Potency of nitrogen fixing bacteria isolated from POME disposal pond and their effect on the growth of *Caesalpinia pulcherrima* (L) Sw

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**Abstract.** Nitrogen fixing bacteria (NFB) play an important role to promote plant growth through nitrogen fixation and IAA hormone production. The objective of this research was to obtain the functional bacteria (nitrogen fixing bacteria) isolated from POME disposal pond that show the ability in nitrogen fixation and the potential as plant growth promoting rhizobacteria (PGPR), as well as to investigate the potential of these bacteria through in vitro and in vivo growth of *Caesalpinia pulcherrima* (L) Sw, and its effect on bacterial populations in media contaminated by POME. In vitro plant growth promoting activity characterization included N-fixing activity, Indole Acetic Acid production, and *C. pulcherrima* germination (inoculant + POME). In vivo plant growth promoting activity used sterile sand inoculated with NFB and watered with POME, whereas the control was not inoculated and watered with aquadest. Five strains were found as potential PGPR, namely *Azotobacter* sp., *Azospirillum* sp1-3., and *Rhizobium* sp. In addition, *Azospirillum* sp.1 is the best PGPR that produced highest seed vigour index (SVI), seed germination in vitro, and seed germination in vivo respectively. *Azospirillum* sp.1 and *Rhizobium* sp. have the ability to promote growth of *C. pulcherrima* in vitro and in vivo with bacterial populations as much as 10⁷cfu mL⁻¹ in POME contaminated media. The bacteria and *C. pulcherrima* are potential for reclaiming infertile soil.

1. **Introduction**

Indonesia held the largest number of palm oil plantation in the world, for about 7.796 million hectares in 2010 [1]. Palm oil processing plants are spread out in several cities alongside various waste concerns. The waste impacts negatively on natural resources and the environment, for instance pollution and depletion of natural resources that degrade the quality of the environment. The resulting waste is classified into three types: solid waste, liquid waste and gas. The characteristics of palm oil plant liquid waste are brown, acidic, viscous, odorous, and non-toxic in nature. It is called POME or Palm Oil Mill Effluent [2].

Currently POME and sludge are used as fertilizer. Direct use can indeed increase phosphorus, nitrogen, calcium and magnesium in the soil [3], but blockages and puddles occur in the soil [4]. Whereas indirect use is by isolating bacteria from POME disposal pond. Thompson *et al.* [5] reported that correct and precise bacterial isolation determined the suitability of bacteria in the selected waste water remediation process. Therefore, the principle of the bacterial isolates selection provides the optimal reduction of pollutant level. Naturally, the preferred bacteria are available in small amounts
and unwanted bacteria are present in large numbers. Isolation process must be done in order to multiply the number of preferred bacteria [6].

Bacterial isolates obtained from palm oil waste usually have microscopic morphological characteristics of cells belonging to Gram-positive [7] and Gram-negative [8]. Gram-negative heterotrophic rods such as Zoogloe, Pseudomonas, Chromobacter, Azorhobacter, Alcaligenes and Flavobacterium [9]. Moreover, it is found the functional bacteria that can fix N, dissolve P, produce IAA, and effective as a bio-stimulant for the growth of chili plants [8].

The roles of bacteria in wastewater treatment and their ability in maintaining the ecological balance of aquatic environments have been extensively studied. However, the research on the isolation of microbes from POME storage pond was scarce. It is the bases to further study about functional bacteria, particularly the plant growth promoting rhizobacteria (PGPR) that has the effective role in the process of soil health rehabilitation, which will be able to optimize plant growth, especially in waste-contaminated soil.

Functional bacteria are bacteria that could work synergically with organic fertilizers. These bacteria possess the ability to stimulate plant growth (bio-stimulant). The procedure is by synthesizing and regulating the concentrations of various Plant Growth Regulators (PGRs) such as indole-3 acetic acid or IAA [10], producing ACC-deaminase, and providing nutrients as bio-fertilizers. The process was done by N2 fixing from air through a symbiosis and dissolving P bound nutrients in soil by synthesizing PME-ase (Phosphomonoesterase). These bacteria should also be able to control the activity of pathogens from invasive organisms (bio-control), agrochemical compound modifier [11], and soil originated-pathogens (bio-protectant) by producing antipathogenic compounds or metabolites such as siderophore, chitinase, antibiotics, and cyanide [12].

Functional bacteria that have these abilities are usually classified as PGPR. When the bacteria are inoculated into seeds, sprouts, seedlings, or soil, they will directly colonize the root area of the plant (rhizosphere) aggressively and fertilize the soil, thus increase the supply of nutrients needed by the plant [13]. Some of the bacteria included in PGPR belong to the genus Flavobacterium, Herbaspirillum, Acetobacter, Acinetobacter, Alcaligenes, Azorhobacter, Bacillus, Beijerinckia, Burkholderia, Serratia, Enterobacter, Erwinia, Pseudomonas, Azospirillum, Azotobacter, and Rhizobium [14].

Some bacteria such as Rhizobium, Azotobacter, and Azospirillum are able to nitrogen fixation as well and produce plant growth hormones such as IAA, gibberellins, and cytokines [15]. Therefore, these genus are considered essential components of biological organic fertilizers [16; 17], especially as the fundamental materials of bioorganic fertilizers that are useful to stimulate plant growth in revegetating barren land due to pollution by POME waste.

Isolation, characterization and identification of bacteria isolated from POME disposal pond is one approach to verify that nitrogen fixing bacteria (NFB) is beneficial in promoting soil fertility and plant growth in soil contaminated with POME waste. The effectiveness of NFB is proven by laboratory and field testing on C. pulcherrima (L) Sw plant was used for bacterial affectivity test. Consideration of the use of C. pulcherrima (L) Sw plant, because this plant has the potential for reboisation in urban areas or recreational forests and also for potentially improving soil fertility.

C. pulcherrima (L) Sw to grow in sandy soils, clay soils, acidic and alkaline soils, and produce bioactive compounds that are useful for health purposes such as anti-inflammatory agent [18] and antiviral producers that are modulated by certain flavonoid compounds recognized as quercetin [19].

The objectives of this research were to obtain the functional bacteria isolated from POME disposal pond that show the ability in nitrogen fixation and the potential as PGPR, to investigate the potential of these bacteria on the growth of C. pulcherrima (L) Sw in vitro and in vivo as well as its effect on bacterial populations in media contaminated with POME. So it is expected that in the future the use of POME as an organic fertilizer is not only inexpensive but also as an alternative for excessive chemical fertilizer applications [20].
2. Materials and Methods

2.1. Isolation and identification
Sampling was carried out randomly in the first reservoir of POME waste (soil pond) in PTP VIII palm oil plantation, Cikasungka, Bogor regency, West Java, Indonesia. The first pool is a direct shelter from factory waste, so that the waste conditions are warm. Samples were taken from POME disposal pond in the form of sediment or soft soil. The isolation of bacteria was carried out in a selective media such as yeast mannitol agar / YEMA [18], Caceres [21], and mannitol Ashby [18]. It was prepared by putting ten grams of sediment in an Erlenmeyer flask containing 90 mL of sterile distilled water. The flask was shaken on a rotary shaker (120 rpm in 30 minutes). Serial dilution of $10^1$ to $10^7$ was made from each sample. About 0.2 mL of soil extract was taken and put into sterile Petri dish, then was poured over by selective medium (temperature: 50°C): YEMA (10 g manitol; 0.5 g K$_2$HPO$_4$; 0.2 g MgSO$_4$.7H$_2$O; 0.1 g NaCl; 1.0 g yeast; 20 g agar; 1 L aquadest + 2.5 ml L$^4$ Congo red solution) to obtain Rhizobium, manitol Ashby (20 g manitol; 0.2 g K$_2$HPO$_4$; 0.2 g MgSO$_4$.7H$_2$O; 0.1 g NaCl; 0.1 g K$_2$SO$_4$; 5 g CaCO$_3$; 20 g agar; 1 L aquadest) to obtain Azotobacter, and Caceres medium (0.5 g K$_2$HPO$_4$; 0.2 g MgSO$_4$.7H$_2$O; 0.1g NaCl; 0.5 g yeast extract; 0.02 g CaCl$_2$; 0.01 g FeCl$_3$.6H$_2$O; 5.09 g DL mallic acid, 4.8 g KOH; 20 g Agar; 15 mL L$^1$ Congo red solution) to obtain Azospirillum. All media were made at pH 7.0 and incubated at room temperature for 7 days. The isolate obtained was purified and stored on a Luria-Bertani (LB) slanted media. After 3 days of incubation, the isolates were identified by observing the morphological characters such as cell shape (cococcus, rod, short rod), gram positive / negative, cell motion (motile, spore formation, single, paired or chain), and cell movement (motile, spore formation, single, paired or chain). Biochemical properties of isolates were determined according to the Bergey’s Systemic Bacteriology method [22-24].

2.2. Functional bacteria characterization as PGPR

2.2.1 Nitrogen fixation. The selection of nitrogen fixing bacteria was performed based on the method of Dobereiner [25]. The bacteria obtained from selective media were inoculated into a test tube containing Nitrogen Free Bromthymol blue (NFB) semi solid medium without N elements (0.5 % DL-mallic acid; 0.4 % KOH; 0.05 % K$_2$HPO$_4$; 0.01 % MgSO$_4$.7H$_2$O; 0.005 % MnSO$_4$.H$_2$O; 0.002 % NaCl; 0.001 % CaCl$_2$; 0.005 % FeSO$_4$.7H$_2$O; 0.0002 % Na$_2$MoO$_4$.2H$_2$O; and 0.175 % bacto agar, and 2 ml 0.5 % bromthymol blue), then incubated at room temperature for 5-7 days. The bacterial activity of nitrogen fixing was indicated by the formation of a circular mist-like ring beneath the surface of the medium. Single bacterial cultures were prepared on a Petri dish and on a test tube, both containing tilted Caceres media. It was incubated for 2 x 24 hours at 30°C for further growth observation. The growth was an indication that the bacteria were nitrogen fixing bacteria.

2.2.2. The production of Indole-3 Acetic Acid. The presence of Indole-3 acetic acid produced by bacteria is one of significant indication of PGPR group. Isolates were inoculated into a 50 mL flask containing King B broth with 200 ppm tryptophan as physiological precursors of auxin biosynthesis in the IAA analysis. After that, it was incubated at room temperature for 24, 48, and 72 hours. About 2 mL of culture suspension was taken from each incubation period and centrifuged for 5 min. The supernatant was transferred into a test tube, and then added with 4 mL of Salkowski reagent. The production of IAA was indicated by the pink colour on the bacterial extraction [26]. Quantitative analysis of IAA production was measured using spectrophotometer at $\lambda$ 540 nm with interpolation on IAA calibration curve, afterwards.

2.3. The potency of NFB as PGPR during the Germination of C. pulcherrima (in vitro)

2.3.1. Bacterial augmentation on seed. A total of 30 swollen seeds after soaking in sterile aquadest were taken with tweezers and placed into one of the nitrogen fixing bacteria cultures on LB liquid
medium. Seeds were incubated in bacterial culture for 1 hour. The same procedure was repeated for soaking seeds in other bacterial isolates. A total of 450 seeds were augmented with 5 different nitrogen-fixing bacteria and the rest (90 seeds) were not augmented (control). All treatments replicated 3 times.

2.3.2. Germination. Based on previous research (Unpublished), a total of 30 seeds, swollen by soaking in sterile aquadest, were collected and planted in Petri dish covered by filter paper moistened with 5 mL of inoculant and 5 mL of 10 mL POME in 100 mL sterile distilled water solution. In contrast, the controls were not mixed with bacteria and POME. The characteristics of seed germination observed were germination percentage and sprout length after 7-10 days incubation. Parameters in germination studied were percentage of germination and vigour index [% Germination x (Average of shoot length + root length)]. The percentage of germination is the total number of germinated seeds divided by the total number of the seeds multiplied by 100 %. After that, the number of bacterial population that infected the sprouts root was calculated. One sprouts from each Petri dish (both control and treatment) was transferred into a test tube containing 9 mL of sterile aquadest (dilution to 10⁻¹), and mashed. The test tube was then pressed into a vortex for 2 minutes to mix the content. About 1 mL extract was diluted up to 10⁻⁵ dilution series. As much as 0.2 mL of extract was taken from each dilution series 10⁻², 10⁻³, and 10⁻⁵ and placed in a sterile Petri dish. After that, selective media was poured over into the Petri dish to attain the Azotobacter, Azospirillum, and Rhizobium. The culture was homogenized and incubated for 3 days at room temperature. The number of growing colony was counted with a colony counter to obtain colony-forming units.

2.4. The potency of BNF during the germination of C. pulcherrima (in vivo)

2.4.1. Seedling planting. Germination took place in Petri dish filled with sterile aquadest. After 3 days of incubation, the seeds that had germinated into sprouts were put into the Baker glass containing the liquid inoculants of the NFB (Rhizobium, Azotobacter, and Azospirillum). Two sprouts were planted on pots packed with sterile sand media (300 g). The sand media was dampened beforehand with a sterile distilled water (20 mL per pot) up to field capacity, then inoculated with 5 mL of liquid inoculant from each of the nitrogen-fixing bacterial isolates, and incubated for 5 days before planting. After planting, the moisture of the media and the maintenance of the potted plants were ensured by watering based on the treatment. POME solution with a ratio of 100 mL in 1000 mL was applied to potted plants containing Rhizobium, Azotobacter, and Azotobacter isolates 1 to 5, and for comparison watering with aquadest containing macro and micro elements or Müller’s method were done as well. Control plants were watered with sterile aquadest. Three replicates were prepared for each treatment. In the age of five weeks, the plants were harvested. Some data were measured, such as plant height, root length, number of branches, wet weight, dry weight, presence of embryo, and bacterial population per pot of all treatments by Total Plate Count.

3. Results and Discussion

3.1. Isolation and identification
Bacterial isolation from POME disposal pond on selective media (YEMA Congo red, Caceres, and Mannitol Ashby) resulted in 15 isolates and 5 isolates were identified manually. The identified isolates were Rhizobium sp. (1 isolate), Azotobacter sp. (1 isolate), and Azotobacter sp. (3 isolates).

3.2. Functional bacteria characterization as PGPR

3.2.1. Nitrogen fixation. The growth of 5 strains inoculated in a test tube containing semi solid Nitrogen Free Bromthymol Blue (NFB) medium showed that all the strains could form a ring-like circular mist below the media surface after 3 days incubation at room temperature (Figure. 1).
The ring was formed due to the production of nitrogenase by nitrogen fixing bacteria. Comparable results were reported by Baldani et al. [27], Kusumawati et al. [28], which stated that the formation of nitrogenase by nitrogen fixing bacteria was indicated by the presence of a circular fog beneath the surface of a semi-solid NFB medium. The formation of nitrogenase signified that the nitrogen fixating bacteria were absolutely motile. Caceres [21] reported that the formation of ring-like white mist beneath the surface of a semi-solid NFB media implies that the bacteria, especially Azospirillum sp. are motile. Further results confirmed that the bacteria belong to the nitrogen fixing bacteria group with asymmetrical circle, pink, flat, glossy, and smooth colony with flat edges (Figure 2). The pink colour of the bacterial colony was affected by Congo red in Caceres media that was absorbed into the cells by simple diffusion and fused to the protein component of cell membrane [29, 30].

3.2.2. The production of Indole Acetic acid. The ability of bacteria (Azospirillum sp.1-3, Azotobacter sp., and Rhizobium sp.) to produce of IAA growth hormone can be seen in Figure 3. The IAA test resulted in pink colonies of all 5 strains tested on NFB medium containing L-Tryptophan 200 ppm as precursor, after the addition of Salkowski reagent. Quantitatively, IAA test showed positive results as well, where the average IAA level reached the optimum level after 48 hours incubation. The IAA produced by bacteria was varied on the range from 0.117 – 0.443µg mL⁻¹ and the highest yield of IAA were observed in Azospirillum sp.1 (0.443 ppm).

This occurred because the 48-hour incubation was a logarithmic phase in which the effect of the enzymes used in the bioconversion of tryptophan to IAA such as tryptophan monoxygenase, IAA hydrolase, indol-pyruvate decarboxylase and IAA dehydrogenase, was sufficient and parallel to the growth rate. Therefore, IAA production reached the optimum level. After that, IAA production decreased at 72 hours incubation phase since the bacteria passed into the phase of death, so that IAA production decreased. The highest level of IAA production in the 48-hour incubation phase was also obtained in Gusniar [31] and Kresnawaty [32] studies. According to Bhattacharyya and Dey [33], the decrease in IAA production at 72 hours was due to the release of IAA degrading enzymes such as oxidase and peroxidase.
Shilev [33] reported that some bacteria from NFB group (symbiotic and non-symbiotic) are capable to generate growth hormone of IAA to stimulate lateral roots growth. The lateral roots can produce exudates and the nutrients that were absorbed by the roots thus increased bacterial population for enhanced inoculation effect [34].

3.3. The potency of NFB as PGPR during the germination of C. pulcherrima (in vitro)

The results of the potency of NFB as PGPR during the germination of C. Pulcherrima (in vitro), showed that the values of the ridicle length, hypocotyls length, germination percentage, index vigour, and bacteria population in infected root are statistically significant if compared among control and bacterial plus POME treatments with bacterial plus aquades treatments (Table 1). Shankar [35] reported that the value of vigour index on germination is important information to know the ability of growth of sprouts, normally under optimal and sub-optimal conditions. The vigour index is also an indication of seed strength for the seed growth and for dealing with environmental conditions that affect it [36]. The statement can strengthen the results of this study.

![Figure 3. The production of IAA by bacteria (µg mL⁻¹)](image)

### Table 1. The potency of nitrogen fixation bacteria during germination of C. pulcherrima (in vitro)

| Treatment               | Germination (%) | Redicule length (Cm) | Hypocotyl length (Cm) | Total length (Cm) | Index vigour | Σ Bacteria (10⁷Cfu mL⁻¹) |
|-------------------------|-----------------|----------------------|----------------------|------------------|--------------|------------------------|
| Control (uninoculated)  | 98±0.6          | 1.70±0.1             | 2.80±0.1             | 4.5±0.0          | 441.00±2.6  | 0                      |
| Azospirillum sp.1 + POME| 76±0.9          | 2.54±0.0             | 3.00±0.6             | 5.54±0.6         | 421.04±50.2 | 2.83±0.3               |
| Azospirillum sp.2 + POME| 70±1.7          | 1.52±0.3             | 2.40±0.3             | 3.92±0.3         | 274.47±22.5 | 2.33±0.3               |
| Azospirillum sp.3 + POME| 70±0.6          | 1.50±0.3             | 2.41±0.3             | 3.92±0.3         | 274.23±24.1 | 2.37±0.3               |
| Azotobacter sp. + POME  | 70±1.2          | 1.52±0.0             | 2.19±0.2             | 3.80±0.1         | 265.69±4.9  | 2.47±0.3               |
| Rhizobium sp. + POME    | 75±0.6          | 2.52±0.6             | 3.04±0.2             | 5.56±0.4         | 417.42±31.0 | 2.6±0.6                |
| Azospirillum sp.1 + aquades| 98±0.0         | 4.14±0.0             | 5.51±1.0             | 9.65±0.0         | 945.70±3.4  | 4.6±0.6                |
| Azospirillum sp.2 + aquades| 82±0.6         | 3.99±0.0             | 5.24±0.6             | 9.23±0.6         | 757.53±53.2 | 3.6±0.6                |
| Azospirillum sp.3 + aquades| 80±0.0         | 3.98±0.0             | 5.13±1.0             | 9.11±1.0         | 728.8±6.9  | 2.6±0.6                |
| Azotobacter sp. + aquades| 80±0.6         | 4.00±0.3             | 5.33±0.6             | 9.33±0.9         | 745.40±63.9 | 3.3±0.6                |
| Rhizobium sp. + aquades  | 86±1.2          | 4.02±0.0             | 5.22±0.0             | 9.39±0.1         | 807.39±5.4  | 3.5±0.6                |

Notes: ± SD is value from 3 replications. The numbers followed by the same letter are not significantly different at (p<0.05) level of Duncan’s test.

The results of germination in this study show that the germination of C. pulcherrima in Azospirillum sp.1-3, Azotobacter sp., and Rhizobium sp. inoculation treatment watered with aquades were better than in watered with POME and control treatment (germination without bacteria and POME); whereas the percentage of germination index vigour of C. pulcherrima in control were better than in Azospirillum sp.1-3, Azotobacter sp., and Rhizobium sp. inoculation treatment watered
with POME, except parameters of radicles length, hypocotyl length, total length, and bacterial population. The highest percentage of germination (98%) and vigour index (945.70) of C. pulcherrima was produced by seeds inoculated with Azospirillum sp.1 plus aquades and the highest value of other germination is also found in the control (98%), but other values of parameters are very low. The values of the parameters with the lowest and highest effects of all treatment on the treated C. pulcherrima are shown in sequence i.e. Azotobacter sp. plus POME (percent of germination = 70%; radicle length = 1.52 cm; hypocotyl length = 2.19 cm; Total length = 3.8 cm, index vigour = 265.69; bacteria population = $2.47 \times 10^7$ cfu mL$^{-1}$); Azospirillum sp.1 plus aquades (percent of germination = 98%, radicle length = 4.14 cm; hypocotyl length = 5.51 cm; Total length = 9.85 cm, index vigour = 945.7; bacteria population = $4.60 \times 10^7$ cfu mL$^{-1}$). This experiment showed the same results (low vigour index value), specially on the germination of C. pulcherrima in Azospirillum sp., Azotobacter sp., and Rhizobium sp. inoculation treatment watered with POME. This may be due to the inoculums concentration of Azospirillum sp., Azotobacter sp., and Rhizobium sp. plus POME that inhibits the imbibitions process, thus lowering the value of the vigour index and progress of germination process. This phenomenon occurred possibly due to the concentration of inoculants plus POME on the seeds that inhibited the process of seed imbibitions. The imbibitions stage is an early stage that determines the success of germination in which the fusion of fluid into the seeds is very important for the regulation and activation of growth hormone in the seeds.

Kuswanto [36], stated that the presence of inhibitors in seeds, seed exterior, solutions with high osmotic pressure, metabolic pathways inhibitors or respiratory rate inhibitors inhibits seed germination, for example by the competition of oxygen (O$_2$) are an inhibitor of growth germination. These conditions restrain the germination or slow down the process, therefore germination will continue despite the suboptimal or stressed condition resulting in the low value of the vigour index.

According to Harper and Lynch [37] germination was inhibited due to the competition between Azospirillum sp., Azotobacter sp., and Rhizobium sp. all which are viable to the seeds of C. pulcherrima in the use of oxygen (O$_2$) for respiration. Competition occurred due to the Azospirillum sp. and Azotobacter sp. are aerobic that requires oxygen (O$_2$). These bacteria are also able to live independently in nature so that these bacteria will not be bound to the growth of sprouts from C. pulcherrima to survive. Sprouts at the beginning until the growth of first leaves are able to perform photosynthesis for the growth comes from the reaction of aerobic respiration that breaks down food stored on the seeds [38].

Thus presence of competition indicated by the vigour index was affected by the characteristic of each bacterial isolate in the competition of O$_2$ which was determined from the respiration rate of the bacteria and the ability to colonize during germination by sticking to the root surface and infecting the roots. Bacteria capable of colonizing during germination are bacteria that can be associated and symbiotic with C. pulcherrima (in vitro). Thus the result of the in vitro germination selection of bacterial potency showed that isolate Azospirillum sp.1 and Rhizobium sp. were effectively associated and symbiotic with C. pulcherrima plant.

The results of bacterial population calculations showed that all isolates can be associated and symbiotic with C. pulcherrima plant. The highest bacterial population in root germination of C. pulcherrima was observed on Azospirillum sp.1 and Rhizobium sp. According to Dennis et al. [39], the number of bacterial populations associated with a plant is based on the bacteria characteristic and exudates preference and the characteristics of different plant exudates.

3.4. The potency of functional bacteria during the germination of C. pulcherrima (in vivo)

Table 4 showed the potency of Azospirillum sp., Azotobacter sp., and Rhizobium sp. as PGPR in C. Pulcherrima in vivo seedling. It was shown the germination of C. pulcherrima in Azospirillum sp., Azotobacter sp., and Rhizobium sp. inoculation treatment watered with POME was better than in watered with aquades and both treatment was better than control treatment (germination without bacteria and POME).

The treatment of bacteria inoculation as PGPR on C. pulcherrima had presented any difference in all parameters measured six weeks after planting, except for number of leaf branches. The best
bacteria as PGPR on the plant of *C. pulcherrima* watered with POME and aquades are belonging to *Azospirillum* sp.1 and *Rhizobium* sp. The highest value of parameter (shoot length, root length, fresh weight, dry weight, and bacteria population) generated by *Azospirillum* and *Rhizobium* bacteria in pot contaminated POME are respectively 10 and 9.9 cm, 6.63 and 6.60 cm, 0.57 and 0.57 g, 0.10 and 0.09 g, 32.67 x 10^7 and 14.67 x 10^7 cfu g^-1. Thus POME watering has a better impact of *C. pulcherrima* growth and bacteria population than aquadest watering. The same results with the same treatment (POME watering) and the same test plant (*C. pulcherrima*) were obtained on the results of the study. This proves that the organic waste could be renewed into fertilizer, citric acid, bioethanol, biohydrogen, bioplastic, and low cost hydrolytic enzymes [40]. According to Basiron and Wang [41], it occurred due to the transformation of POME into fertilizer by functional bacteria. Other researchers also found functional bacteria isolated from POME. Alias and Tan [42] isolated bacteria from POME and identified it as *Burkholderia cepacia* that have the ability to produce poly- (3) -hydroxybutyrate. Whereas Alam et al. [43] obtained several species of microbes that were able to break down organic matter. These results supported the results of this study, because of the result of all studies on in vitro and in vivo germination selection of bacterial potency showed that *Azospirillum* sp.1 and *Rhizobium* sp. were effectively associate and symbiotic with *C. pulcherrima* plant in pot-infected waste of POME.

**Table 2.** Potency of PGPR during the germination of *C. pulcherrima* (in vivo) and effect of watering type on bacterial population

| Treatments                  | Shoot length (Cm) | Root length (Cm) | Fresh weight (Gram) | Dry weight (Gram) | Number of leaf branches | Σ Bacteria (10^7 Cfu g^-1 sand) |
|----------------------------|-------------------|------------------|---------------------|-------------------|-------------------------|-------------------------------|
| Control (uninoculated)     | 5.6±0.4^a         | 3.33±0.2^a       | 0.17±0.2^a          | 0.02±0.0^a        | 3±0.58^ab               | 0                             |
| *Azppirillum* sp.1+POME    | 10±0.2^d          | 6.63±0.4^c       | 0.57±0.1^c          | 0.10±0.0^f        | 4±0.58^ab               | 32.67^b                       |
| *Azppirillum* sp.2+POME    | 8.3±0.4^de        | 4.80±0.1^cd      | 0.40±0.1^bc         | 0.06±0.0^bde      | 4±1.2^ab                | 3.67a                         |
| *Azppirillum* sp.3+POME    | 7.2±0.6^d         | 5.10±0.1^d       | 0.39±0.1^bc         | 0.06±0.0^bde      | 4±1.2^ab                | 2.40a                         |
| *Azotobacter* sp.+POME     | 8.1±0.5^d         | 6.53±0.0^e       | 0.32±0.1^ab         | 0.05±0.0^bc       | 4±1.0^b                 | 5.80a                         |
| *Rhizobium* sp.+POME       | 9.9±0.1^f         | 6.60±0.3^c       | 0.57±0.1^c          | 0.09±0.0^ef       | 4±1.2^ab                | 14.67a                        |
| *Azppirillum* sp.1+aquades | 9.2±0.1^f         | 5.0±0.0^d        | 0.35±0.0^ab         | 0.08±0.0^def      | 4±0.6^b                 | 2.00a                         |
| *Azppirillum* sp.2+aquades | 7.1±0.1^f         | 3.9±0.1^ab       | 0.23±0.1^ab         | 0.04±0.0^ab       | 3±0.6^ab                | 1.83a                         |
| *Azppirillum* sp.3+aquades | 6.2±0.1^ab        | 3.7±0.2^ab       | 0.23±0.0^ab         | 0.04±0.0^ab       | 3±1.0^b                 | 1.63a                         |
| *Azotobacter* sp.+aquades  | 7.0±0.0^bc        | 4.2±0.2^bc       | 0.57±0.0^c          | 0.04±0.0^ab       | 3±0.6^b                 | 2.00a                         |
| *Rhizobium* sp.+aquades    | 9.1±0.1^ef        | 6.3±0.6^c        | 0.37±0.1^abc        | 0.08±0.0^def      | 3±0.0^b                 | 1.21a                         |

Notes: ± SD is value from 3 replications. The numbers followed by the same letter are not significantly different at (p<0.05) level of Duncan’s test.

4. Conclusion

All the functional bacteria (NFB) isolated from POME disposal pond showed the potency to be plant growth promoting rhizobacteria (PGPR). *Azospirillum* sp.1 and *Rhizobium* sp. are the best PGPR (highest level of IAA production) and the ability to promote growth of *C. Pulcherrima* (L) Sw in in vitro and in vivo with as much as 10^7 bacterial populations in POME contaminated media.

5. References

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