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Properties of Bacteria Beneficial to the Promotion of Plant Growth

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Abstract. Plant associated bacteria are wide spread, common and non-harmful in environments. The 20 isolates of plant associated bacteria were isolated from local plants in Northern part of Thailand. The bacterial strains were screened for plant growth promoting activity. They showed variation in enzymatic activity. There were 15 and 13 isolates of acetoin and ammonia production, respectively. Phosphate solubilization was detected in 12 isolates and only 3 isolates exhibited a good indole acetic acid (IAA) production which could tolerance to stress conditions. The IAA production was associated with the bacterial growth in medium supplemented with L-tryptophan. It was tryptophan-dependent production. The antimicrobial activity exhibited well against phytopathogens, *Xanthomonas campestris* pv. *vesicatoria*, *Sclerotium rolfeii* and *Fuzarium oxysporum* using well diffusion method. These properties showed benefits of plant associated bacteria in the induction of plant growth.

Keywords: Plant growth promotion, Indole acetic acid (IAA), Antimicrobial activity

1. Introduction

Plant growth promoting bacteria (PGPB) are defined as free-living soil, rhizosphere, rhizoplane and phylosphere bacteria that, under some conditions, are beneficial for plants [1]. Plant associated bacteria can contribute to the health, growth and development of plants. Plant growth promotion by bacteria may result either from indirect effects such as the biocontrol of soil borne diseases through competition for nutrients, siderophore-mediated competition for iron, antibiosis or the induction of systemic resistance in host plants [2], or from direct effects such as the production of phytohormones, enzymes involved in growth regulator metabolism, such as ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, auxins, indole acetic acid (IAA), acetoin, 2,3-butanedial, cytokinins [3], providing host plants with a fixed nitrogen supply, the solubilization of soil phosphorus and iron. Indole acetic acid (IAA) is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity [4]. IAA has been shown to have an important role not only in plant development but also in activation of the plant defense system [5]. Moreover, several recent reports indicate that IAA can also be a signaling molecule in bacterial communication [6]. Plant growth promoting bacteria (PGPB) are associated with many, if not all, plant species and are commonly present in many environments [7]. The aim of this study, was investigation of biological properties of 20 isolates of plant associated bacteria which were isolated from local plants in Northern part of Thailand. Their properties were determined for production of plant growth promoting substances, tolerance in stress conditions and antimicrobial activity.

2. Materials and Methods

2.1. Screening of Plant Associated Bacteria for Production of Plant Growth Promoting Substances

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All plant associated bacteria were tested enzymatic activity, such as esterase, lipase, protease and amylase by plate method using tween-80, tween-20, skim milk and starch as respective substrates. All of the bacterial suspensions were dropped on specific plates for each enzyme and then the plates were incubated at 30˚C for 48 hr. The clear zone was detected by measurement of diameter. Additionally, these isolates were spot inoculated on Pikovskaya's agar medium and incubated at 30˚C for 48 hr. After incubation, the presence of clear zone around the growth was measured the diameter indicating phosphate solubilization [8]. For the screening of ammonia production, all the isolated bacteria were inoculated into 5 ml of sterile peptone broth and incubated at 30˚C for 48 hr. After incubation, 0.5 ml of Nessler's reagent was added into the tubes. The production of ammonia was indicated by the appearance of yellow colour. The Voges-Proskauer test was used as a qualitative method for the detection of acetoin production. The bacteria were inoculated into 2 ml of MR-VP broth individually and incubated at 30˚C for 48 hr. After incubation, 1 ml of bacterial culture, 3 ml of freshly prepared α-naphthol (5%) in absolute ethanol and 1 ml of 40% KOH were mixed and stirred vigorously. The formation of a red colour indicated the presence of acetoin. For the screening of Indole acetic acid (IAA), the bacteria were inoculated into 5 ml of nutrient broth supplemented with 3 mg/ml of L-tryptophan and incubated at 30˚C for 48 hr [9]. After incubation, 1 ml of Salkowski's reagent was added into 1 ml of the sample culture and the mixture was left in the dark for 30 min at room temperature. The development of pink colour indicated the production of indole acetic acid.

2.2. Determination of Plant Associated Bacteria for Tolerance in Stress Conditions

All plant associated bacteria were inoculated into 5 ml of nutrient broth at 30˚C for 48 hr. The cell cultures were treated with 3% hydrogen peroxide (H_2O_2) and 3% sodium chloride (NaCl) for 2 hr. After that 10 µl of the treated cell were dropped on NA and incubated at 30°C for 24 hr [10]. For metal ion stress, 10 µl of cell culture were dropped on NA supplemented with 1 mM of ferric chloride ($FeCl_3$), mercury chloride ($HgCl_2$), copper sulfate ($CuSO_4$), zing sulfate (ZnSO4), manganese sulfate ($MnSO_4$), nikel sulfate ($NiSO_4$), aluminium sulfate ($AlSO_4$), silver sulfate ($AgSO_4$), cobalt sulfate ($CoSO_4$), cadmium sulfate ($CdSO_4$) and lead nitrate ($Pb(NO_3)_2$). The plates were incubated at 30°C for 48 hr. The presence of colonies indicated tolerant growth to stress conditions.

2.3. Determination of Plant Associated Bacteria for Antimicrobial Activities

All plants associated bacteria were cultured in LB medium at 30˚C for 72 hr under agitation. The cell-free supernatant was obtained by centrifugation (10,000 rpm x 10 min.) and filtered through a 0.45 µm Millipore filter. The well diffusion method was used to determine. The 50 µl of filtrate were dropped in the well (0.6 cm.) on nutrient agar which contained about 10^8 CFU/ml of *Xanthomonas campestris* pv. *vesicatoria* in upper layer. The plates were incubated at 30˚C for 48 hr. The antibacterial activity was determined by measurement of the diameter of clear zone. Antifungal activities against *Sclerotium rolfeii* and *Fuzarium oxysporum*, the mycelial growth was spotted in the center on potato dextrose agar (PDA), the 50 µl of filtrate were dropped in the well around the mycelial spot in an equal distance. The plates were incubated at 30˚C for 72 hr. Percent inhibition was calculated: % inhibition = [1- (distance of tested fungal growth/distance of control fungal growth)] x 100.

2.4. Growth and IAA Production of Plant Associated Bacteria

The plant associated bacteria were inoculated into 60 ml of the nutrient broth supplemented with 3 mg/ml of L-tryptophan or without L-tryptophan and were incubated at 30˚C. The cultures were collected at incubation time of 0, 12, 24, 36, 48, 60 and 72 hr. The bacterial growth was monitored using spectrophotometer by measuring the optical density at 600 nm. The cultures were centrifuged at 10,000 rpm for 10 min. One ml of supernatant was added into 1 ml of Salkowski's reagent and the mixture was left in the dark for 30 min at room temperature. The production of IAA was monitored by measuring the optical density at 530 nm.

3. Results and Discussion

3.1. Properties of Plant-Associated Bacteria Suitable for Promoting in Plant Growth

Enzymatic activities of all plant associated bacteria are shown in Table 1. The esterase, lipase and protease are hydrolytic enzymes which are involved in the suppression of pathogenic growth and subsequent reduction in damage to plants [1]. These enzymes were found more than 10 bacterial isolates. According to hydrolytic enzymes, the antimicrobial activity is perhaps related to mechanisms promoting plant defence to phytopathogens. The filtrate and supernatant of the bacteria were determined the antimicrobial activity against *Xanthomonas campestris* pv. *vesicatoria, Sclerotium rolfeii* and *Fuzarium oxysporum* (Fig 1). The results showed that the UD 136 had a good antifungal activity while the UD 15 gave a good antibacterial activity (Table 1). The UD 288 showed high activities against both bacteria and fungi, and also gave high enzymatic activity. However, the UD 317 produced 3 hydrolytic enzymes but did not have antimicrobial activity. It seems that the antimicrobial activity play their own roles to inhibit pathogens; it may not involved in enzymatic mechanisms. For plant growth promoting substances, most of bacterial isolates could produce these substances individually (Table 1). Amylase enzyme which promote early germination and increase in availability of starch assimilation [11] showed high activity in the most producing strains. Acetoin is volatile substance responsible for plant growth promotion [3, 8] and ammonia production has a signaling role in the interaction between bacteria and plants [12]. The 12 of bacterial isolates could converse insoluble forms of phosphate to plant available forms as shown in phosphate solubilization. These indicate that most of the bacterial isolates represented plant associated bacteria could promote plant growth. Additionally, the bacterial properties of tolerance in stress conditions were determined. These properties may be help host plants living in stress conditions by interactions of bacteria and plants. All bacterial isolates were tolerant with 3% sodium chloride and 1mM of all tested metal ions, except nikel sulfate. Only UD 44 and UD 317 showed the growth on 1mM nikel sulfate; also UD 247 and UD 270 were tolerant with 3% hydrogen peroxide (data not shown). The UD 44, UD 247 and UD 270 were IAA producing strains that showed tolerance to stress. It has been reported that strains of IAA production had more active metabolism resulting in tolerance to stress environments [10].

3.2. Growth and IAA Production of Plant-Associated Bacteria

The UD 44, UD 247 and UD 270 were good producers for IAA production. The IAA is a plant hormone with no apparent function in bacterial cell and it could be speculated that IAA production may improve the fitness of the plant-bacterium interaction [2]. The quantitative analysis of IAA production was monitored during the growth of three bacteria in nutrient broth with and without L-tryptophan. The IAA production was not observed in the absence of L-tryptophan; this showed the same results in previous reports [13]. The IAA production was associated with the bacterial growth (Fig 2). The bacteria produced high IAA amount after 24 hr, particularly UD 270. This implied that greater bacterial growth coincided with greater IAA production.

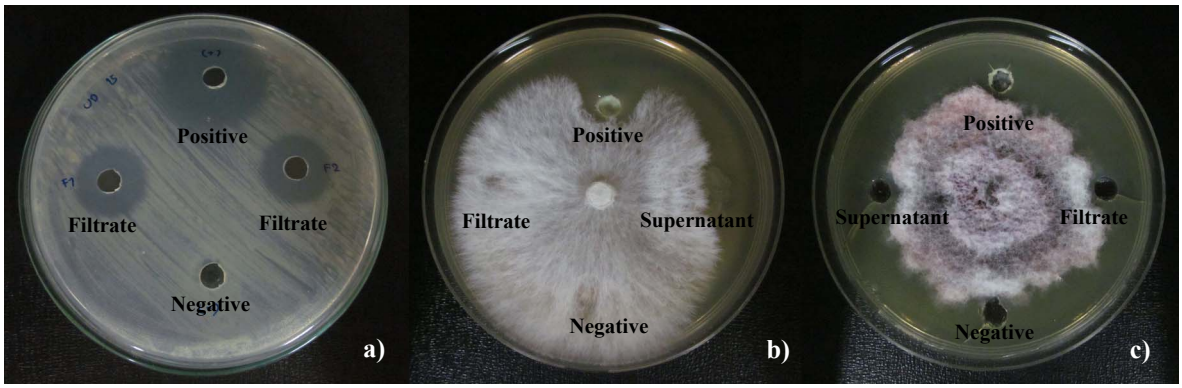

Fig. 1: Antimicrobial activity of plant associated bacteria against phytopathogens. a) UD 15 against *Xanthomonas campestris* pv. *vesicatoria*, b) UD 136 against *Sclerotium rolfeii*, c) UD 288 against *Fuzarium oxysporum*. Positive is Tetracycline (20 µg/ml for bacteria) and Nystatin (100,000 units/ml for fungi); Negative was bacterial medium

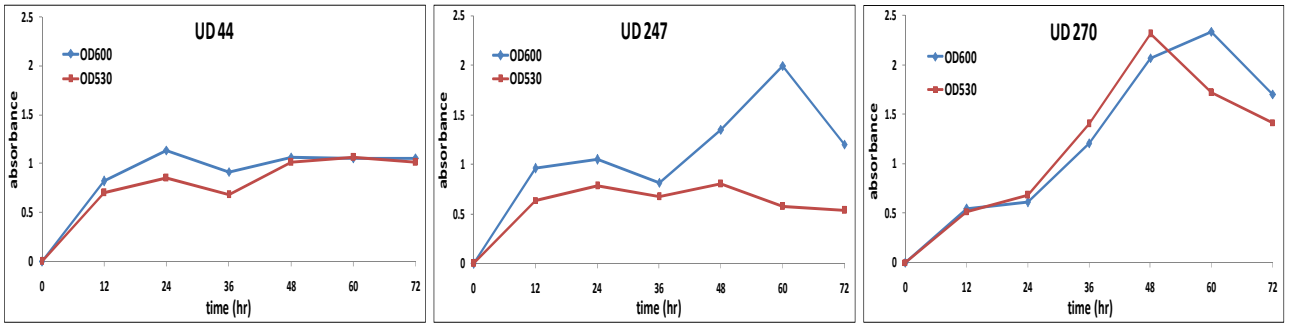

Fig. 2: Growth and indole acetic acid production of plant associated bacteria in nutrient broth with 3 mg/ml of L-tryptophan

Table 1: Characteristics of 20 plant associated bacteria for plants growth promoting

Isolates	Enzymatic activity[a]				Plant growth promoting substances				Antimicrobial activities		
	Esterase	Lipase	Protease	Amylase	Phosphate solubilize[b]	Acetoin production[c]	Ammonia production[c]	Indole acetic acid (IAA)[c]	*Xanthomonas*[d]	*Sclerotium*[e]	*Fuzarium*[e]
UD 15	++	+++	-	++++	++	++	-	-	21.25	32	-
UD 25	-	+	+++	+++	++	++	+	-	15.25	35	-
UD 41	-	+	-	+++	++	++	+	-	18.50	30	-
UD 44	+++	+	++	-	-	-	+	++	-	-	-
UD 50	-	+++	++	++	-	-	++	+	-	-	-
UD 57	+	-	++	+++	++	++	+	-	-	38	-
UD 66	-	-	++	+++	+	++	+	-	16.75	34	-
UD 136	+	-	++	++++	++	++	+	-	16.00	40	18
UD 169	-	-	+++	+++	++	+	-	-	16.50	36	-
UD 205	-	-	+	++++	++	++	+	-	20.00	38	-
UD 247	-	+++	-	-	-	-	++	++	-	-	-
UD 270	++	+++	+	-	-	-	-	++	-	-	-
UD 288	-	+++	++	+++	++	++	++	-	21.00	34	33
UD 292	++	++++	+	++++	-	++	-	-	-	-	15
UD 306	-	+	++	++	++	++	+	-	15.00	36	14
UD 317	++	++	+++	-	++	++	+	-	-	-	-
UD 343	+++	++	+	-	+	++	++	-	-	-	-
UD 392	+++	-	-	-	-	-	-	+	-	-	-
UD 405	++	+++	+	++	-	+	-	-	-	-	-
UD 412	-	++	+	++	-	+	-	+	-	-	-

[a] Zones of enzymatic activity: - no activity, + 0.1 – 1.0 cm, ++ 1.1 – 2.0 cm, +++ 2.1 – 3.0 cm, ++++ 3.1 – 4.0 cm.
[b] Zones of phosphate solubilization: - no activity, + 0.1 – 1.0 cm, ++ 1.1 – 2.0 cm.
[c] Plant growth promoting substances synthesis : - represents no synthesis, + represents synthesis, ++ represents greatly synthesis
[d] Zones of inhibition (mm.)
[e] Percent inhibition = [1- (distance of tested fungal growth/distance of control fungal growth)] x 100

4. Conclusions

All of the 20 plant associated bacteria showed different properties of plant growth promoting activity. The UD 15, UD 136 and UD 288 had a potential property of antimicrobial activity against phytopathogens. This group may be involved in the induced systemic resistance (ISR). Moreover, the UD 288 could produce amylase, acetoin, ammonia and phosphate solubilization that were involved in the induction of plant growth. Like, the UD 44, UD 247 and UD 270 showed high IAA production which is a hormone for plant growth.

These strains also were tolerant with metal ions, sodium chloride and hydrogen peroxide. The characterization of the potential strains will be more investigated for properties beneficial to agriculture.

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