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In vitro Screening and Optimization of IAA Production from Plant Growth Promoting Rhizobacteria *Burkholderia cepacia* UPMB3

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Key words: Burkholderia cepacia UPMB3, Optimisation, *In vitro*, Oil palm

Abstract

Burkholderia cepacia UPMB3 is an important plant growth promoting rhizobacteria isolated from oil palm rhizosphere which is considered to promote plant growth directly or indirectly. The IAA was extracted, purified, detected and confirmed by thin layer chromatography analyses from the strain UPMB3 of *B. cepacia*. R_f value was compared with the authentic IAA. Maximum 50 µg/ml IAA was produced in the medium supplemented with 4 mg/ml L-tryptophan, under shaken conditions at 150 rpm in seven days incubation at pH 7. The bacterial extract significantly influenced the growth of oil palm seedlings producing shoot, root, leaf and leaf length compared to control.

Introduction

The *Burkholderia cepacia* complex (Bcc) is a diverse group of bacteria commonly found in soil, water, and the rhizosphere. It comprises 19 species, which includes soil and rhizosphere bacteria as well as plant and human pathogens (Paulina et al. 2001). Different *Burkholderia* species have been emerged as a potentially promising group of plant growth-promoting rhizobacteria (PGPR). Approximately 80% of rhizosphere bacteria can secrete IAA (Bhavdish et al. 2003). It is likely that plant growth promotion by rhizobacteria is the result of combined action of several ways. But the production of phytohormones (specially IAA) is considered as a direct mechanism used by bacteria to

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increase the growth and yield of plants (Idris et al. 2007). The phytohormone auxins play a central role in plant growth and development as a regulator of numerous biological processes. IAA is the member of this group of phytohormones and a product of L-tryptophan metabolism in microorganisms. Bacteria that inhabit the rhizosphere may influence plant growth by contributing to a host plant's endogenous pool of bioactive compounds such as phyto-hormones, antibiotics, siderophores. IAA is one of the most physiologically active auxins and a common product of L-tryptophan metabolism by several microorganisms including PGPR. PGPR could be a great ecological and agricultural importance, if they are reliably used in place of chemical fertilizers and pesticides without being pathogenic to plants. Bacterial production of IAA has been studied not only regarding its physiological effects on plants but also regarding its possible role as a phytohormone in plant - microbe interaction. The roles of PGPR have been extensively studied as biofertilizers to increase the yield of agronomically important crops. This study was conducted to detect and optimise the IAA production by *Burkholderia cepacia* UPMB3 and its influence on oil palm seedling growth.

Materials and Methods

The strain *Burkholderia cepacia* UPMB3 (Fig.1) was isolated from roots of oil palm and was confirmed by the Biolog® identification system (version 4.2) (Zaiton et al. 2006). The bacterium was collected from Plant Protection Department, Universiti Putra Malaysia.

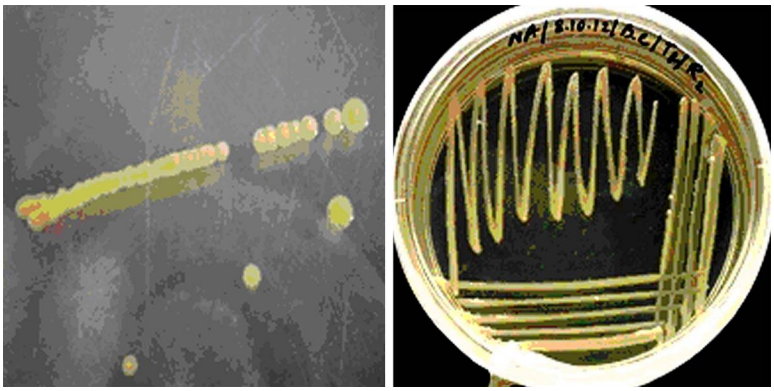

Fig. 1. Rhizobacterial strain *Burkholderia cepacia* UPMB3.

It was cultured in NB supplemented with L-tryptophan, adjusted pH to 7.0 and incubated at 28 ± 2°C on a rotary shaker at 150 rpm for seven days. The bacterial cell-free supernatant was harvested by centrifugation at 10,000 rpm

for 15 min at 4ºC and used for extraction of IAA. For screening of IAA, the bacterial supernatant was mixed with ortho-phosphoric acid and Salkowski's reagent. Mixtures were incubated at room temperature for 30 min and observed for pink color production. The colour change was recorded in the spectrophotometer at 530 nm.

IAA was extracted and purified following the method described by Tien et al. (1979) with some modifications. The bacterial supernatant was reduced by rotatory evaporator and acidified. The supernatant was extracted with ethyl acetate then evaporated to dry at 40°C and dissolved in methanol. After filtering through 0.45 µm membrane filter the extract was kept at –20°C. For the confirmation of IAA production, it was detected by thin-layer chromatography analysis. Silica gel thin layer chromatography (TLC) was found to be a powerful technique in purification, separation and possible identification of natural and synthetic indole derivatives. Thirty ml of extracted samples was plated on TLC plates and was run by the solvent system benzene : acetone : acetic acid (6 : 3 : 1) and sprayed with Ehrlich's reagent. Spots with R_f values identical to authentic IAA were identified under UV light (254 and 365 nm). The effect of L-tryptophan (0 - 5 mg/ml) and different pH level (3, 5, 7 and 10) on IAA production in NB medium was optimised. One per cent inoculum of $O.D._{600nm}$ was incubated at 28 ± 2°C for 3, 5, 7 and 10 days, respectively under static and shaken (150 rpm) conditions in an incubator shaker. After incubation the broth was centrifuged at 10,000 rpm for 10 min. Supernatant was collected. Two ml of Salkowski's reagent was added to 1 ml supernatant and extent of pink colour showing IAA production was measured spectrophotometrically at 530 nm.

The effect of IAA on germinated oil palm seeds was studied. The germinated oil palm seeds were surface sterilized with 95% ethanol for 2 min and then with 10% Clorox for 15 min. Successive washing was done with sterile distilled water to remove the chemicals completely. The seeds were separately soaked in 250 ml of bacterial culture filtrate based on the production of IAA by the strain UPMB3 and synthetic IAA. Sterile distilled water was used as non-treated control treatment. Plastic pot experiment was carried out to observe the effect of IAA produced by *B. cepacia* UPMB3 strain on oil palm seedling growth. The seeds were soaked in each treatment for 48 hrs and then planted in pots filled with sterile soil. The pots were kept in partial sunlight and observed daily including watering to maintain soil moisture. After 4 weeks, the plants were carefully uprooted and recorded data subsequently.

The experiment was arranged as completely randomized design. Three replications were used for each treatment and repeated twice. Data were

analysed using statistical analysis system (SAS v9.3) and means were statistically compared using LSD test. The significance level was set up at $p < 0.05$.

Results and Discussion

The bacterial strain *B. cepacia* UPMB3 showed pink colour reaction with Salkowski's reagent indicating their ability to produce IAA (Fig. 2A). IAA production was confirmed by the appearance of blue bands with that of authentic IAA bands on pre-coated silica gel plates under UV (365 nm). The solvent system benzene : acetone : acetic acid was used to detect the IAA production by the bacterial strain. The R_f value was found 0.88 for *B. cepacia* UPMB3 and was similar to the standard IAA R_f value (Fig. 2B).

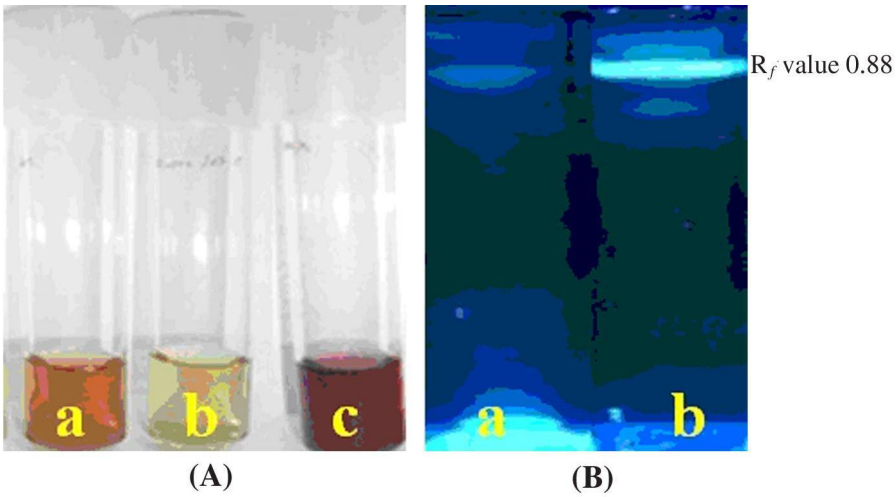

Fig. 2. (A) Screening of IAA production. a = *B. cepacia* UPMB3 (pink colour), b = *B. cepacia* UPMB3 (Control), c = Standard IAA. (B) Thin layer chromatography analysis. a = *B. cepacia* UPMB3, b = Standard IAA.

Ritika et al. (2012) reported that different isolates of *Pseudomonas* sp produced auxins like substances in the stationary phase of growth at 72 hrs of incubation period at 28°C. The homogeneity of the partially purified auxins was checked by thin layer chromatography and auxins gave the maximum R_f value of 0.81 in solvent system isopropanol: water (3:2). Pink spots corresponding to auxins or auxins like substances were visible when sprayed with Salper reagent. The IAA produced by the *B. licheniformis* MML2501 was confirmed by the appearance of blue bands with that of authentic IAA bands on TLC plates with R_f value 0.66 in solvent system isopropanol : ammonia : water (8 : 1 : 1) and sprayed with Ehrlich's reagent (Prashanth and Mathivanan 2010).

B. cepacia UPMB3 was found to be the efficient producer of IAA. The different concentrations of L-tryptophan in the culture medium influenced the IAA production. From the result, it was observed that the bacterial strain was able to produce a lower amount of IAA, without L-tryptophan. The maximum IAA production by *B. cepacia* UPMB3 was recorded 50.88 μg/ml at 4 mg/ml L-tryptophan concentration (Fig. 3). IAA production was found to be decreased when the media were supplemented with 5 mg/ml L-tryptophan.

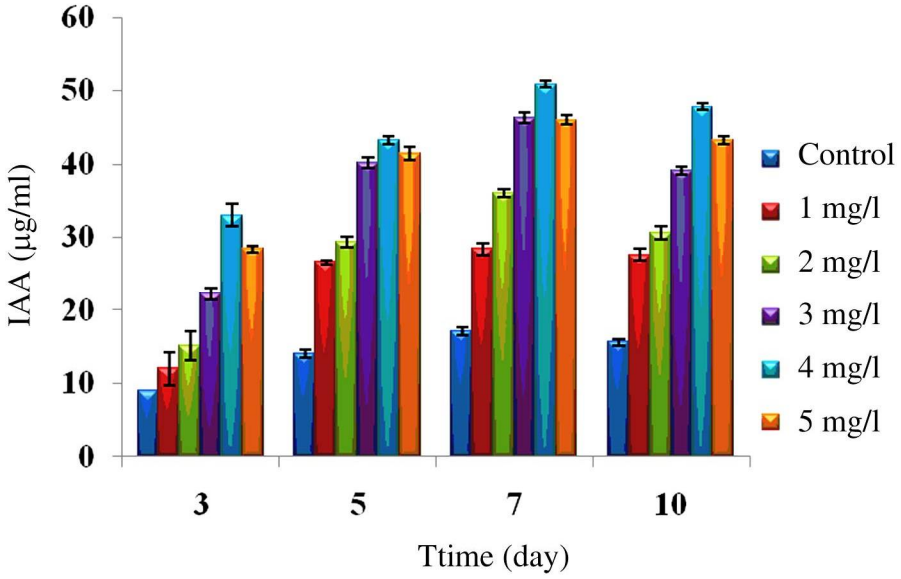

Fig. 3. Production of IAA by *B. cepacia* UPMB3 at various concentrations of L - tryptophan. Each value is the mean of 3 replications. Vertical bars represent standard error.

IAA production was found to be decreased when the media were supplemented with 5 mg/ml L-tryptophan in 10 days incubation. This decrease might be due to the release of IAA degrading enzymes such as IAA oxidase and peroxidase as reported earlier in *Rhizobium* sp. from *Cajanus cajan* (Datta and Basu 2000). Some other workers also observed in variable IAA production ability of bacteria. Ahmad et al. (2005) reported that rhizosphere *Azotobacter* spp. and *Pseudomonas* spp. produced a high level of IAA when these bacteria were cultured in a nutrient broth amended with 1, 2 and 5 mg/ml of L-tryptophan. The concentration of IAA in *P. fluorescens* AK1 and *P. aeruginosa* AK2 isolates without L-tryptophan was 3.1 and 3.3 pmol/ml. A further increase in IAA production was observed in the presence of different concentrations of L-tryptophan (100, 200 and 500 μg/ml). A significant increase in the production of IAA was recorded in

P. fluorescens AK1 and *P. aeruginosa* AK2 in the presence of 100, 200 and 500 μg/ml of L-tryptophan, i.e. 3.8, 5.2 and 6.9, 3.9, 4.0 and 4.2 pmol/ml, respectively (Karnwal 2009).

To optimize the different levels of pH on IAA production, the bacterial strain was inoculated in NB medium supplemented with optimized L-tryptophan concentration (4 mg/ml). Different pH levels such as 3, 5, 6, 7, 8 and 9 were maintained at 3, 5, 7 and 10 days incubation period. The bacterial strain produced minimum amount of IAA at pH 3. Maximum IAA production was achieved 39.12 μg/ml at pH 7 of the culture medium (Fig. 4).

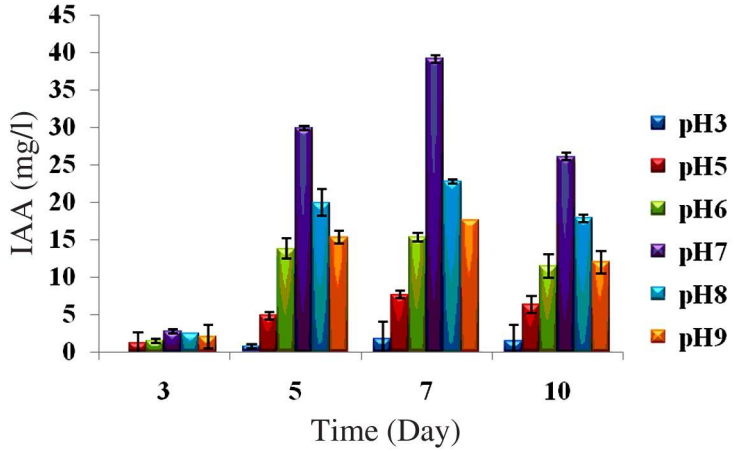

Fig. 4. Production of IAA by *B. cepacia* UPMB3 at different pH. Each value is the mean of 3 replications. Vertical bars represent standard error.

IAA production decreased at pH 9.0. Acidic or high alkaline pH level is not suitable for IAA production of *B. cepacia* UPMB3 due to its poor growth. Shirokikh et al. (2007) reported the distribution of *Streptomyces* sp. from acidic soils is lower than neutral soils. A significant correlation was also observed between bacterial growth and IAA production. According to Madhuri (2011), different *Rhizobium* strains produced maximum IAA at pH level 7.0.

The bacterial strain *B. cepacia* UPMB3 was influenced by the culture conditions and incubation periods as well. Both the static and shaken conditions were tested for the production of IAA. The maximum production of IAA for *B. cepacia* UPMB3 (50 μg/ml) was achieved in shaken condition on seven days of incubation period, which was statistically significant compared to the static condition (Fig. 5). The reason hypothesized that during shaken condition, the bacteria might be able to get maximum L-tryptophan supplied in the culture medium, which could result in more IAA production.

Ahmad et al. (2005) reported that the production of IAA in fluorescent *Pseudomonas* isolates increased with an increase of L- tryptophan concentration from 1 to 5 mg/ml in the majority of isolates. In presence of 5 mg/ml of L-tryptophan, 5 isolates of *Pseudomonas* produced high levels (41.0 to 53.2 µg/ml) of IAA at seven days incubation period. Production of IAA, in *Bacillus licheniformis* MML2501 with a maximum of 23 µg/ml under optimised conditions such as pH 7.0, temperature 35°C, L-tryptophan at a concentration of 16 mM and at 200 rpm shaken conditions (Prashanth and Mathivanan, 2010).

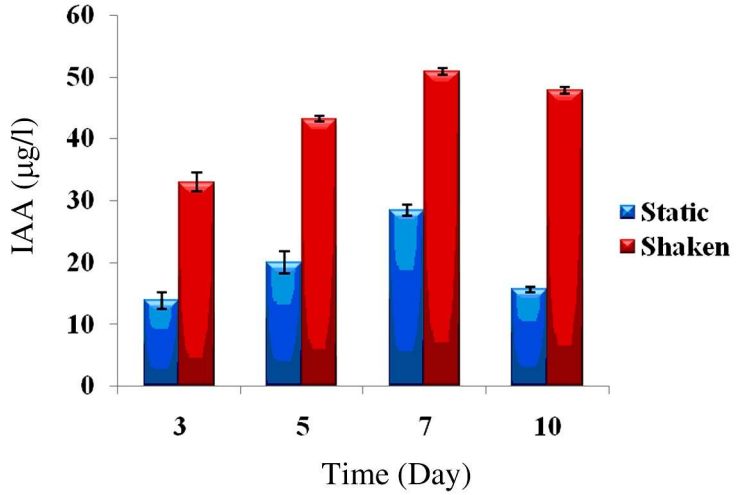

Fig. 5. Effect of culture conditions and incubation period on IAA production by *B. cepacia* UPMB3. Each value is the mean of 3 replications. Vertical bars represent standard error.

A pot experiment was conducted to study the influence of the bacterial extract (UPMB3) on growth promotion of oil palm germinated seedlings. The results of the pot experiment revealed that among the treatments, T1 (germinated seeds inoculated with *B. cepacia* UPMB3) and T3 (germinated seeds inoculated with synthetic IAA) showed a positive effect on growth promotion in oil palm seedlings compared with control treatment T2 (non-inoculated germinated seeds). It was observed that the oil palm seedlings treated with *B. cepacia* UPMB3 produced average 15.66 roots per seedlings respectively whereas the synthetic IAA produced average 9.55 roots per seedling after 4 weeks of planting. The average number of leaves, the shoot, roots and leaf length per seedling also found higher than the control treatment. Results are presented in Table 1 and Fig. 6.

Table 1. Morphogenic response of oil palm seedlings inoculated with *B. cepacia* UPMB3 supernatant after 4 weeks.

Different parameters	Treatments		
	T1 (*B. cepacia* UPMB3 + GOPS)	T2 (Control)	T3 (Synthetic IAA + GOPS)
Root length* (cm)	17.00 ± 0.83[a]	9.86 ± 1.75[b]	18.11 ± 0.92[a]
Shoot length* (cm)	1.86 ± 0.35[cd]	1.73 ± 0.12[cd]	1.90 ± 0.12[cd]
Leaf length*(cm)	4.50 ± 1.39[cd]	1.33 ± 0.14[d]	3.07 ± 0.32[cd]
No. of leaves*	2.00 ± 0.00[cd]	1.33 ± 0.28[d]	1.66 ± 0. 16[cd]
No. of roots*	15.66 ± 1.04[a]	9.00 ± 1.32[b]	9.55 ± 1.07[a]

*Average GOPS = Germinated oil palm seeds. Values followed by the same letter are not significantly different according to LSD test at $p < 0.05$ level.

Fig. 6. Influence of phytohormones in different treatments on morphogenic response of oil palm seedlings in pot experiment. T1: *B. cepacia* UPMB3 + oil palm seedling, T2: Untreated oil palm seedling, T3: Synthetic IAA + oil palm seedling.

This result confirmed that IAA produced by the bacterial strain *B. cepacia* UPMB3 in the culture filtrate plays a role in plant growth promotion of oil palm seedlings. Seed treatment of *Bacillus licheniformis* MML2501 in groundnut showed a significant increase in seed germination, plant growth and yield under potted plant experiments (Prashanth and Mathivanan 2010). El-Tarabily (2008) reported that *Streptomyces* spp. from a tomato rhizosphere had the ability to produce IAA and improved tomato growth by increasing root dry weight. The genus *Burkholderia* has shown to be the most widespread rice growth promoting bacteria able to produce plant hormones. The application of these PGPR in

greenhouse and/or field experiments showed, in most cases, a statistically significant increase in seed germination, weight and length of the plant, which means a better grain production efficiency (Rashedul et al. 2009).

Results showed that the rhizobacterial strain UPMB3 of *Burkholderia cepacia* has the ability to produce IAA which may enrich plant growth promotion.

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References

Ahmad FL and **Khan MS** (2005) Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk. J. Biol. **29**: 29-34.

Bhavdish N, Johri A, Sharma J and **Virdi S** (2003) Rhizobacterial diversity in India and its influence on soil and plant health. Adv. Biochem. Eng. Biotech. **84**: 49-89.

Datta C and **Basu PS** (2000) Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. Microbiol. Res. **155**: 123-127.

El-Tarabily KA (2008) Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. Plant and Soil **308**: 161-174.

Idris EE, Iglesias EJ, Talon M and **Borriss R** (2007) Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. MPMI. **20**: 619-626.

Karnwal A (2009) Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L-tryptophan and rice root exudates. J. Plant Pathol. **91**: 61-63.

Madhuri M (2011) Screening of rhizobia for indole acetic acid production. Ann Biol Res. **2**(4): 460-468.

Paulina EDL, Santos R, Bustillos C, and **Jesu´ SCM** (2001) *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Appl. Environ. Microbiol. **67**(6): 2790-2798.

Prashanth S and **Mathivanan N** (2010) Growth promotion of groundnut by IAA producing rhizobacteria *Bacillus licheniformis* MML2501. Arch Phytopathol Plant Protec. **43**: 191-208.

Rashedul IM, Madhaiyan M, Deka Boruah HP, Yim W, Lee G and **Saravanan VS** (2009) Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. Microbiol. Biotechnol. **19**: 1213-22.

Ritika KR, Ajay K, Amit K, Sandip P, Amit P and **Mohinder K** (2012) Indole acetic acid production by fluorescent *Pseudomonas* isolated from the rhizospheric soils of *Malus and Pyrus*. Recent Res. Sci. Technol. **4**: 06-09.

Shirokikh IG, Zenova GM, Merzaeva OV, Lapygina EV, Batalova GA and **Lysak LV** (2007) Actinomycetes in the prokaryotic complex of the rhizosphere of oats in a soddy-podzolic soil. Eur Soil Sci. **40**: 158-162.

Tien TM, Gaskins MH and **Hubbell DH** (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). AEM **37**: 1016-1024.

Zaiton S, Sariah M and **Zainal MA** (2006) Isolation and characterization of microbial endophytes from oil palm roots: Implication as biocontrol agents against *Ganoderma*. The Planter **82**: 587-597.