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Indole-3-Acetic Acid Producing Yeasts in the Phyllosphere of Legumes: Benefits for Chili Growth

Sopitar Soponputtaporn[1], Moltira Srithaworn[1], Yaowanoot Promnuan[1], Piyamat Srirat[2,3] and Orawan Chunhachart[1,3,*]

[1]Division of Microbiology, Department of Science and Bioinnovation, Faculty of Liberal Arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
[2]Division of Biology, Department of Science and Bioinnovation, Faculty of Liberal Arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
[3]Microbes for Agriculture Research Unit, Faculty of Liberal Arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

([*]Correspondence author's e-mail: orawan.chu@ku.th)

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Abstract

Plant growth-promoting yeast (PGPY), which is associated with plants, has demonstrated the ability to enhance plant growth and crop yield and provide an effective alternative strategy for reducing the extensive use of chemical fertilizers. However, plant development and yield effects of the phyllosphere yeast have received comparatively little research. Therefore, 95 phylloplane yeast were isolated from the leaves of plants in *Leguminosae*, and their capability to produce indole-3-acetic acid (IAA) in yeast extract peptone dextrose (YPD) broth supplemented with L-tryptophan was evaluated in this research. Forty-two isolates were selected, due to their ability to produce IAA. Among these isolates, 7 showed high IAA production of more than 40 mg/g DW in YPD with tryptophan as the precursor. These strains were identified as *Candida tropicalis*, *Pichia kudriavzevii* and *Tortispora caseinolytica* based on morphological and D1/D2 domain of LSU rDNA sequence analysis. Other plant growth promotion traits, including the solubilization of phosphate, production of ammonia and siderophores and ACC deaminase activity, were also investigated. The results revealed that *Candida tropicalis* KPS2219 exhibited maximum IAA production of 54.10 mg/g DW, high ammonia production at 1.16 mg/mL and siderophore production. In a greenhouse experiment, the ability of *C. tropicalis* KPS2219 to enhance the growth of chili seedlings was investigated. The results revealed that seed priming followed by foliar spraying with *C. tropicalis* KPS2219 significantly increased the root length, shoot length, root dry weight and stem dry weight of the seedlings by 17.72, 29.15, 60 and 46.15 %, respectively, in comparison to those of the uninoculated control. These findings indicate the possibility of *C. tropicalis* KPS2219 as a bioinoculant to promote plant development and the effectiveness of foliar application. The efficacy of employing yeast consortiums to enhance growth will be investigated for further study.

Keywords: Phylloplane yeast, Legumes, Plant growth promotion, Indole-3-acetic acid

Introduction

Thailand is the world's top producer and exporter of important economic food crops, fruits, and vegetables [1]. Therefore, the demand for chemical fertilizers has increased because of the expansion of the agricultural sector. The Office of Agricultural Economics estimates that Thailand's imports of chemical fertilizer increased from a value of over 1.35 million USD in 2020 to more than 3.02 million USD in 2022 [2]. Increased long-term usage of chemical fertilizers has adverse impacts on the environment, human health, and soil quality [3]. To avoid the adverse effects of chemical fertilizers used in agricultural farming, biofertilizer has been used as a potential solution [4]. Additionally, the use of microorganisms that promote plant development has taken on a significant role as an alternative approach for reducing the usage of chemical fertilizers [5]. The benefits of utilizing plant growth-promoting microorganisms, including bacteria, actinomycetes and yeast, have been widely reported [6,7].

Yeast are eukaryotic, unicellular microorganisms classified under the phylum Ascomycetes or Basidiomycetes of the kingdom Fungi. Yeasts are involved in a variety of ecologically relevant activities, such as fermentation, decomposition, biodegradation, and the colonization of microhabitats [8]. In recent years, diverse groups of yeast have become more significant in agricultural sectors for promoting plant

growth and yeast nitrogen-based biofertilizer [7,9]. Plant growth-promoting yeast (PGPY) are a beneficial group of yeasts, and the main sources of isolation are the rhizosphere and phyllosphere of a number of plant species [10,11]. Plant development can be promoted by yeast in both direct and indirect ways. PGPY employs a direct mechanism to produce phytohormones such as auxins [9] and cytokinin [12]. An indole derivative of the auxin family known as indole-3-acetic acid (IAA), a plant growth hormone, has undergone substantial studies and has been found to be the most prevalent auxin type in plants. IAA is synthesized, not only in plants but also in bacteria [13], yeast [10,11], actinomycetes [14] and filamentous fungi [15], and different levels of IAA production have been observed among microbial groups. IAA-producing yeasts include both Ascomycetous yeasts such as *Candida* spp., *Cryptococcus* spp., and *Torulaspora* spp. [11] and Basidiomycetous yeast, including *Rhodosporidiobolus fluvialis*. [16] have been reported. PGPY can improve nutrient absorption in addition to producing phytohormones through several different mechanisms. Phosphate-solubilizing as well as zinc-solubilizing yeast enhance plant growth by solubilizing insoluble phosphates and zinc in the soil, which is absorbed by plant roots [10,11]. Indirectly, siderophores generated by microorganisms assisted in the buildup of Fe in environments poor in iron, and siderophore-producing yeasts have been reported [17]. The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which transforms the plant ethylene precursor ACC into ammonia and α-ketobutyrate, is also known to be produced by PGPY. PGPY prevents growth inhibition and protects plants from a number of environmental stressors by lowering ethylene levels [18]. The nitrogen fixation or production of ammonia (NH_3) is one of the major characteristics of PGPY because plants may receive nitrogen, the most important nutrient, as either nitrate or ammonium [11,19]. Furthermore, biocontrol activity against plant pathogens is also one of the indirect actions of PGPY [20]. The major application strategy of PGPY uses soil inoculation techniques, either independently or in combination with chemical fertilizers [21,22]. According to their plant growth-promoting traits, the impact of PGPY on the growth and development of plants has been widely studied. However, isolation of PGPY mainly originated from plant rhizospheres. PGPY derived from the plant phyllosphere is very occasionally reported [9].

Phylloplane or phyllosphere is the term used to describe the parts of plants that are aboveground and mainly made up of leaves, a niche habitat for microorganisms [8,23]. For growth, phylloplane microorganisms need both the organic chemicals made by the plant and the organic substances received from outside sources [24]. There have been reports of yeasts from both phyla, Ascomycota and Basidiomycota, colonizing the phylloplane of plants in temperate and tropical climates [25]. The diversity of yeast isolated from the phylloplane of corn [26] and rice [27] has been reported. Fabaceae or Leguminosae is one of the largest and most economically important plant families and is widely planted in Thailand. Additionally, the plant family with the greatest number of medicinal species in Thailand is the legumes, which are a natural supply of key substances used in medicine [28, 29]. Therefore, it is conceivable that the phylloplane of legumes provides a habitat for beneficial yeast. However, isolation of yeast from the phylloplane of legumes is infrequent [20].

Chili is an important horticultural crop that is commonly used in the cuisine of Thailand, southern India, and southeast Asia. Thailand is the 4 largest exporter of chili [30]. The Department of Agricultural Extension estimates that in Thailand, chili is grown on approximately 11 % of the country's total vegetable cultivation area [31]. Conventional agriculture relies on a high input of chemical fertilizers, both in the soil and as foliar sprays during cultivation, which has a variety of adverse environmental consequences and increases production costs [2]. Currently, agricultural development is focused on environmentally friendly agricultural systems to ensure the sustainability of food production. As previously mentioned, it has been demonstrated how a variety of soil and root microorganisms work as stimulants to enhance plant development. Plant-associated yeast benefits the growth and development of plants; therefore, using yeast from the phylloplane that promotes plant growth has the potential to minimize the high input of synthetic or inorganic fertilizers and benefits for organic agriculture. Although being in close contact with the plant during the whole growing season, the impact of the phyllosphere yeast on plant development and yield has received comparatively little research [9]. Thus, the purpose of this study was to isolate yeast from the phylloplane of plants in the Leguminosae family and to evaluate their plant growth-promoting traits. This research also aimed to identify PGPY at the molecular level and to determine how they influence the development of a model chili plant (*Capsicum frutescens* L.) cultivated in a greenhouse.

Materials and methods

Sampling sites and sample collection

Sixty-one leaf samples were collected from *Leguminosae* in different areas of Chachoengsao, Nakhon Pathom and Kanchanaburi Province, Thailand (**Table 1**). Plant leaves were collected in sterile, dried polythene bags, kept in an icebox and transferred to the laboratory within 6 h for further analysis. The leaves were kept at 4 ± 2 °C until screening, which was not more than 2 days after sample collection.

Yeast isolation and culture conditions

Five g of healthy leaves were cut with sterilized scissors and forceps. Surface sterilization was carried out using a 2 % (v/v) sodium hypochlorite solution mixed with 0.1 % (v/v) Tween 20 for 3 min followed by washing with 70 % (v/v) ethanol for 1 min. Then, the leaves were washed in sterile distilled water 3 times [11]. The plant leaves were placed in yeast extract peptone dextrose (YPD) broth supplemented with 0.02 % (w/v) streptomycin (10 g yeast extract, 20 g potato dextrose broth and 20 g peptone per liter of water) and incubated at 25 ± 2 °C with shaking at 150 rpm for 48 h. The suspensions were spread on YPD agar supplemented with 0.02 % (w/v) streptomycin and incubated at 25 ± 2 °C for 48 h. The colony was chosen and cross streaked on YPD agar supplemented with 0.02 % (w/v) streptomycin to obtain pure yeast culture.

Screening of indole 3-acetic acid (IAA) production

The colorimetric study reported by [32] was carried out to assess the production capabilities of IAA. A pure yeast that had been grown at 25 ± 2 °C for 24 h was inoculated into 5 mL of YPD broth with and without 0.1 % (w/v) L-tryptophan added, and it was then incubated at 25 ± 2 °C with shaking at 150 rpm for 5 days. The culture suspensions were centrifuged at 8,000×g for 5 min, and the supernatants were collected. One milliliter of supernatant was mixed with 1 mL of Salkowski reagent containing 12 g $FeCl_3$ and 7.9 M H_2SO_4 [33] followed by a 30 min incubation in the dark. Pink coloring indicated that IAA was being produced. For quantification of indole-3-acetic acid production, purified yeast cultures were inoculated in 50 mL of YPD broth supplemented with or without 0.1 % (w/v) L-tryptophan and incubated at 25 ± 2 °C for 7 days. IAA production was determined using a spectrophotometer (Thermo Scientific, GENESYS 10S UV-VIS, USA) set at 530 nm. Standard IAA concentrations of 0 - 100 g/mL were used to create the calibration curve. Growth was determined as dry weight by drying cells at 100 °C until weight was constant [34].

Phosphate solubilization

Yeasts were grown in 5 mL of YPD broth medium at 25 ± 2 °C for 48 h with shaking at 150 rpm. Three microliters of the inoculum adjusted to an optical density at 600 nm of 0.10 was spotted on Pikovskaya's agar according to [35] (3 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot H_2O$, 0.001 g $FeSO_4$, 0.1 g $MnSO_4$, 0.2 g KCl, 10 g dextrose, 0.5 g yeast extract, 15 g agar per liter of water). The culture was incubated at 25 ± 2 °C for 5 days. Each treatment was performed in 3 replicates. A strain that can dissolve phosphate is indicated by a clear zone around the colony. The phosphate solubilization efficiency (SE) was calculated as a ratio between the diameter of the halo zone and the diameter of the colony [11].

Siderophore production

Siderophore production was investigated using Chrome azurol S (CAS) agar (1.21 g Chrome azurol S (CAS), 100 mL iron (III) solution (1 mmol $FeCl_3 \cdot 6H_2O$, 10 mmol HCl), 1.82 g hexadecyltrimetyl ammonium bromide (HDTMA), 0.3 % (v/v) methylene blue, 15 g agar per liter of water). The medium was prepared according to the method described by [36]. Yeasts were grown on YPD agar at 25 ± 2 °C for 24 h, and then a single colony was picked and point inoculated onto CAS agar. The cultures were incubated at 25 ± 2 °C for 5 days. The isolate showed colonies surrounded by light orange zones, indicating the presence of iron carriers.

1-aminocyclopropane-1-carboxylate (ACC) deaminase activity

The ACC deaminase activity was determined as described by [10,37]. Yeasts were grown in 5 mL of YPD broth at 25 ± 2 °C for 24 h with shaking at 150 rpm. The cultured suspension was inoculated into 5 mL of yeast carbon base (YCB) broth supplemented with 3.0 mM ACC (filter sterile) as the nitrogen source. YCB broth without ACC was used as a control. The cultures were incubated at 25 ± 2 °C for 14 days. The ACC deaminase activity was determined by culture turbidity comparing with the uninoculated

control. The culture turbidity indicates the potential of yeast to use ACC as their N source through deamination.

Quantitative analysis of ammonia production

Ammonia production by yeast isolates was tested in peptone water. A sample of 100 µl of yeast suspension 10^8 CFU/ mL was inoculated in 5 mL of peptone water broth and incubated at 25 ± 2 °C with shaking at 150 rpm for 5 days. The culture suspension was centrifuged at 8,000×g for 5 min. One milliliter of supernatant was mixed with 100 µl of Nessler's reagent and incubated for 5 min until a yellow-to-brown color developed. Ammonia production was determined by a UV-Vis spectrophotometer (Thermo Scientific, GENESYS 10S UV-VIS, USA) set at 450 nm. Standard ammonium sulfate was used to create the calibration curve [19].

Growth ability under N-free conditions

Yeasts were grown in 5 mL of YPD broth medium at 25 ± 2 °C for 48 h with shaking at 150 rpm. On an N-free plate (0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.1 g $CaSO_4$, 0.003 g $FeCl_3$, 0.001 g Na_2MoO_4 and 15 g agar per liter of water), the inoculum was spotted after being adjusted to an optical density at 600 nm of 0.10. After incubation at 25 ± 2 °C with shaking at 150 rpm for 7 days. The nitrogen fixation capability of the isolates was determined by growth on N-free medium.

Yeast identification

The purified yeast strains were checked for colony morphology by cross streaking on YPD agar with 0.02 % (w/v) streptomycin at 25 ± 2 °C for 48 h. A single colony was observed. Cell morphology was observed by wet mount staining and optical microscopy (Olympus, CX31RBSFA, USA) to study the morphology of the cells. Based on the analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene sequences, yeasts were classified using molecular taxonomy. Methods for genomic DNA isolation of the D1/D2 domain were extracted with GF-1Plant DNA Extraction Kit II, (Vivantis, Malaysia) according to the manufacturer's protocol. The amplification of the D1/D2 domain was performed with primers NL1 (5′GCATAT CAATAAGCGGAGG AAAAG-3′) and NL4 (5′GGTCCGTGTTTCAAGAC GG-3′) as described by Kurtzman and Robnett [38]. The PCR products were checked by agarose gel electrophoresis and purified with a GF-1 AmbiClean Kit (Vivantis, Malaysia). The purified products were submitted to ATGC Co., Ltd. for sequencing. The sequences were submitted to the nucleotide database of GenBank by BLAST software from NCBI. Using Bio Edit sequence alignment Editor 7.2.5, the sequences of closely related type strains from the GenBank database were multiple aligned. In MEGA ver. 11.0.11 [39], a phylogenetic tree was generated using the maximum likelihood technique [40].

Assessment of growth promotion of phylloplane yeasts on chili seedlings under greenhouse conditions

Candida tropicalis KPS2219 was chosen for a greenhouse experiment to examine its capacity to promote chili seedling growth based on its high IAA production ability. The experiment was performed in the greenhouse of Kasetsart University at the Kamphaeng Saen campus, Nakhon Pathom, Thailand, during March-May 2022 with an average temperature of 37 ± 2 °C. Chili seeds (*Capsicum frutescens* Linn. cv. TVRC758), purchased from the Tropical Vegetable Research Center, Kasetsart University Kamphaeng Sean campus, were used in this experiment. For the experimental design, a completely randomized design (CRD) was used. Chili seeds were surface sterilized using 15 % (v/v) sodium hypochlorite solution with 0.1 % (v/v) Tween 20 for 15 min and washed 3 times with sterilized water [11]. The seeds were then separately soaked in a cell suspension of *C. tropicalis* KPS2219 at a concentration of 10^8 CFU/mL and continuously shaken at 150 rpm for 6 h. Then, the seeds germinated in sterilized peat moss for 14 days. After that, the seedlings were transplanted into 12 cm. pots containing soil and cocopeat at a ratio of 1:1 (w/w). The experiment was carried out in triplicate, with each treatment using 15 pots. Then, 10 mL of yeast suspension (10^8 CFU/mL) was sprayed on the leaves of plants in each pot every 2 weeks. Sterilized deionized water was used as a control in both the soaking and spraying steps. After 30 days of growth, the plants were harvested, and the roots were washed carefully with running water. The lengths of the shoots and roots, and the shoot and root wet weights and dry weights were measured. For determination of dry weight, all samples were dried at 100 °C until constant weight at approximately 2 days.

Statistical analysis

Statistical analysis was carried out using SPSS 16.0 (SPSS Inc., Illinois, USA). Means of different treatments were compared using Duncan's multiple range test at the 0.05 level of probability.

Results and discussion

Isolation of phylloplane yeast and assessment of IAA production

Based on screening, 95 phylloplane yeasts were isolated from 61 leaf samples of 26 legume plant species. All isolates were determined to be able to produce IAA in YPD broth with and without 0.1 % L-tryptophan supplementation. Forty-two IAA-producing phylloplane yeast were obtained, as shown in **Table 1**. The colony and cell morphologies of IAA-producing strains were examined as shown in **Table 2**. All isolates showed the cell morphology of yeast, such as ovoid, cylindrical, and elongated shapes with budding, under a light microscope. Plant growth-promoting yeast (PGPY) have gained importance due to their ability to stimulate plant growth, inhibit plant pathogens, and serve as biofertilizers [9]. For isolation, there are only a few cases of PGPY being isolated from the phyllosphere of plants, compared to being mostly isolated from the rhizosphere of a wide variety of plant species [41]. In this study, 95 phylloplane yeast were isolated from the leaves of legumes by the plating method using YPD agar (**Table 1**). This approach has the benefit of offering pure cultures that may be utilized directly to research organisms' physiology and metabolism as well as their potential for usage in industrial, agricultural, and environmental applications. However, this approach is limited in that it cannot detect species that cannot be cultured on culture media.

Table 1 Leaf from diverse plant species and yeasts isolated from phylloplane of *Leguminosae*.

Location	Geographical coordinate	Plant	Number of isolates	Number of IAA producing isolates
Mung district, Chachoengsao province	13° 4′ 19″ N, 101° 4′ 15″ E	Asian pigeonwings (*Clitoria ternatea*)	3	3
		Black rosewood (*Afzelia xylocarpa*)	2	-
		Burma padauk (*Pterocarpus macrocarpus*)	1	-
		Burma padauk (*Pterocarpus macrocarpus*)	1	-
		Cassod tree (*Senna siamea*)	1	-
		Earleaf acacia (*Acacia auriculiformis*)	1	1
		Peacock flower (*Caesalpinia pulcherrima*)	1	1
		Peacock flower (*Caesalpinia pulcherrima*)	1	-
		Purple orchid tree (*Bauhinia purpurea*)	2	-
		River tamarind (*Leucaena leucocephala*)	2	1
		River tamarind (*Leucaena leucocephala*)	2	2
		Royal poinciana (*Delonix regia*)	1	-
		Sensitive plant (*Mimosa pudica*)	2	2
		Sensitive plant (*Mimosa pudica*)	1	-
		Sesban (*Sesbania sesban*)	1	-
		Tamarind (*Tamarindus indica*)	3	1
Sai Yok district, Kanchanaburi province	14° 7' 1" N, 99° 8' 15" E	Earleaf acacia (*Acacia auriculiformis*)	2	-
		Jewel vine (*Derris scandens*)	2	-
		Red sandandalwood tree (*Adenanthera pavonina*)	2	2
		River tamarind (*Leucaena leucocephala*)	2	2
		Sensitive plant (*Mimosa pudica*)	3	-
		Tamarind (*Tamarindus indica*)	3	-
		Tamarind (*Tamarindus indica*)	1	-
Bo phloi district, Kanchanaburi province	14.3059° N, 99.4784° E	Burma padauk (*Pterocarpus macrocarpus*.)	1	1
		Golden shower tree (*Cassia fistula*)	1	-
		Golden shower tree (*Cassia fistula*)	1	-
		Golden shower tree (*Cassia fistula*)	3	2
		kra phi nang nuan (*Dalbergia cana Grah. ex Kurz*.)	1	1
		Peacock flower (*Caesalpinia pulcherrima*)	1	1
		Rain tree (*Samanea saman*)	2	-
Phanom Thuan district, Kanchanaburi province	14° 7′ 49″ N, 99° 41′ 56″ E	Cassod tree (*Senna siamea*)	1	1
		Manila tamarind (*Pithecellobium dulce*)	1	1
		Manila tamarind (*Pithecellobium dulce*)	1	-
		Peacock flower (*Caesalpinia pulcherrima*)	1	1
		River tamarind (*Leucaena leucocephala*)	1	1
Tha Maka district, Kanchanaburi province	13° 55′ 15″ N, 99° 45′ 56″ E	Golden shower tree (*Cassia fistula*)	2	2
		Pink shower (*Cassia bakeriana* Craib)	2	1
		River tamarind (*Leucaena leucocephala*)	1	-

Location	Geographical coordinate	Plant	Number of isolates	Number of IAA producing isolates
Kasetsart university Kamphaeng Sean campus, Nakhon Pathom province	13° 59′ 2″ N, 99° 59′ 38″ E	Burma padauk (*Pterocarpus macrocarpus*)	1	1
		Burma padauk (*Pterocarpus macrocarpus*)	1	-
		Copper pod (*Peltophorum dasyrrhachis* (Miq.) Kurz)	2	-
		Copper pod (*Peltophorum dasyrrhachis* (Miq.) Kurz)	1	-
		Copper pod (*Peltophorum dasyrrhachis* (Miq.) Kurz)	1	-
		Copper pod (*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne)	3	3
		Earleaf acacia (*Acacia auriculiformis*)	2	2
		Indian coral tree (*Erythrina subumbrans*)	1	1
		Java cassia (*Cassia javanica*.)	1	-
		Java cassia (*Cassia javanica*.)	2	2
		Ma kha num (*Sindora siamensis*)	1	1
		Peacock flower (*Caesalpinia pulcherrima*)	1	-
		Pink shower (*Cassia bakeriana* Craib)	2	-
		Purple orchid tree (*Bauhinia purpurea*)	1	-
		Purple orchid tree (*Bauhinia purpurea*)	1	-
		Rain tree (*Samanea saman*)	2	-
		Red sandandalwood tree (*Adenanthera pavonina*)	2	-
		Royal poinciana (*Delonix regia*)	2	2
		Sesban (*Sesbania sesban*)	1	-
		Tamarind (*Tamarindus indica*)	2	2
		Tamarind (*Tamarindus indica*)	3	-
		Thai rosewood (*Dalbergia cochinchinensis*)	1	1
		Yellow ashoka (*Saraca thaipingensis*)	1	-
			95	42

Quantitative analysis of IAA production

The quantitative analysis of IAA using a colorimetric assay revealed that 42 isolates produced IAA from the medium containing L-tryptophan and 6 isolates produced IAA without L-tryptophan. Forty-two yeast isolates produced IAA at different levels in medium supplemented with 0.1 % L-tryptophan. Seven isolates (KPS2219, KPS2206, KPS2007, KPS2006, KPS2044, KPS2034, and KPS2033) were determined to have remarkably high IAA production since they produced more than 40 mg/g DW of IAA (**Table 2**). Of them, the isolate KPS2219 produced the highest yield of IAA (54.10 mg/g DW). Thirteen isolates had moderate IAA levels ranging from 32.95 - 13.73 mg/g DW, whereas 22 isolates had IAA levels less than 12.0 mg/g DW. However, 6 produced IAA without supplementation with L-tryptophan, and the amount of IAA ranged from 9.73 - 51.09 mg/g DW. Isolates KPS2044 and KPS2078 produced less IAA than when L-tryptophan was added to the medium. The isolates KPS2031, KPS2211, KPS2218, and KPS2117, on the other hand, generated more IAA in the presence of L-tryptophan-containing medium. The first characteristic of a microorganism that promotes plant development is phytohormone production. Indole-3-acetic acid (IAA) is an auxin that plays an important role in cell division and elongation [42]. Several microorganisms, including yeast, can produce IAA through various pathways, which has a considerable impact on plant growth and development. Therefore, the production of IAA by all isolates was examined. In medium containing L-tryptophan, 42 isolates exhibited IAA production ability (44.2 %), whereas 53 isolates (55.7 %) did not, indicating that IAA production is strain dependent. In this study, IAA-producing yeast showed a wide range of IAA production levels (1.34-54.10 mg/g DW), as shown in **Table 2**. To our knowledge, *C. tropicalis* KPS2219 exhibited a remarkably high IAA amount of 54.10 mg/g DW in comparison to prior works. From previous reports, epiphytic yeast isolated from rice and sugar cane leaves produced IAA ranging from 1.2 - 29.3 mg/g DW [10] and 24.3 - 314.3 mg/L [34]. From the results, some isolates can produce low amounts of IAA without L-tryptophan supplementation. Yeast isolated from the rhizosphere and phyllosphere of *Drosera spatulate* produced IAA ranging from 8.63 - 610.63 mg/L in YPD supplemented with L-tryptophan and 1.6 - 236.62 mg/L in YPD without L-tryptophan [11]. IAA can be synthesized via either a tryptophan-independent pathway or tryptophan-dependent pathway [43]. The yeast *Rh. palidigena* DMKU-RP301 has been reported to produce IAA via the indole-3-propionic acid (IPA) pathway, a tryptophan-dependent pathway. *S. cerevisiae* has been shown to have tryptophan-independent IAA biosynthesis [44]. Seven isolates of phylloplane yeast (KPS2219, KPS2206, KPS2007,

KPS2006, KPS2044, KPS2034, and KPS2033) that produced IAA higher than 40 mg/g DW (**Table 2**) were selected for identification at the molecular level and to determine other plant growth-promoting traits.

Table 2 Morphological characteristics and IAA production of phylloplane yeast isolated of *Leguminosae*.

Isolate	IAA production (mg/g DW)		Colony morphology	Cell morphology	Budding cell	Source
	YPD with Trp	YPD w/o Trp	From, Edge, Surface, Elevation			
KPS2219	54.10 ± 3.46	-	Circular, entire, smooth, pulvinate	Round	Pseudohyphae	Copper pod (*Peltophorum pterocarpum*)
KPS2206	50.19 ± 1.94	-	Circular, entire, smooth, pulvinate	Round	Pseudohyphae	Golden shower tree (*Cassia fistula*)
KPS2007	46.71 ± 1.90	-	Circular, entire, smooth, pulvinate	Round	Pseudohyphae	Golden shower tree (*Cassia fistula*)
KPS2006	44.71 ± 1.14	-	Circular, entire, smooth, pulvinate	Round	Pseudohyphae	Golden shower tree (*Cassia fistula*)
KPS2044	43.18 ± 3.31	10.16 ± 11.01	Circular, undulate, smooth, convex	Cylindrical	Multilateral budding	Peacock flower (*Caesalpinia pulcherrima*)
KPS2034	40.86 ± 0.57	-	Circular, entire, smooth, pulvinate	Round	Polar budding	Golden shower tree (*Cassia fistula*)
KPS2033	40.20 ± 1.01	-	Circular, entire, smooth, convex	Ovoid	Monopolar budding	Peacock flower (*Caesalpinia pulcherrima*)
KPS2203	32.95 ± 8.67	-	Circular, entire, smooth, convex	Ovoid	Polar budding	Copper pod (*Peltophorum pterocarpum*)
KPS2237	29.02 ± 3.68	-	Circular, entire, wrinkled, pulvinate	Round	Polar budding	Pink shower (*Cassia bakeriana* Craib)
KPS2078	26.83 ± 4.22	9.73 ± 11.37	Circular, entire, wrinkled, pulvinate	Ovoid	Polar budding	Tamarind (*Tamarindus indica*)
KPS2040	26.76 ± 2.04	-	Circular, filamentous, smooth, convex	Ovoid	Polar budding	Asian pigeonwings (*Clitoria ternatea*)
KPS2207	26.37±0.90	-	Circular, entire, smooth, convex	Ovoid	Polar budding	Copper pod (*Peltophorum pterocarpum*)
KPS2131	25.73 ± 4.29	-	Circular, entire, wrinkled, convex	Ovoid	Pseudohyphae	Red sandandalwood tree (*Adenanthera pavonina*)
KPS2031	23.42 ± 2.57	28.90 ± 11.54	Circular, filamentous, smooth, umbonate	Ovoid	Multilateral budding	River tamarind (*Leucaena leucocephala*)
KPS2118	19.13 ± 0.90	-	Circular, filamentous, smooth, convex	Cylindrical	Pseudohyphae	Earleaf acacia (*Acacia auriculiformis*)
KPS2042	16.44 ± 0.51	-	Circular, filamentous, smooth, pulvinate	Ovoid	Bipolar budding	Asian pigeonwings (*Clitoria ternatea*)
KPS2105	15.85 ± 4.48	-	Circular, entire, smooth, umbonate	Lemon	Polar budding	Asian pigeonwings (*Clitoria ternatea*)

Isolate	IAA production (mg/g DW)		Colony morphology	Cell morphology	Budding cell	Source
	YPD with Trp	YPD w/o Trp	From, Edge, Surface, Elevation			
KPS2030	15.62 ± 0.65	-	Circular, entire, smooth, pulvinate	Round	Polar budding	River tamarind (*Leucaena leucocephala*)
KPS2223	15.50 ± 1.32	-	Rhizoid, undulate, rough, convex	Ovoid	Pseudohyphae	Peacock flower (*Caesalpinia pulcherrima*)
KPS2225	13.73 ± 1.12	-	Circular, entire, smooth, pulvinate	Round	Pseudohyphae	Cassod tree (*Senna siamea*)
KPS2227	12.03 ± 2.70	-	Circular, entire, smooth, convex	Ovoid	Polar budding	Tamarind (*Tamarindus indica*)
KPS2029	10.22 ± 1.83	-	Circular, entire, smooth, umbonate	Ovoid	Polar budding	Burma padauk (*Pterocarpus macrocarpus*)
KPS2226	9.24 ± 1.31	-	Circular, entire, smooth, convex	Round	Pseudohyphae	Tamarind (*Tamarindus indica*)
KPS2112	8.95 ± 0.92	-	Irregular, undulate, rough, convex	Ovoid	Pseudohyphae	River tamarind (*Leucaena leucocephala*)
KPS2240	8.86 ± 0.80	-	Circular, entire, wrinkled, convex	Ovoid	Polar budding	Indian coral tree (*Erythrina subumbrans*)
KPS2148	6.74 ± 0.25	-	Rhizoid, filamentous, rough, umbonate	Elongated	Fission	Sensitive plant (*Mimosa pudica*)
KPS2111	6.61 ± 0.16	-	Irregular, undulate, rough, pulvinate	Lemon	Pseudohyphae	River tamarind (*Leucaena leucocephala*)
KPS2211	5.88 ± 0.20	23.29 ± 7.02	Circular, entire, wrinkled, pulvinate	Ovoid	Polar budding	Java cassia (*Cassia javanica*.)
KPS2080	5.67 ± 1.27	-	Rhizoid, filamentous, rough, pulvinate	Elongated	Fission	Earleaf acacia (*Acacia auriculiformis*)
KPS2114	5.25 ± 0.43	-	Rhizoid, lobate, rough, convex	Elongated	Fission	River tamarind (*Leucaena leucocephala*)
KPS2132	5.05 ± 0.08	-	Circular, entire, wrinkled, convex	Ovoid	Monopolar budding	Red sandandalwood tree (*Adenanthera pavonina*)
KPS2224	5.03 ± 0.05	-	Circular, entire, smooth, convex	Ovoid	Pseudohyphae	Manila tamarind (*Pithecellobium dulce*)
KPS2243	4.63 ± 0.54	-	Circular, entire, smooth, umbonate	Ovoid	Polar budding	Ma kha num (*Sindora siamensis*)
KPS2218	4.35 ± 0.56	15.27 ± 0.55	Circular, entire, smooth, convex	Ovoid	Pseudohyphae	Royal poinciana (*Delonix regia*)
KPS2117	4.13 ± 1.19	51.09 ± 1.58	Circular, filamentous, rough, convex	Cylindrical	Pseudohyphae	Earleaf acacia (*Acacia auriculiformis*)
KPS2113	3.84 ± 0.06	-	Irregular, undulate, rough, convex	Round	Pseudohyphae	River tamarind (*Leucaena leucocephala*)
KPS2210	3.82 ± 1.14	-	Circular, entire, wrinkled, convex	Ovoid	Polar budding	Java cassia (*Cassia javanica*.)
KPS2241	3.52 ± 2.24	-	Circular, entire, smooth, umbonate	Ovoid	Polar budding	kra phi nang nuan (*Dalbergia cana Grah. ex Kurz.*)

Isolate	IAA production (mg/g DW)		Colony morphology	Cell morphology	Budding cell	Source
	YPD with Trp	YPD w/o Trp	From, Edge, Surface, Elevation			
KPS2220	2.98 ± 0.03	-	Circular, entire, wrinkled, convex	Cylindrical	Multilateral budding	Royal poinciana (*Delonix regia*)
KPS2214	2.91 ± 0.86	-	Circular, entire, wrinkled, convex	Ovoid	Polar budding	Burma padauk (*Pterocarpus macrocarpus*)
KPS2205	1.69 ± 0.80	-	Circular, entire, smooth, umbonate	Ovoid	Pseudohyphae	Thai rosewood (*Dalbergia cochinchinensis*)
KPS2149	1.34 ± 1.51	-	Irregular, undulate, rough, raised	Elongated	Fission	Sensitive plant (*Mimosa pudica*)

Plant growth-promoting characteristics

Seven phylloplane yeast isolates that produced IAA levels higher than 40 mg/g DW were selected to examine other plant growth promotion traits, including phosphate solubilization, ammonia production, growth under nitrogen-free conditions, ACC deaminase activity and siderophore production. The isolates KPS2206 and KPS2006 exhibited phosphate solubilizing ability with solubilizing efficiencies of 1.05 ± 0.01 and 1.06 ± 0.01, respectively (**Table 3**). The isolates KPS2044 and KPS2033 showed positive results for ACC deaminase activity. For siderophore production, 3 isolates (KPS2219, KPS2034 and KPS2033) were able to produce the zone ranging from 2 - 8 mm. The isolate KPS2033 produced the widest yellow zone at 8 mm. Ammonia production was also examined in peptone water broth. The results revealed that the isolates KPS2219, KPS2206, KPS2207, KPS2006 and KPS2034 can produce ammonia ranging from 0.84 - 1.16 mg/L. All isolates showed the ability to grow in both nitrogen-free broth and agar medium. None of the IAA-producing isolates exhibited all plant growth promotion traits tested in this study.

Table 3 Plant growth-promotion characteristics of high IAA.

	Phosphate solubilization (SE)	NH_3 production (mg/L)	Growth ability[a] under N-free condition	ACC deaminase[a] activity	Siderophore[b] production	Closest species	% Identities	Result of identification (Accession no)
KPS2219	-	1.16 ± 0.01	+	−	2	*Candida tropicalis* DM KUJC47-1	97 %	*Candida tropicalis* (ON935766)
KPS2206	1.05 ± 0.01	1.03 ± 0.01	+	−	-	*Candida tropicalis* DM KUJC47-1	93 %	*Candida tropicalis* (ON935764)
KPS2007	-	1.13 ± 0.01	+	−	-	*Candida tropicalis* DM KUJC47-1	97 %	*Candida tropicalis* (ON935768)
KPS2006	1.06 ± 0.01	0.84 ± 0.01	+	−	-	*Candida tropicalis* DM KUJC47-1	95 %	*Candida tropicalis* (ON935765)
KPS2044	-	-	+	+	-	*Pichia kudriavzevii* YSP-546	92 %	*Pichia kudriavzevii* (ON935759)
KPS2034	-	1.02 ± 0.01	+	−	5	*Candida tropicalis* DM KUJC47-1	81 %	*Candida tropicalis* (ON935769)
KPS2033	-	-	+	+	8	*Tortispora caseinolytica* CBS:7781	99 %	*Tortispora caseinolytica* (ON935770)

SE: Solubilization efficiency = (halo zone/diameter of colony)
[a] + Positive result, − Negative result
[b] Siderophore production, Diameter of halo zone (mm) = [halo zone - colony zone]

Phosphate-solubilizing microorganisms solubilize phosphate via key mechanisms, including the synthesis of enzymes, the secretion of organic acids, and the release of siderophores that may chelate metal ions and form complexes [45]. Although the phosphate solubilizing activity of the isolates KPS2206 and KPS2006 was present, the solubilization efficiency (1.05 and 1.06) was not very high. The phosphate solubilizing index (PSI) ranges for phosphate solubilizing yeast from Teff rhizosphere soil were 1.72 to 3.35. A high capacity of phosphate solubilization was observed in *Phichia norvegensis* and *Cryptococcus albidus var. aerius*, which obtained 3.35 and 3.2 SPI, respectively [46]. In the present study, the selected strains

(KPS2219, KPS2206, KPS2006, KPS2007, and KPS2034) showed a high amount of ammonia (NH_3) in peptone water medium. Ammonia can be absorbed by plant leaves. It has been suggested that plants may absorb and assimilate atmospheric NH_3-N through quick uptake with immediate assimilation and fast storage with progressive metabolism [47]. Therefore, NH_3 produced by these phylloplane yeasts will benefit plant growth. Siderophore production, one important trait of antagonistic yeast against plant pathogens, is also found in the isolates KPS2219, KPS2033 and KPS2034. However, the activity was relatively small in comparison to that of *Wickerhamomyces anomalus* DMKU-RP25 and *W. anomalus* DMKU-RP04, which generated zones between 2.9 and 3.0 cm. These strains were isolated from the phylloplane of rice leaves and showed antagonistic activity against rice pathogenic fungi [27].

Yeast identification

Seven yeast isolates that exhibited high IAA production (> 40 mg/g DW) were identified in the D1/D2 domain of the LSU rRNA gene sequence. All yeast strains were identified to be in the phylum Ascomycota and subphylum Saccharomycotina (3 genera) with *Candida* 1 species, *Pichia* 1 species and *Tortispora* 1 species (**Table 3**). The phylogenetic position of yeast species obtained in this study showed that strains KPS2006, KPS2007, KPS2206, KPS2219 and KPS2034 have 99 % DNA sequence similarity to *Candida tropicalis* DMKUJC47-1, followed by KPS2033 and KPS2044, which showed 100 % similarity to *Tortispora caseinolytica* CBS:7781 and *Pichia kudriavzevii* YSP-546, respectively (**Figure 1**). The gene sequences of the isolates were submitted to NCBI GenBank under accession numbers ON935766, ON935764, ON935768, ON935765, ON935759, ON935769 and ON935770. Seven isolates total, 5 of which (71.4 %) were identified as *Candida tropicalis*. The majority (80 %) of the *Candida tropicalis* isolates came from *Cassia fistula*, often known as the golden shower tree. The other 2 isolates were obtained from *Caesalpinia pulcherrima* or Peacock flowers. *Candida* species (27.8 %) and *Pichia* (22.3 %) were both present in the phyllosphere of corn leaves; however, *C. tropicalis* and *Tortispora* were not [26]. *Candida* species also appeared in rice leaves at an average prevalence of 15.6 %, with *C. tropicalis* showing up at 4.5 %. Neither *Pichia* nor *Tortispora* were found [27]. The predominance of yeast species might vary depending on plant species, habitats, and ecosystems.

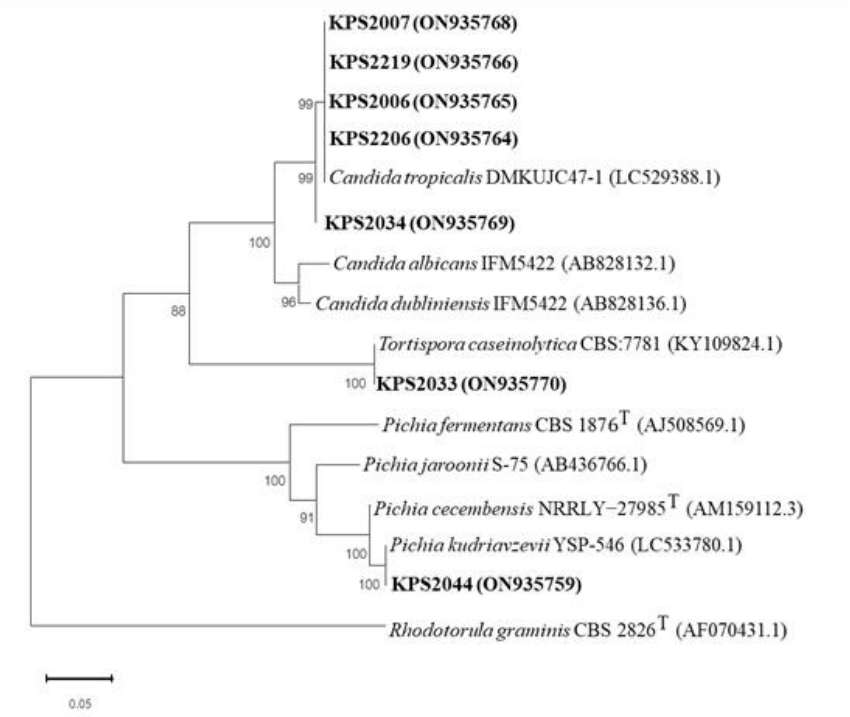

Figure 1 Phylogeny of yeasts isolated from phylloplane of *Leguminosae* and related species constructed by the the maximum likelihood technique based on the D1/D2 domains of LSU rRNA gene. Bootstrap values based on 1,000 replications were indicated at branch nodes. The scale bar indicates 0.05 substitutions per nucleotide position.

Effects of growth-promoting yeasts on the growth of chili seedlings under greenhouse conditions

As a result, *C. tropicalis* KPS2219, the highest IAA-producing strain, was selected for a greenhouse experiment. To better understand how IAA-producing yeast affects plant growth and development, we used chili as a plant model. Since the phyllosphere of leaves is the habitat of *C. tropicalis* KPS2219, foliar treatment of the chili plant was the main approach. Additionally, seed priming or coating with plant growth-promoting microorganisms is an effective approach to enhance plant growth at the early emergence of seedlings [48]. Therefore, the inoculation of *C. tropicalis* KPS2219 by soaking the seeds and then spraying cell suspension on the chili plant's leaves was used in the greenhouse experiment. Seeds were soaked in a cell suspension of *C. tropicalis* KPS2219 before germination. The root and shoot lengths of the 14-day seedlings were not affected after seed priming with *C. tropicalis* KPS2219. However, after the seedlings were moved to 12-cm pots, the foliar application of the *C. tropicalis* KPS2219 cell suspension significantly increased the root length, shoot length, root dry weight, and stem dry weight of the chili seedlings by 17.72, 29.15, 60 and 46.15 %, respectively, when compared to the control (**Table 4**). Additionally, chili seedlings sprayed with *C. tropicalis* KPS2219 had greater robustness and longer root hairs in comparison to the uninoculated control (**Figure 2**). A previous study on the germination and development of maize seeds after priming with the IAA-producing rhizospheric yeast *Clavispora lusitaniae* Y35 and the diazotrophic bacterial strain *Serratia liquefaciens* 385 was examined by using the paper method. The results revealed that plumule growth and the radical system architecture of the seedlings were improved [49]. Our findings, on the other hand, showed that seed priming with *C. tropicalis* KPS2219 had no effect after germination in sterilized peat moss for 14 days, suggesting that the environment or germination testing method may have an impact on yeast survival or activity. After foliar application of *C. tropicalis* KPS2219, shoot and root length as well as root and stem dry weight of chili significantly improved by 17.72, 29.15, 60 and 46.15 %, respectively, in comparison with the control, indicating that *C. tropicalis* KPS2219 had a beneficial effect on plant development compared to the uninoculated control (**Table 4**). Our finding is consistent with other previous works that studied the effect of plant growth-promoting yeast on plant growth [50,51] Additionally, foliar spray is an effective method to introduce phyllospheric yeast to plants. In the phyllosphere's microecosystem, yeast and bacteria are more common in the grooves, around the trichomes, and in the stomata of epidermal cells [52]. Rapid nonpolar transport in the phloem and slower cell-to-cell polar auxin transport in diverse tissues are the 2 physiologically distinct and geographically independent transport mechanisms that carry out auxin distribution throughout a plant. Radio-labeled auxin was mostly found in the vascular cambium and xylem parenchyma in the aerial section of a plant (stem), indicating that auxin was primarily carried downward, although it was also found in the more peripheral cell layers [53,54]. It is possible that *C. tropicalis* KPS2219 colonized the phyllosphere of chili leaves and produced IAA, which was translocated downward to other parts, such as the root tip. In roots, auxin is also generated to some degree, with the meristematic zone of the primary root tip and emerging lateral roots serving as the main sources of auxin [55]. From our results, an increase in lateral root development was observed (**Figure 2**), which benefits plants by increasing soil water and nutrient absorption and lowering the requirement for fertilizer application in the field. *C. tropicalis* KPS2219 produces not only IAA but also NH_3, which is an important macronutrient for plants. Previous studies have shown that foliar N fertilizer treatment improved plant development faster and more efficiently than soil application. Most of the nitrogen (N) provided by roots or leaves was originally distributed equally in the fibrous roots and leaves. N was subsequently transferred to the newly emerging leaves and flower organs during vegetative development [56,57] and then to the expanding organs and reserves [58]. These prospective phylloplane yeasts will be applied in consortiums or in combination with chemical fertilizer in further research to promote plant growth and productivity. High IAA-producing strains will also undergo optimization for greater IAA production.

Table 4 Effects of plant growth-promoting yeasts on growth of *Capsicum annuum* TVRC 758 seedlings.

Day	Treatment	Root length (cm)	Shoot length (cm)	Dry weight of roots (g)	Dry weight of stems (g)
14	Control	2.58 ± 0.26^{a}	4.75 ± 0.51^{ab}	N/A	N/A
	C. tropicalis KPS2219	2.82 ± 0.26^{a}	5.67 ± 0.62^{a}		
30	Control	9.82 ± 0.26^{b}	9.57 ± 1.55^{b}	0.10 ± 0.01^{b}	0.39 ± 0.06^{b}
	C. tropicalis KPS2219	11.56 ± 0.37^{a}	12.36 ± 0.72^{a}	0.16 ± 0.02^{a}	0.57 ± 0.06^{a}

N/A: Not applicable

Different superscripts in a column differ significantly ($p < 0.05$) according to Duncan's multiple range test.

Figure 2 Effects of plant growth promoting yeasts on growth and development of *Capsicum annuum* TVRC 758 seedlings after 30 days: A) uninoculated control and B) inoculated with *C. tropicalis* KPS2219 strain.

Conclusions

Plant growth-promoting yeast (PGPY) was isolated and screened from the phyllosphere of Leguminosae. *C. tropicalis* KPS2219 exhibited remarkably high IAA production in L-tryptophan supplement medium and NH_3 production. Foliar spray of *C. tropicalis* KPS2219 on chili seedlings significantly increased shoot length, root length and biomass compared to the uninoculated control. Our results suggested that *C. tropicalis* KPS2219 could be used as a bioinoculant to improve the growth of plants and that foliar spraying is an effective method to apply PGPY to plants. Further studies are required to examine the effect of inoculation of *C. tropicalis* KPS2219 and other potential strains in consortiums to improve the growth and yield of chili in greenhouse experiments and field trials.

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