing nitrogen and produce siderophore.

Based on the results from this experiment, it is clearly indicated that rhizobacterial strains especially SA 25 and SN 40 are potential biofertilisers. The characteristics of these two isolates which have multitasking activity (phosphate solubiliser, IAA producer and as nitrogen fixing bacteria) can be really promising to become the bests strain as biofertilisers. As PGPR the inocula tested had also enhanced growth and development of roots (root dry weight, volume and primary root numbers) and tops (total dry matter, top dry weight) of the host plants.

Enhancement in root growth and development were significantly better compared to the control supplied with chemical fertiliser. The inoculation process done at two monthly intervals was proven to give a better result compared to oil palm seedling that was supplied with chemical fertiliser. These strains are suitable for oil palm nurseries as the result from the inoculation of these two strains were really promising.

Acknowledgements
The authors wish to thank the Faculty of Plantation and Agrotechnology and University of Malaya for providing facilities for this research.

References
[1] Basiron Y and Weng C K 2004 The oil palm and its sustainability Journal of Oil Palm Research 16
[2] Pande G, Akoh C C and Lai O-M 2012 Palm Oil: Elsevier) pp 561-86
[3] Sumathi S, Chai S and Mohamed A 2008 Utilization of oil palm as a source of renewable energy in Malaysia Renewable and sustainable energy reviews 12 2404-21
[4] Norhidayu A, Nur-Syazwani M, Radzil R, Amin I and Balu N 2017 The production of crude palm oil in Malaysia Int. J. Econ. Manag 11 591-606
[5] Kumar A and Verma J P 2018 Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiological research 207 41-52
[6] Vejan P, Abdullah R, Khadiran T, Ismail S and Nasrulhaq Boyce A 2016 Role of plant growth promoting rhizobacteria in agricultural sustainability—a review Molecules 21 573
[7] Ajmal M, Ali H I, Saeed R, Akhtar A, Tahir M, Mehboob M Z and Ayub A 2018 Biofertilizer as an alternative for chemical fertilizers Journal of Agriculture and Allied Sciences 7 1-7
[8] 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 8 191-213
[9] Salleh N I, Phan T-P, Lau S, Yeoh C-B and Tay M-G 2019 Monitoring of Ammoniacal Nitrogen and Phosphate in the Leachates When Diluted Palm Oil Mill Effluent was Used as a Fertilizer Borneo Journal of Resource Science and Technology 9 56-64
[10] Tao H-H, Slade E M, Willis K J, Caliman J-P and Snaddon J L 2016 Effects of soil management practices on soil fauna feeding activity in an Indonesian oil palm plantation Agriculture, Ecosystems & Environment 218 133-40
[11] Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A and Tribedi P 2017 Biofertilizers: a potential approach for sustainable agriculture development Environmental Science and Pollution Research 24 3315-35
[12] Pesonen J, Kuokkanen T, Rautio P and Lassi U 2017 Bioavailability of nutrients and harmful elements in ash fertilizers: Effect of granulation Biomass and Bioenergy 100 92-7
[13] Hamaki T, Suzuki M, Fudou R, Jojima Y, Kajiura T, Tabuchi A, Sen K and Shibai H 2005 Isolation of novel bacteria and actinomycetes using soil-extract agar medium Journal of bioscience and bioengineering 99 485-92
[14] Mehta S and Nautiyal C S 2001 An efficient method for qualitative screening of phosphate-solubilizing bacteria Current microbiology 43 51-6
[15] Gupta A and Gopal M 2008 Siderophore production by plant growth promoting rhizobacteria Indian Journal of Agricultural Research 42 153-6
[16] Joshi S and Bohra A 2011 Screening of Rhizobacteria for their plant growth promotion abilities and their interaction with Rhizobium of Mung bean Journal of Applied and Natural Science 3 323-8
[17] Tamura K, Dudley J, Nei M and Kumar S 2007 MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0 Molecular biology and evolution 24 1596-9
[18] Bodhankar S, Grover M, Hemanth S, Reddy G, Rasul S, Yadav S K, Desai S, Mallappa M, Mandapaka M and Srinivasaraao C 2017 Maize seed endophytic bacteria: dominance of antagonistic, lytic enzyme-producing Bacillus spp 3 Biotech 7 232
[19] Sawma J T and Mohler C L 2002 Evaluating seed viability by an unimbibed seed crush test in comparison with the tetrazolium test Weed Technology 16 781-6
[20] Hariprasad P and Niranjana S 2009 Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato Plant and soil 316 13-24
[21] Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park S-C, Jeon Y S, Lee J-H and Yi H 2012 Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species International journal of systematic and evolutionary microbiology 62 716-21