

# Isolation of potential fluorescent pseudomonads from Kuini (*Mangifera odorata*) planted soil and their potential as biofertilizer

Seng Heng Jeffrey Lim,  
Nur Samahah Mohd Zainual

Green Technology Program,  
Agrobiodiversity and Environment  
Research Center, Malaysian  
Agricultural Research and Development  
Institute, Persiaran MARDI-UPM,  
Serdang, Selangor, Malaysia

## Abstract

*Pseudomonas* sp. are known to be good Plant Growth Promoting Rhizobacteria (PGPR). In this study, *Pseudomonas* sp. were isolated from soil planted with kuini (*Mangifera odorata*) using soil dilution method and spread onto King's B media. Five isolates of *Pseudomonas* sp. were observed to give promising results in the phytohormone and antimicrobial test conducted. These isolates are *Pseudomonas* sp. isolate K24pf, K29pf, K32pf, K33pf and K37pf. From the 5 potential isolates, *Pseudomonas* sp. isolate K29pf was chosen because it showed potential activity in producing marked amounts of Indole-3-Acetic Acid and gibberellic acid. *Pseudomonas* sp. isolate K29pf also produced antimicrobial activities towards *Ralstonia solanacearum*, *Erwinia caratovora*, *Erwinia mallotivora* and *Colletotrichum gloeosporioides*. Seed germination test showed that *Pseudomonas* sp. isolate K29pf was able to promote approximately 90% growth of *Brassica chinensis* seeds. Pot trial conducted showed that Treatment 3 (+OF+PGPR) was able to increase *Brassica chinensis* root by 36.5% and 28.4% of its biomass compared to treatment using Treatment 1 (+OF).

## Introduction

Soil is the most complex and heterogeneous of natural system composed of solid, liquid and gaseous phase. Soil is the habitat for many beneficial and pathogenic microorganisms. Bacteria inhabiting the rhizosphere can influence plant growth by producing certain bioactive compounds beneficial to the plants such as phytohormones and antibiotics.<sup>1,2</sup> These bacterial groups are known as Plant Growth

Promoting Rhizobacteria (PGPR).<sup>3</sup>

Plant Growth Promoting Rhizobacteria include the species in the genera of *Bacillus*, *Pseudomonas*, *Azotobacter* and *Rhizobium*. An effective PGPR should have these characteristics; ability of root colonization, phyto-stimulator and biocontrol agent against certain phytopathogens.<sup>3</sup> *Pseudomonas* sp. has been known as one of the most important members of PGPRs that showed all these three characteristics of PGPR.<sup>3</sup> Wahyudi *et al.*<sup>4</sup> and Vasanthakumar and McManus,<sup>5</sup> showed that some *Pseudomonas* sp. such as *Pseudomonas gingeri* and *Pseudomonas alcaligenes* has the ability to produce Indole Acetic Acid (IAA), which is important for the root growth.<sup>5</sup> Studies by Pandey and Desai<sup>6</sup> showed that *Pseudomonas* sp. also has the ability to produce gibberellic acid which is important to promote the growth and of yield of plants. Dey *et al.*<sup>7</sup> showed that *Pseudomonas* sp. isolated were effective to produce phyto-activities toward phytopathogens such as *Aspergillus niger* and *A. flavus*. In another study, Wahyudi *et al.*<sup>4</sup> showed that *Pseudomonas* sp. isolated from rhizosphere of soybean field have the ability to inhibit the growth of *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*, the causal agent of root rot disease.

In this study, our objectives are to isolate pseudomonas from kuini (*Mangifera odorata*) planted soil, characterize their potential bioactivities and analyzed their efficacy on a pot trial. As this is the pioneer work regarding isolation of PGPR from *Mangifera odorata* in Malaysia, we will focus on the best known PGPR which is the *Pseudomonas* sp.

## Materials and Methods

### Isolation of soil microbes

*Pseudomonas* sp. were isolated from kuini (*Mangifera odorata*) planted soil collected from MARDI Sintok, Kedah, Malaysia (Latitude: 6°29'6.98" Longitude: 100°29'2.27"). Soil samples were collected at the depth of 15 cm from the rhizosphere. These soils were from the soil type of Siri Napai a type of laterite soil. The collected soil samples were kept in ice box when transporting back to MARDI Headquarter located at Serdang, Selangor, Malaysia. The samples were crushed into smaller pieces and were processed by diluting 10 g of the soil sample in 100 ml of sterile distilled water (sH<sub>2</sub>O). The soil suspension was vigorously agitated at 200 rpm for 1 h. After 1 h, 150 µl of the soil suspension was pipetted onto King's B agar.<sup>8</sup> Agar plates were then

Correspondence: Seng Heng Jeffrey Lim, Green Technology Program, Agrobiodiversity and Environment Research Center, Malaysian Agricultural Research and Development Institute, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.  
Tel.: +603.8953.7243 - Fax: +603.8953.7200.  
E-mail: shlim@mardi.gov.my

Key words: Plant Growth Promoting Rhizobacteria; Fluorescent pseudomonads; Biofertilizer; *Mangifera odorata*.

Acknowledgements: the authors would like to thank Ms. Siti Noor Ashikin and Dr. Razean Haireen for helping out with the collection of soil samples and also everybody that has contributed directly or indirectly in this project.

Contributions: JLSH: sample collection, Polymerase Chain Reaction of bacteria, data analysis, pot trial and writing of article; NS: bacterial isolation, fluorescent pseudomonads characterizations, pot trial and co-writer of paper.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: this work was supported by Malaysian government under the 11th Malaysian Plan (RMK11-PGB-407).

Received for publication: 23 August 2018.

Revision received: 13 February 2019.

Accepted for publication: 24 February 2019.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright S.H. Jeffrey Lim and N.S. Mohd Zainual, 2019  
Licensee PAGEPress, Italy  
Microbiology Research 2019; 10:7844  
doi:10.4081/mr.2019.7844

incubated at 28 ± 2°C for 48 h. After that, the agar plates were exposed to UV light at 365 nm for selection of any fluorescent colonies. The emerging colonies were selected and streaked onto a fresh new agar plate for further usage.

### Screening of Indole-3-Acetic Acid

For Indole-3-Acetic Acid (IAA) estimation, method used by Iqbal and Hasnain,<sup>9</sup> was followed with a little modification. Fluorescent pseudomonads isolated were incubated at 30 ± 2°C for 48 hrs. The density of the culture broths were then adjusted to 10<sup>6</sup> cfu/mL for each test isolates. Two milliliter of the culture broth was pipetted into a new Eppendorf tube and centrifuged at 10,000 rpm for 30 min. After centrifugation, 1 ml of the supernatant was mixed with 2 mL of Salkowski's reagent in a test tube

**Table 1. Treatment used in pot trial of *Brassica chinensis* var *parachinensis*.**

Treatment	Representation	Amount added
Treatment 1: Baba organic fertilizer (4:2:6)	+OF	3 g/ application
Treatment 2: <i>Pseudomonas</i> sp. strain K29pf	+PGPR	50 ml/application
Treatment 3: Baba organic fertilizer (4:2:6) + <i>Pseudomonas</i> sp. strain K29pf	+OF+PGPR	3g + 50 ml /application
Treatment 4: No fertilizer added	Control	Nil

PGPR, plant growth promoting rhizobacteria.

and allowed to stand in dark for 30 min. Development of pink color indicated IAA production and the amount of IAA was measured using Nanodrop spectrophotometer at wavelength of 530 nm. A standard curve was constructed using synthetic IAA (Sigma, Seelze, Germany) with purity of 99.3% to determine the amount of IAA secreted by each fluorescent pseudomonads.

### Screening of gibberellic acid

Screening of gibberellic acid (GA<sub>3</sub>) producing fluorescent pseudomonad was done according to method proposed by Panday and Desai.<sup>6</sup> Standard curve was constructed using synthetic GA<sub>3</sub> (Sigma) and was measured at 254 nm using Nanodrop spectrophotometer.

### Screening for nitrogen fixer and phosphate solubilizer activity

For the screening of nitrogen fixer from fluorescent pseudomonads, Burk's Nitrogen free medium was used (10.0 g glucose, 0.41 g KH<sub>2</sub>PO<sub>4</sub>, 0.52 g K<sub>2</sub>HPO<sub>4</sub>, 0.05 g Na<sub>2</sub>SO<sub>4</sub>, 0.2 g CaCl<sub>2</sub>, 0.1 g MgSO<sub>4</sub> 7H<sub>2</sub>O, 0.005 g FeSO<sub>4</sub> 7H<sub>2</sub>O, 0.0025 g Na<sub>2</sub>MoO<sub>4</sub> 2H<sub>2</sub>O and 15 g agar-agar in 1 L of sdH<sub>2</sub>O).<sup>10</sup> The pH of the medium was adjusted to 7.0±0.2 prior autoclaving at 121°C for 15 min. The fluorescent pseudomonads were screened for their phosphate solubilizing ability using Pikovskaya's agar (10.0 g glucose, 5 g Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 0.5 g yeast extract, 0.5 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 0.2 g NaCl; 0.2 g KCl; 0.1 g MgSO<sub>4</sub>, 0.005 g FeSO<sub>4</sub> 7H<sub>2</sub>O MnSO<sub>4</sub> H<sub>2</sub>O 0.001 g and 15.0 g agar-agar in 1 L of sdH<sub>2</sub>O).<sup>11</sup> The pH was adjusted to 7.0±0.2 autoclaving at 121°C for 15 min. Isolates of fluorescent pseudomonads were then grown on the each of the plates in triplicates and halo zones formed indicate the positive activity for the tested bacteria.

### Screening of antimicrobial activities

Screening of antimicrobial activities were conducted based on the culture overlay method. The phyto-pathogens used were *Ralstonia solanacearum* (MMCC10019), *Erwinia caratovora* (MMCC10018), *Erwinia mallotivora* (MMCC10032), *Colletotrichum capsicii* (MMCC20012) and *Colletotrichum gloeosporioides* (MMCC20013). Anti-bacterial test was

**Table 2. Arrangement of the treatment in Randomized Complete Block Design.**

R1	R2	R3	R4	R5
(+OF)	Control	(+OF+PGPR)	(+PGPR)	(+PGPR)
(+PGPR)	(+OF)	Control	(+OF+PGPR)	(+OF)
(+OF+PGPR)	(+PGPR)	(+OF)	Control	(+OF+PGPR)
Control	(+OF+PGPR)	(+PGPR)	(+OF)	Control

conducted by lawning the test pathogenic bacteria onto the media plate and the agar block with pseudomonas was overlaid on top of the phytopathogens. For anti-fungal test, a dual cultures agar test was performed, where the pathogenic fungi was inoculated at the center of the agar plate and flanked by tested fluorescent pseudomonads on left and right side of the agar. Clear zone produced indicates the ability to secrete antimicrobial compound against the tested pathogens. Antimicrobial activity was evaluated after 24 h of incubating and the clear zones were rated using the rating scheme suggested by Baniyadi *et al.*<sup>12</sup> from the modification of rating by Lee and Hwang,<sup>13</sup> whereby; 0-4 mm= no inhibition, 5-9 mm= weak inhibition, 10-19 mm= moderate inhibition and ≥20 mm= strong inhibition.

### Identification of beneficial microbes

Isolation of DNA was conducted using Qiagen DNeasy DNA extraction protocol as suggested by the manufacturer. PCR parameters and conditions used for this study followed the optimization made by Jeffrey<sup>14</sup> with the modification on the primers used. The 16S rRNA gene region was amplified using universal primers F8 (AGA GTT TGA TCM TGG CTC) and rP2 (ACG GCT ACC TTG TTA CGA CTT).<sup>15</sup> PCR products obtained were subjected to purification using Vivantis GF-1 Gel DNA recovery kit following the protocol provided by the manufacturer. The purified PCR products were sent for sequencing at First Base Laboratories Sdn. Bhd., Selangor. Results obtained from the sequencing were then compared with the databases from National Center for Biotechnology Information.

### Germination test

Germination test was conducted using 20 seeds of *Brassica chinensis* var *parachinensis*

for each treatment. Mean of 20 seeds weighted at 0.0464±0.002 g. The seeds were pre-soaked with 10 ml of selected fluorescent pseudomonads culture broths for 30 mins while control seeds were soaked with 10 ml of water for the same duration. After 30 min, seeds from each treatment were dried in laminar flow for 2 hrs before being transferred onto filter paper placed in the 90×15 mm petri dishes. The filter papers were then wetted with 5 ml of fluorescent pseudomonads broths while sterile distilled water of 5 ml was used as control for 5 days. Number of seed germinated in 5 days were counted and represented as percentage of germination. Tests were conducted in room temperature 28±2°C and all tests were conducted in triplicates.

### Pot trial

Soil used for the planting of *Brassica chinensis* var *parachinensis* was cocoa peat obtained from local market. Three seeds of *Brassica chinensis* var *parachinensis* were planted in each pot. A total 50 ml broth containing *Pseudomonas* sp. strain K29pf with the concentration of 10<sup>6</sup> were inoculated onto the soil for each pot in treatment 2 and 3, while 3g of organic fertilizer was added around the plant in each pot in treatment 1 and 3 (Table 1). No artificial lighting were provided to induce the plant growth. The plants were watered manually every morning and evening during the experiment. Temperature was recorded twice a day in the morning (around time 0900) and in the evening (around time 1700). The treatments used are shown in Table 1.

Treatments were applied twice throughout the experiment on 1<sup>st</sup> and 15<sup>th</sup> day after planting. *B. chinensis* var *parachinensis* was harvested on day 30 after planting. The fresh weight, dry weight and root length of the plants for each treatment were recorded. The tests were conducted with 5 replicates for each treatment and in a randomized complete block design method (Table 2).

Fresh weight of the plants were taken immediately after harvesting. While the dry weight of the plants were taken after oven drying at 55°C overnight. The dried plants were place in a ziplock bag once it is removed from the oven to prevent the plant from contacting with the surrounding moisture. The dry weight was determine by subtracting the weight of the dry plant with the ziplock bag with the emptied ziplock bag.

Dry weight (g) = (weight of dried plant in ziplock bag) – (weight of emptied ziplock bag).

### Statistical data analysis

All data for fresh plant weight, dry plant weight and root elongation was done using one way analysis of variance was done using SAS 9.3 software. The Tukey's honest significant difference at P=0.05 was used to determine the significance among the samples.

## Results

### Isolation of soil fluorescent pseudomonads and screening of IAA and GA<sub>3</sub> activities

A total of 25 *Pseudomonas* sp. was isolated soil of kuini plot from MARDI Sintok. The average colony forming unit per gram soil (cfu/g) for *Pseudomonas* sp. isolated was  $7.3 \times 10^5$ . We observed that 100% of the fluorescent pseudomonads presented the ability to produce IAA but only 20% showed the ability to produce GA<sub>3</sub>. The results are shown in Table 3.

### Screening of nitrogen fixation, phosphate solubilization, antimicrobial activities and identification of fluorescent pseudomonads

Secretion of bioactivities by microbes was known to be an important characteristic

for the microbes to stay viable in their ecosystem. From the total of 25 fluorescent pseudomonads isolated, 60% of the *Pseudomonas* sp. showed antimicrobial activity towards *Ralstonia solanacearum* (MMCC10019), 20% for *Erwinia caratovora* (MMCC10018), 18% towards *Erwinia mallotivora* (MMCC10032), 4% *Colletotrichum capsicii* (MMCC20012) and 8% for *Colletotrichum gloeosporioides* (MMCC20013). Antimicrobial activity of selected fluorescent pseudomonads were tabulated in Table 3.

Screening for phosphate solubilizing and nitrogen fixation ability of 25 isolates of fluorescent pseudomonads isolated showed that only 8% of the fluorescent pseudomonads isolates gave positive reaction to the phosphate solubilizing media used, while no nitrogen fixation activities was observed for all 25 isolates of fluorescent pseudomonads (Table 3). The 5 poten-

**Table 3. Phytohormone, antimicrobial and enzymatic activities produced by 25 isolates of *Pseudomonas* sp. isolated form kuini soil.**

Isolate no.	Phytohormone activity (Mean±SE mm)		Antimicrobial activity (Mean±SE mm)					Nitrogen fixation	Phosphate solubilizing
	IAA (µg/ml)	GA <sub>3</sub> (µg/mL)	<i>Ralstonia solanacearum</i> (MMCC10019)	<i>Erwinia caratovora</i> (MMCC10018)	<i>Erwinia mallotivora</i> (MMCC10032)	<i>Colletotrichum capsicii</i> (MMCC20012)	<i>Colletotrichum gloeosporioides</i> (MMCC20013)		
K1pf	1.24±0.03	-	8.40±0.53	6.40±0.27	-	-	-	-	
K2pf	0.53±0.10	-	10.20±0.43	-	-	-	-	-	
K5pf	0.82±0.03	-	-	-	-	-	-	-	
K8pf	1.55±0.03	60.32±0.06	7.50±0.85	-	-	-	-	-	
K9pf	3.21±0.02	-	-	14.50±0.57	-	-	-	-	
K13pf	2.72±0.06	-	7.30±0.55	-	-	-	-	-	
K14pf	1.92±0.03	-	9.80±0.73	15.40±0.50	-	-	-	-	
K18pf	0.58±0.02	-	-	-	-	-	-	-	
K21pf	0.56±0.01	-	11.30±0.65	-	-	-	-	-	
K24pf	7.86±0.01	-	10.00±0.58	-	15.00±0.58	-	-	-	
K25pf	4.31±0.03	-	-	-	-	-	-	-	
K28pf	2.60±0.01	-	-	-	-	-	-	-	
K29pf	8.52±0.02	72.41±0.01	15.00±0.58	20.30±0.87	21.50±0.69	-	12.00±0.98	-	
K32pf	3.79±0.07	91.86±0.03	13.20±1.73	-	-	13.70±0.64	15.60±0.69	-	
K33pf	6.52±0.02	73.00±0.01	12.50±0.98	-	15.60±0.35	-	-	-	
K34pf	3.18±0.04	-	-	-	-	-	-	-	
K35pf	1.63±0.01	-	8.90±0.65	-	-	-	-	-	
K37pf	6.34±0.03	75.66±0.01	13.50±0.98	-	10.30±0.35	-	-	-	
K38pf	0.93±0.01	-	-	10.00±0.49	-	-	-	-	
K41pf	0.82±0.03	-	-	-	-	-	-	-	
K43pf	2.45±0.12	-	5.90±0.65	-	-	-	-	-	
K44pf	1.75±0.08	-	-	-	7.50±0.60	-	-	-	
K49pf	0.98±0.06	-	10.50±0.65	-	-	-	-	-	
K50pf	2.53±0.03	-	8.50±0.65	-	-	-	-	-	
K51pf	3.25±0.05	-	-	-	-	-	-	-	

tial isolates were identified based on their 16S rRNA sequence. The identity of the 5 isolates were tabulated in Table 4.

### Seed germination test

Microbial benefit of seed germination can be an index of its potential benefit for agricultural, including propagation. Here, *Pseudomonas* sp. strain K29pf allowed a 90% germination of the seeds while *Pseudomonas putida* strain K33pf and *Pseudomonas* sp. K37pf had the lowest seeds germination rate of 70% (Table 5).

### Efficacy of *Pseudomonas* sp. strain K29pf on pot trial

The mean temperature recorded of the glass house was 27°C during the morning and 34°C during the evening throughout the experiment. From all the test conducted, *Pseudomonas* sp. strain K29pf was chosen for the pot trial. It was observed that Treatment 3 gave the highest wet weight with the average wet weight of 75.6 g whereas Treatment 4 gave the lowest average wet weight of 28.5 g (Table 6). The observation on the root length indicated that Treatment 3 stimulate the longest roots elongation with the average of 13.1 cm. The results also indicated that Treatment 2 stimulated the elongation of roots, however does not gave significant increase in the weight of *Brassica chinensis* var *parachinensis* compared to Treatment 1 (Table 6).

## Discussion

Indole-3-Acetic Acid which is an auxin, can stimulate cell elongation and division and also promote plant growth and development. However, high concentrations of IAA will cause toxicity to the plant such prevention of shoot and root growth.<sup>16</sup> Study done by Rodrigues *et al.*<sup>17</sup> indicated that 80% of the *Pseudomonas* sp. isolated from sugarcane showed the ability to produce IAA. In our study, all the 25 isolates of fluorescent pseudomonads isolated were observed to produce IAA. From this, we observed that

**Table 4. Identification of selected fluorescent pseudomonads.**

Isolate no	Identity	Similarity (%)
K24pf	<i>Pseudomonas</i> sp.	99
K29pf	<i>Pseudomonas</i> sp.	99
K32pf	<i>Pseudomonas aeruginosa</i>	99
K33pf	<i>Pseudomonas putida</i>	98
K37pf	<i>Pseudomonas</i> sp.	99

the highest IAA produced was only 8.52 µg/ml which was considered low compared to IAA produced by *Pseudomonas putida* UB1 (591.8 µg/ml) is a study conducted by Bharucha *et al.*<sup>18</sup> This observation indicated that the IAA reading obtained in this study were significantly low. However, study done by Dagnaw *et al.*<sup>19</sup> stated that it is normal to obtained plant growth promoting rhizobacteria with low production of IAA due to the differences in species, strains and also the influence of different culturing condition.

Gibberellin acid is an endogenous hormone that function as plant growth regulators and influencing the developmental process of plants such as stem elongation, seed germination and sex expression. In this study, we observed that *Pseudomonas aeruginosa* strain K32pf produced the highest GA<sub>3</sub> (91 µg/ml). This was higher compare to GA<sub>3</sub> produced by *Pseudomonas* isolate K8 (70 µg/ml) isolated by Desai.<sup>20</sup> However comparison of GA<sub>3</sub> production from *Pseudomonas* sp. done by Sharma *et al.*,<sup>21</sup> GA<sub>3</sub> production by *Pseudomonas* sp. isolated in this study were very low. Bottini *et al.*,<sup>22</sup> noted that production of GA<sub>3</sub> might be influenced by factors such as the media used, the supply of O<sub>2</sub> and N.

In this study, *Pseudomonas* sp. isolate K29pf showed the most potential with the 15.0, 20.3 and 21.5 mm respectively of clear zones produced towards *Ralstonia solanacearum*, *Erwinia carotovora* and *Erwinia mallotivora* respectively. In a study conducted by Zhou *et al.*,<sup>23</sup> *Pseudomonas brassicacearum* J12 was observed to inhibit

the growth of *Ralstonia solanacearum*. *Pseudomonas putida* ICCF 391 on the other hand showed antagonistic activity

against *E. carotovora*.<sup>24</sup> Soare *et al.*,<sup>24</sup> mention that *E. carotovora* have the ability to produce bacteriocine, a compound that enable them to grow and inhibit other microbial growth. With the compound, *E. carotovora* can be seen as a microbes that are resistant to many other microbes. The formation of clear zone by only *Pseudomonas* sp. strain K29pf showed that this *Pseudomonas* sp. strain K29pf might have the ability to neutralize the bacteriocine produced by *E. carotovora*.<sup>24</sup> Apart from producing antibacterial activity, *Pseudomonas* sp. strain K29pf also showed its ability to inhibit the growth of *Colletotrichum gloeosporioides*. These findings suggested that *Pseudomonas* sp. isolate K29pf has the potential to be develop into a beneficial biocontrol agent due to its effectiveness and board range of antimicrobial spectrum.

The ability of *Pseudomonas* sp. to fix nitrogen are considered to be rare.<sup>25</sup> Indeed, in the study conducted by Lin *et al.*,<sup>25</sup> *Pseudomonas stutzeri* was the only known *Pseudomonas* sp. that fixed nitrogen. Unlike its rareness in fixing nitrogen, *Pseudomonas* sp. was well known for its ability to solubilize phosphate. In a study conducted by Dipak and Sankar,<sup>26</sup> *Pseudomonas* sp. was noted as a good phosphate solubilizer and most *Pseudomonas* sp. have the ability to solubilize phosphate<sup>26-28</sup> from the environment. However, in the present study we only isolated 2 pseudomonas

**Table 5. Seed germination test of fluorescent pseudomonads.**

Isolate	Percent of seed germinated
<i>Pseudomonas</i> sp. strain K24pf	80.30*
<i>Pseudomonas</i> sp. strain K29pf	90.50*
<i>Pseudomonas aeruginosa</i> strain K32pf	85.40*
<i>Pseudomonas putida</i> strain K33pf	70.80*
<i>Pseudomonas</i> sp. strain K37pf	70.40*
Control	95.80*

\*The mean value significantly differ according to Tukey's honest significant difference at P=0.05 was showed.

**Table 6. Plants dry weight, wet weight, dry weight percentage and root elongation vs treatments.**

Treatment	Weight (g)		Dry weight %	Root elongation (cm)
	Fresh weight	Dry weight		
+OF	62.7*	12.5*	19.9	11.0*
+PGPR	54.8*	15.3*	27.9	11.5*
+OF+PGPR	75.6 *	21.5*	28.4	13.1*
Control	28.5 *	5.4*	18.9	9.6*

\*The mean value significantly differ according to Tukey's honest significant difference at P=0.05 was showed.

with phosphate solubilizing properties. This could be due to the fact that fluorescent pseudomonads in this study were isolated from laterite soil which is well known for its low content of phosphorous.

A study by Tiwari and Singh<sup>29</sup> indicated that *Triticum aestivum* (L) and *Zea mays* (L) seeds were inhibited by *Pseudomonas aeruginosa*. The infected seeds shown the character of soft and was covered by bio-film of the bacteria. However, in this study the seeds that did not germinate showed none of these attributes. However, Tabatabaei *et al.*,<sup>30</sup> stated that IAA levels, unknown metabolites produced by the bacteria and stress induced by the bacteria might also be one of the reason that seeds germination was inhibited.

Application of the potential microbes to the real world is away to test its functionality after completion of lab tests. From the study, +PGPR increased the weight of *Brassica chinensis* var *parachinensis* but did not surpassed the wet weight of +OF. This showed that by adding the *Pseudomonas* sp. alone, the microbes might not be able to provide the plants with the required nutrients from the soil for its growth. In +OF+PGPR, we saw an increased of 20% in the plant biomass compared to +OF only. This showed that combination of organic fertilizer and *Pseudomonas* sp. strain K29pf provide the plant with the needed nutrients and thus boosting the weight increment. This argument above is supported by finding from Antonius and Agustiyani,<sup>31</sup> where they noted that application of beneficial microbes to the compost and application thereafter is the effective method to increase the biomass of the plants. Previous studies show that a biofertilizer prepared by combining PGPR with composts enhance growth-promoting effects and bio-control of the microbes towards the plants.<sup>32</sup> Saia *et al.*<sup>33</sup> also observed that soil under fertilized condition with addition of PGPR increased the density of bacteria on the rhizoplane and also the N concentration of the plant.

## Conclusions

*Pseudomonas* sp. are well known for their PGPR ability. In this study, *Pseudomonas* sp. K29pf isolated from kuini (*Mangifera odorata*) soil had showed its potential to produce phytohormones such as Indole-3-Acetic Acid and gibberellic acid, antimicrobial activities against *Ralstonia solanacearum*, *Erwinia caratovora* and *Erwinia mallotivora* and *Colletotrichum gloeosporioides* and also the ability to solubilize phosphate. This shows that *Pseudomonas* sp. K29pf can act as a good

source of PGPR; further studies are needed to fully understand the efficacy of this bacteria on field.

## References

- Patten CL, Glick BR. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl Environ Microbiol* 2002;68:3795-801.
- Mubarik NR, Mahagiani I, Anindyaputri A, et al. Chitinolytic bacteria isolated from chili rhizosphere: Chitinase characterization and its application as biocontrol for whitefly (*Bemisia tabaci* Genn.). *Am J Agr Biol Sci* 2010;5:430-5.
- Bloemberg GV, Lugtenberg BJJ. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr Opin Plant Biol* 2011;4:343-50.
- Wahyudi AT, Astuti RP, Widyawati A, et al. Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. *J Microbiol Antimicrobiol* 2011;3:34-40.
- Vasanthakumar A, McManus PS. Indole-3-acetic acid-producing bacteria are associated with cranberry stem gall. *Phytopathology* 2004;94:1164-71.
- Pandya ND, Desai PV. Screening and characterization of GA3 producing *Pseudomonas monteilii* and its impact on plant growth promotion. *Int J Curr Microbiol Appl Sci* 2014;3:110-5.
- Dey R, Pal KK, Bhatt DM, Chauhan SM. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol Res* 2004;159:371-94.
- King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanine and fluorescein. *J Lab Clin Med* 1945;44:301-7.
- Iqbal A, Hasnain S. Auxin producing *Pseudomonas* strains: Biological candidates to modulate the growth of *Triticum aestivum* beneficially. *Am J Plant Sci* 2013;4:1693-700.
- Wilson PW, Knight, SC. Experiments in bacterial physiology. Minneapolis, USA: Burgess; 1952. p 49.
- Pikovskaya RI. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* 1948;17:362-70.
- Baniasadi F, Shahidi Bonjar GH, Baghizadeh A, et al. Biological control of *Sclerotinia sclerotiorum*, causal agent of sunflower head and stem rot disease, by use of soil borne actinomycetes isolates. *Am J Agr Biol Sci* 2009;4:146-51.
- Lee JY, Hwang BK. Diversity of anti-fungal actinomycetes in various vegetative soils of Korea. *Canad J Microbiol* 2002;48:407-17.
- Jeffrey LSH. Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. *Afr J Biotechnol* 2008;7:3700-5.
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16 S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697-703.
- Hansen H, Grossmann K. Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. *Plant Physiol* 2000;124:1437-48.
- Rodrigues AA, Forzani MV, Soares RS, et al. Isolation and selection of plant growth-promoting bacteria associated with sugarcane. *Pesquisa Agropecuária Tropical* 2016;46:149-58.
- Bharucha U, Patel K, Trivedi UB. Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effects as plant growth-promoting rhizobacteria on Mustard (*Brassica nigra*). *Agr Res* 2013;2:215-21.
- Dagnaw F. Characterization of plant growth promoting bacteria from sugarcane (*Saccharum officinarum* L.) rhizosphere of Wonji-Shoa Sugar Estate and farmers landraces of Ethiopia. *Biotechnology* 2015;14:58-64.
- Desai SA. Isolation and characterization of gibberellic acid (GA3) producing rhizobacteria from sugarcane roots. *Biosci Discov* 2017;8:488-94.
- Sharma S, Sharma A, Kaur M. Extraction and evaluation of gibberellic acid from *Pseudomonas* sp.: Plant growth promoting rhizobacteria. *J Pharmacognosy Phytochem* 2018;791:2790-5.
- Bottini R, Cassan F, Piccoli P. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Appl Microbiol Biotechnol* 2004;65:497-503.
- Zhou T, Chen D, Li C, et al. Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. *Microbiol Res* 2012;167:388-94.
- Soare MG, Caterina T, Maria P, et al. Antimicrobial activity of newly isolated *Bacillus* sp. and *Pseudomonas* sp. strains and their potential use as biocontrol agents. *Scientific Bulletin. Series F.*

- Biotechnol 2017;XXI.
25. Lin M, Yan Y, Lu W, et al. Regulatory coupling of nitrogen and carbon metabolism in nitrogen-fixing *Pseudomonas stutzeri* A1501. In de Bruijn FJ, ed. *Biological Nitrogen Fixation*. Hoboken, NJ, USA: John Wiley & Sons, Inc; 2015.
  26. Dipak P, Sankar NS. Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. *Ann Agr Sci* 2017;15:130-6.
  27. Parani K, Saha BK. Prospects of using phosphate solubilizing *Pseudomonas* as bio fertilizer. *Eur J Biol Sci* 2012;4:40-4.
  28. Trivedi P, Sa T. *Pseudomonas corrugate* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production and plant growth at lower temperatures. *Curr Microbiol* 2008;56:140-4.
  29. Tiwari P, Singh S. A plant growth promoting rhizospheric *Pseudomonas aeruginosa* strain inhibits seed germination in *riticum aestivum* (I) and *Zea mays* (L). *Microbiol Res* 2017;8:73-9.
  30. Tabatabaei S, Ehsanzadeh P, Etesami H, et al. Indole-3-acetic acid (IAA) producing *Pseudomonas* isolates inhibit seed germination and  $\alpha$ -amylase activity in durum wheat (*Triticum turgidum* L.). *Spanish J Agr Res* 2016;14:e0802.
  31. Antonius S, Agustiyani D. Effects of biofertilizer containing microbial of N-fixer, P-solubilizer and plant growth factor producer on cabbage (*Brassica oleracea* var. capitata) growth and soil enzymatic activities: A greenhouse trial. *J Biol Res* 2011;16:149-53.
  32. Chen LH, Tang XM, Raze W, et al. *Trichoderma harzianum* SQR-T037 rapidly degrades allelochemicals in rhizospheres continuously cropped cucumbers. *Appl Microbiol Biotechnol* 2011;89:1653-63.
  33. Saia S, Rappa V, Ruisi P. Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient expression in durum wheat. *Front Plant Sci* 2015;6:815.

Non-commercial use only