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IDENTIFICATION OF SOIL BACTERIA ISOLATED FROM NASINUAN COMMUNITY FOREST WITH POTENTIAL APPLICATION IN AGRICULTURE

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Abstract: This work aims to isolate and identify bacteria from unexplored resources in the Nasinuan Community Forest in Kantarawichai district, Maha Sarakham province, Thailand, with potential applications in agriculture. These bacteria are able to produce chitinase and phytase, besides fixing nitrogen and solubilizing phosphate. Selective media were used for screening bacteria and point inoculation was used in determining the capacity of interest in bacteria. The 16S rRNA gene sequencing was performed to identify bacterial strains. The results showed that PSB1.2.1, PSB2.1.1 and PSB3.4.3 isolates were phosphate solubilizing strains that are identified as *Enterobacter hormaechei*, *Enterobacter* sp. and *Enterobacter ludwigii*, respectively. PT1.1.3, as phytase-producing bacteria, showed the closest relationship to *Burkholderia cenocepacia*, while PT1.2.1, PT1.3.4, PT2.1.3 and PT3.3.1 are related to *Burkholderia* sp., *Acinetobacter pittii*, *Bacillus cereus* and *Burkholderia gladioli*, respectively. N1.11, N1.12, N3.3 and N3.4 are nitrogen-fixing bacteria identified as *Bacillus thuringiensis*, *Pantoea dispersa*, *Pantoea dispersa* and *Achromobacter xylosoxidans*. The isolates C2.21 and C2.22 are chitinase-producing bacteria identified as *Bacillus amyloliquefaciens* and *Bacillus amyloliquefaciens,* while C2.23 was identified as *Achromobacter spp*. This is the first report of bacterial identification with potential agricultural application from the Nasinuan Community Forest. These bacteria may be used as bioinoculants to promote growth of local plants, enhance yields and reduce the use of chemical fertilizers.

Keywords: Chitinase; Nasinuan Forest; Nitrogen fixation; PSB; Phytase.

Introduction

A plethora of bacteria in the plant rhizosphere, which are capable of stimulating plant growth, has been reported in literature. Among them are chitinase-producing bacteria that can weaken and disintegrate the cell wall of pathogens and pests, and thus, exert anti-fungal, nematicidal, insecticidal and antibacterial properties (Edreva, 2005). The application of chitinase-producing bacteria in agriculture is deemed to mitigate the negative impact of using synthetic fungicides and pesticides on the environment. Thus, chitinase-producing microbes are sought after as green bio-control agents to replace the use of hazardous chemicals.

In addition, plant growth-promoting bacteria (PGPB) have been reported to enhance the level of phosphorus, nitrogen, amino acids and vitamin B in the rhizosphere due to its role as a phosphate-solubilizing bacteria (PSB) (Nautiyal, 1999; Rózycki *et al.*, 1999). Availability of phosphorus-based nutrients may be improved by mobilizing insoluble inorganic polyphosphates and phytates (Richardson *et al.*, 2001). In literature, PSB isolated from soil include *Bacillus circulans*, *Agrobacterium* spp. and *Pseudomonas* spp. (Babalola & Glick, 2012). Others include those from the bacillus (Jahan *et al.*, 2013), azotobacter (Kumar *et al.*, 2014), burkholderia (Istina *et al.*, 2015), erwinia, enterobacter (Chakraborty *et al.,* 2009) and kushneria (Zhu *et al.*, 2011) species.

Moreover, nitrogen-fixing bacteria, a well-known commensal of legume plants, have the ability to express the nitrogenase enzyme complex, which facilitates the binding of

atmospheric nitrogen (N_2) into ammonia (NH_3), which is vital source of nutrient for the plants. Thus, the legumes are able to access nitrogen directly from the atmosphere to meet its demand for nutrition (de Bruijn, 2015). The nitrogen-fixing bacteria generas include *Azospirillum* and *Azotobacter* (Bhattacharyya & Jha, 2012). The bio-inoculation of plants with nitrogen-fixing bacteria is regarded as an environmental friendly alternative agrochemical regime that saves cost in terms of using chemical fertiliser, besides being a sustainable form of agriculture. The use of these bacteria as biofertilizers, bio-inoculants and biocontrol agents in agriculture is gaining immense attention among scientists. In several countries, microbial inoculants have been implemented to enhance crop growth, yields and increase resistance to pathogens. Azotobacter, Azospirillum, Burkholderia and Rhizobium are among several soil microbes that have been commercialized as biofertilizer for crops in India, which also happens to be the top country in using bio-inoculants (Sruthilaxmi & Babu, 2017).

Thus far, no studies have been reported on chitinase-producing, phytase-producing, nitrogen-fixing and phosphate-solubilizing bacteria from soil in the Nasinuan Community Forest of Kantarawichai district in Mahasarakham province, Thailand. The Nasinuan Community Forest has low-to-medium soil salinity and is rich in plant biodiversity. Therefore, we also expected a diverse microbial population with specific properties to be found on forest the soil. The forest is publicly accessible to locals and is being maintained for sustainable uses under the royal initiative of Her Royal Highness Princess Maha Chakri Sirinhorn. Thus, this study aims to isolate and identify the various species of bacteria from the Nasinuan Community Forest and identify their potential application in agriculture.

Materials and Method

Soil Samples

A total of 16 soil samples were randomly obtained within 24 acres of the Nasinuan Community Forest in Kantarawichai district, Maha Sarakham province, Thailand (coordinates: 16.340941, 103.210799), as shown in Figure 1. Soil was collected 15 cm below the surface and stored in polystyrene bags as described by Yotchaisarn *et al.* (2018).

Figure 1: Location of Maha Sarakham province in Thailand (A) and the Nasinuan Community Forest (Phase 2 area with 24 acres), where soil samples were randomly collected from zone 1 to 3 (B). Figure 1B was modified from Yotchaisarn *et al.* (2018)

The properties of soil samples, such as electroelectricity (µS/cm) and salt content (%) were measured by mixing the soil in water in a 1:1 ratio and using an electroelectricity meter. The pH was measured using a pH electrode.

Isolation of Bacteria

A total of 10 g soil sample was suspended in 90 mL of sterile 0.85 % NaCl solution. The suspension was diluted serially and 100 µL of each dilution was spread on petri dishes containing four selective media mixed as stated below:

1. Pikovskaya's agar for phosphate-solubilizing bacteria (Nautiyal, 1999) (g/L); glucose (10), $Ca_3(PO_4)_2$ (5), $MnSO_4$ (0.01), $MgSO_4 \cdot 7H_2O$ (0.1), KCl (0.2), $(NH_4)_2SO_4$ (0.5), $FeSO_4$ (0.01), Yeast extract (0.5), agar (15) pH 7.0.
2. Phytase-specific agar for phytase-producing bacteria (Quan *et al.*, 2001) (g/L); glucose (15), $C_6H_6Ca_6O_{24}P_6$ (1), NH_4N_3 (2), $MgSO_4 \cdot 7H_2O$ (0.5), KCl (0.5), $MnSO_4$ (0.3), $FeSO_4$ (0.3), agar (15) and pH 5.5.
3. Nitrogen-free agar for nitrogen-fixing bacteria (Rosemary *et al.*, 2013) (mg/L); $CoCl_26H_2O$ (0.004), H_3BO_3 (2.86), $MnCl_24H_2O$ (1.81), $ZnSO_47H_2O$ (0.22), $CuSO_45H_2O$ (0.08), $H_2MoO_4H_2O$ (0.09), $MgSO_47H_2O$ (492.96), K_2HPO_4 (174.18), KH_2PO_4 (136.09), $CaCl_2$ (110.99), bromothymol blue (0.025), agar (15) and pH 6.8.
4. Chitin agar for chitin-producing bacteria (Vincy *et al.*, 2014) (g/L); chitin (10), peptone (5), NaCl (5), agar (15) and pH 6.5.

The plates were incubated at 37 °C for seven days. Chitinase-producing bacteria, phytase-producing bacteria, and phosphate-solubilizing bacteria were isolated from colonies with clear zones, while nitrogen-fixing bacteria were isolated from colonies that had turned blue in their respective agar. The isolated colonies were further streaked at least three times to obtain pure bacterial isolates as confirmed by Gram staining and viewing under 1000X light microscope. After that, the pure bacterial isolates were subcultured in corresponding broths overnight at OD_{600nm} = 0.1, and point inoculated on the corresponding agars and incubated at 37 °C for seven days. The diameter of the clear zones over the diameters of the colonies were calculated as the halo to colony ratio to determine the capacity of each isolate as previously reported (Premono, 1996).

Identification of Bacteria by PCR-based 16S rRNA Gene Sequencing

The PCR-based 16S rRNA gene sequencing was carried out according to Yotchaisarn *et al.* (2018). The 16S rRNA gene sequences were then compared with the National Center for Biotechnology Information (NCBI) database using the neucleotide-neucleotide Basic Local Alignment Search Tool (BLASTN) to identify the bacterial species (Altschul *et al.*, 1990).

The phylogenetic tree was created using Muscle method for sequence alignment using the Maximum Likelihood method based on Tamura three-parameter model (Nei & Kumar, 2000). The percentage of replicate trees in the bootstrap test (1,000 replicates) was shown next to the branches. The bar represents a distance of 0.2 substitutions per site. Evolutionary analyses were performed in MEGA X (Kumar, 2018) and the phylogenetic tree was drawn by FigTree (Rambaut, 2014). All 16S rRNA partial sequences of our bacterial isolates were assigned accession numbers and deposited on NCBI database.

Results

Soil Properties at Sample Collection Points

Soil samples from seven collection sites out of the total 16 contained the bacterial isolates of interest. The soil properties of seven sites are stated in Table 1. Salt (%) in the soil was mostly found to be <0.1 %, indicating the lack of salinity (salty soil needed to have >2 % salt). Most soil sample sites were surrounded by indigenous trees or termite hills with electroelectricity values ranging from 38.5 to 72.9 µS/cm, confirming the low salinity characteristic. The pH values ranged from 4.87 to 7.95 (Table 1).

Table 1: Information on seven locations of soil samples taken in Nasinuan Community Forest

Location	Surrounding	Coordinates	Electro electricity (µS/cm)	Salt (%)	pH
1.1	 Under the tree	6.328140 103.209130	70.3	0.1	7.88
1.2	 Under the tree	6.322267 103.209620	42.4	0.1	7.95
1.3	 Near termite hill under the tree	6.317450 103.208468	38.5	0.1	7.11
2.1	 Under the tree	6.324101 103.211128	42.5	0.1	5.46
2.2	 Under the tree	6.331806 103.215295	57.4	0.1	5.18
3.3	 Near termite hill under the tree	6.317144 103.268742	72.9	0.1	4.87
3.4	 Dry land	6.322554 103.209020	40.8	0.1	4.98

Isolation of Bacteria on Selective Media

Initially, 21 positive colonies were identified on Pikovskaya's agar, 16 on phytase-specific agar, 11 on nitrogen-free agar and 12 on chitin agar. These colonies were further isolated using the streak plate method to obtain pure bacterial isolates.

These isolates were then determined for halo to colony ratio corresponding to the property of interest, and only high-capacity isolates were presented in this work. The results showed that phosphate-solubilizing bacteria PSB1.2.1, PSB2.1.1, PSB3.4.3 isolates displayed halo to colony ratios of 1.41, 1.22 and 1.54, respectively.

All three isolates were Gram negative and rod-shaped with creamy yellowish, white and creamy yellowish colonies, respectively (Figure 2).

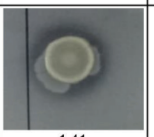
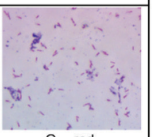
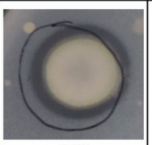
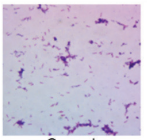
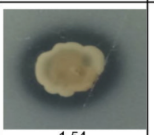
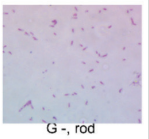
Isolate	Halo:colony ratio	Colony morphology	Gram-staining
PSB1.2.1	 1.41	Creamy, yellowish, rough surface	 G -, rod
PSB2.1.1	 1.22	White, smooth surface	 G -, rod
PSB3.4.3	 1.54	Creamy, yellowish, rough surface	 G -, rod

Figure 2: Phosphate-solubilizing bacterial (PSB) isolates

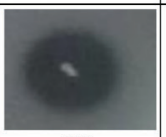
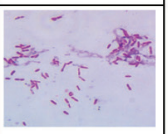
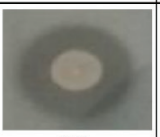
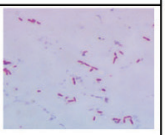
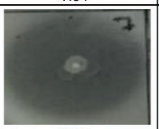
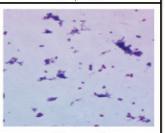
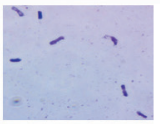
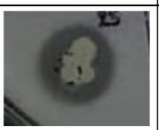
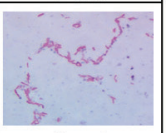
Isolate	Halo:colony ratio	Colony morphology	Gram-staining
PT1.1.3	 3.11	White, smooth surface	 G -, rod
PT1.2.1	 1.91	Creamy, smooth surface	 G -, rod
PT1.3.4	 3.78	White, rough surface	 G +, rod
PT2.1.3	 2.00	White, rough surface	 G +, rod
PT3.3.1	 1.77	White, rough surface	 G -, rod

Figure 3: Phytase-producing (PT) bacterial isolates

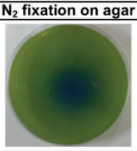
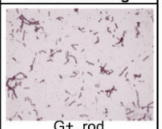
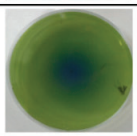
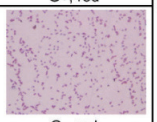
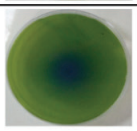
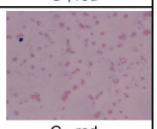
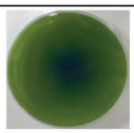
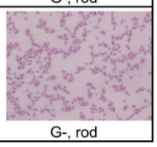
Isolate	N_2 fixation on agar	Colony morphology	Gram-staining
N1.11		Slimy, white, smooth edge	 G+, rod
N1.12		Slimy, white, smooth edge	 G-, rod
N3.3.		Slimy, white, smooth edge	 G-, rod
N3.4		Slimy, white, smooth edge	 G-, rod

Figure 4: Nitrogen-fixing (N) bacterial isolates

Phytase-producing isolates PT1.1.3, PT1.2.1, PT1.3.4, PT2.1.3 and PT3.3.1 displayed halo to colony ratios ranging from 1.77 to 3.78. The three isolates PT1.1.3, PT1.2.1 and PT3.3.1 were Gram negative and rod-shaped, whilst isolates PT2.1.3 and PT1.3.4 were Gram-positive and also rod-shaped (Figure 3).

Nitrogen-fixing bacterial isolates N1.11, N1.12, N3.3 and N3.4 were able to fix nitrogen and transform it into ammonia as observed by turning the green nitrogen-free agar to blue. The three isolates N1.12, N3.3 and N3.4 were Gram negative and rod-shaped, whilst N1.11 was Gram-positive and rod-shaped (Figure 4).

Chitinase-producing isolates C2.21, C2.22 and C2.23 displayed halo to colony ratios of around 1.90. The two isolates C2.21 and C2.22 were Gram-positive and rod-shaped, whilst C2.23 was Gram-negative and rod-shaped (Figure 5).

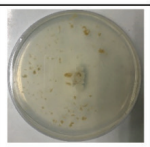
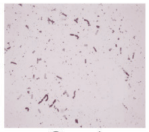
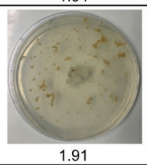
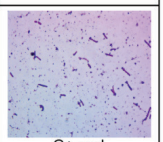
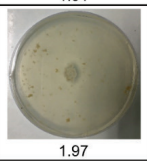
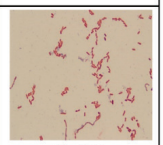
Isolate	Halo:colony ratio	Colony morphology	Gram-staining
C2.21	 1.94	Slimy, white with rough edge	 G+, rod
C2.22	 1.91	Slimy, white with rough edge	 G+, rod
C2.23	 1.97	Slimy, white with rough edge	 G-, rod

Figure 5: Chitinase-producing (C) bacterial isolates

Bacterial Identification

The PCR product of 16S rRNA gene of each isolate was analyzed using agarose gel electrophoresis and the product size was found to be around 1,500 bp (Figure 6). Identification of bacterial strains was carried out by PCR-based 16S rRNA gene sequencing.

Afterwards, a search for the closest relatives was conducted on BLAST website. The results (Table 2) showed that PSB1.2.1, PSB2.1.1 and PSB3.4.3 isolates appeared as phosphate-solubilizing bacteria, which were identified (% identity) as *Enterobacter hormaechei* (99.9 %), *Enterobacter* sp. (99.9 %) and *Enterobacter ludwigii* (99.9 %), respectively. The isolate PT1.1.3 was a phytase-producing bacteria that was found to be the closest relative to *Burkholderia cenocepacia* (99.9 %). Meanwhile, isolates PT1.2.1, PT1.3.4, PT2.1.3 and PT3.3.1 were related to *Burkholderia* sp. (99.9 %), *Acinetobacter pittii* (99.8 %), *Bacillus cereus* (100 %) and *Burkholderia gladioli* (100 %), respectively. Nitrogen-fixing bacteria isolate N1.11 was identified as *Bacillus thuringiensis* (99.4 %), while N1.12 and N3.3 were identified as two different strains of *Pantoea dispersa* (99.8 % and 100%), and N3.4 was identified as *Achromobacter xylosoxidans* (100%).

Chitinase-producing bacterial isolates C2.21 and C2.22 were identified as *Bacillus amyloliquefaciens* strains (100% and 99.9%, respectively), while C2.23 was identified as *Achromobacter* sp. (100%). Their closest relatives were found in Bangladesh, Korea, China, India, Australia, Brazil, Indonesia, Panama and Philippines (Table 2).

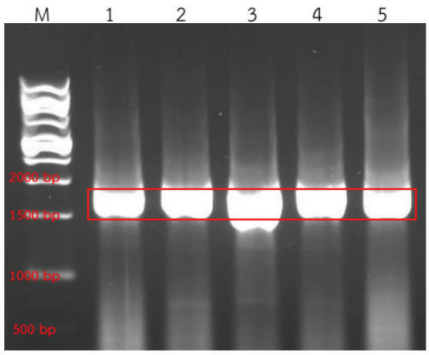


Figure 6: PCR products of 16S rRNA genes of each isolate. M is 1 kb DNA molecular weight ladder (Vivantis); Lane 1-5 = PCR products (~ 1,500 bp) of 16S rRNA genes from pure isolate representatives.

Table 2: Identification of 15 bacterial isolates by 16S rRNA sequencing

Isolate	Accession no.[a]	Closest relative[b] (Accession no.[b])	% identity[c]	Gaps[c]	Max score[c]	Origin[c]
PSB1.2.1	MT001456.1	*Enterobacter hormaechei* FRM31 (KX233851.1)	99.9	0/1122(0%)	2065	*Macrobrachium rosenbergii*, Bangladesh
PSB2.1.1	MT001457.1	*Enterobacter* sp. WCHEs120003 (MK567958.1)	99.9	0/1058(0%)	1949	Sputum, China
PSB3.4.3	MT001458.1	*Enterobacter ludwigii* SNSK244 (MK576153.1)	99.9	0/1079(0%)	1988	Neonatal blood samples, India

PT1.1.3	MT001451.1	*Burkholderia cenocepacia* MSMB384WGS (CP013450.1)	99.9	0/1029(0%)	1895	Water, Australia
PT1.2.1	MT001454.1	*Burkholderia* sp. AMF3725 (JQ316406.1)	99.9	0/880(0%)	1620	Soil, Brazil
PT1.3.4	MT001452.1	*Acinetobacter pittii* BJ6 (MH667652.1)	99.8	0/1067(0%)	1958	Genomic DNA, China
PT2.1.3	MT001455.1	*Bacillus cereus* SKA1.2 (MK694749.1)	100	0/1042(0%)	1925	Well water, Indonesia
PT3.3.1	MT001453.1	*Burkholderia gladioli* Co14 (CP033430.1)	100	0/1014(0%)	1873	Fermented corn meal, China
N1.11	MT001461.1	*Bacillus thuringiensis* AA1 (MG384803.1)	99.4	0/888(0%)	1613	Korea
N1.12	MT001462.1	*Pantoea dispersa* Ovo15 (MN709235.1)	99.8	0/1051(0%)	1700	Dona Chagas farm, Brazil
N3.3	MT001463.1	*Pantoea dispersa* 40 (MF462942.1)	100	0/1138(0%)	1770	Nest, Panama
N3.4	MT001464.1	*Achromobacter xylosoxidans* ES-6 (MK537386.1)	100	0/1068(0%)	1212	Rhizospheric soil, India
C2.21	MT001459.1	*Bacillus amyloliquefaciens* TBMAX73 (MK834711.1)	100	0/1019(0%)	1756	Rice roots, Philippines
C2.22	MT001460.1	*Bacillus amyloliquefaciens* YW224 (MK034186.1)	99.9	0/1099(0%)	1334	Female insole, China
C2.23	MT459240.1	*Achromobacter* sp. T4-26 (MH074854.1)	100	0/1095(0%)	1342	Isaria cicadae, China

a GenBank accession no. of our bacterial isolates deposited on NCBI website.
b GenBank accession no. of closest relative strains to our bacteria on NCBI website.
c Based on BLAST search results, identity (%) of our strains compared to the closest relatives, gaps, Max score and origin of the closest relatives.

Phylogenetic Tree Analysis

The phylogenetic tree of 15 bacterial strains isolated from Nasinuan Community Forest and six reference strains showed that the isolated phytase-producing bacteria and nitrogen-fixing bacteria were evolutionarily different from the reference strains. *B. subtilis* B.S.46 (HQ234325.1), as a reference bacterium for phytase production, was isolated from rice field soil in Iran (Rocky-Salimi *et al.*, 2016) and *A. xylosoxidans* WM234C-3 (AY873802.1), as a reference bacterium for nitrogen fixation, was isolated from wheat cultivation soil in India (Jha & Kumar, 2009). They evolved differently from *B. cereus* PT2.1.3 and *A. xylosoxidans* N3.4, respectively. However, *P. dispersa* N1.12 and *P. dispersa* N3.3, as nitrogen fixing bacteria identified in this study, were evolutionarily similar to *P. dispersa* g58 (KM019887.1) isolated from soil and *P. dispersa* M1R4 (GQ246183.1) isolated from maize roots (Kawaka *et al.*, 2018) (Figure 7).

Likewise, the phosphate-solubilizing bacteria in this study, including *E. hormaechei* PSB1.2.1, *Enterobacter* sp. PSB2.1.1 and *E. ludwigii* PSB3.4.3, had evolved similarly to the reference strains *E. cloacae* MS32 (LT908013.1) (Suleman *et al.*, 2018). Phytase-producing bacteria, including *B. cenocepacia* PT1.4.1, *B. gladioli* PT3.3.1 and *Burkholderia* sp. PT1.2.1, evolved similarly and were found in the same clade. In addition, chitinase-producing bacterium isolated from Tar desert in India, *B. licheniformis* MY75 (EF635428.1) (Sharma *et al.*, 2013) as a reference strain, appeared to be evolutionarily similar to the chitinase-producing bacteria *B. amyloliquefaciens* C2.21 and *B. amyloliquefaciens* C2.22 identified in this study (Figure 7).

Discussion

This is the first report of 15 bacterial strains with potential applications in agriculture that were isolated from Nasinuan Community Forest in Kantarawichai district, Mahasarakham province, Thailand. From phylogenetic tree analysis, some strains displayed a distinct evolutionary trait from their reference strains with the same activities in the existing database, and some strains had never been reported before. This work had discovered three isolates — PSB1.2.1, PSB2.1.1 and PSB3.4.3 — as *E. hormaechei*, *Enterobacter* sp. and *E. ludwigii*, respectively, with the closest relatives from China, India and Bangladesh, respectively. The isolate PT1.1.3 was a phytase-producing bacterium that was

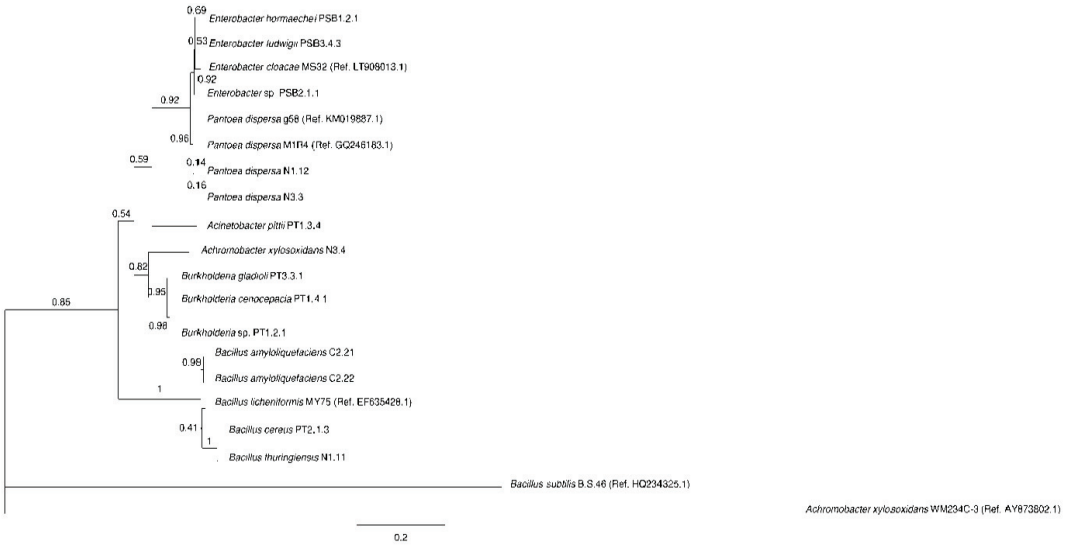


Figure 7: Phylogenetic tree of 15 bacterial strains and six reference strains

closely related to *B. cenocepacia*, whilst isolates PT1.2.1, PT1.3.4, PT2.1.3 and PT3.3.1 were close relatives with *Burkholderia* sp., *A. pittii*, *B. cereus* and *B. gladioli*, respectively. The close relatives were recorded in Australia, China and Brazil.

In accordance with the previous reports, *B. gladioli* and *Enterobacter* sp. were capable of solubilizing phosphates (Gupta *et al.*, 2012) and when they were used in combination with *Pseudomonas synxantha* and *Serratia marcescens*, they were able to increase the phosphorus content in soil, therefore, improving the biomass of *Aloe barbadensis* plants and aloin-A (Gupta *et al.*, 2012). Another work showed that *B. licheniformis* and *B. megaterium* isolated from soil worms in India were also able to solubilize phosphates (Biswas *et al.*, 2018).

Likewise, bacteria of Enterobacteriaceae family were able to digest phytate and used to enhance the growth of pigeon pea (*Cajanus cajan*) (Patel *et al.*, 2010). In addition, phytase-producing *B. cepacia* isolated from soil was used to enhance growth of maize (Pande *et al.*, 2017) and *Acinetobacter* sp. TZ1 isolated from chicken manure was able to produce phytase to digest phytate (Irawan & Nurachman, 2013).

Nitrogen-fixing isolates N1.11, N1.12, N3.3 and N3.4 were identified as *B. thuringiensis*, *P. dispersa*, *P. dispersa* and *A. xylosoxidans,* respectively. Their closest relatives were from Korea, Brazil, Panama and India. Chitinase-producing isolates C2.21, C2.22 and C2.23 were found to be *B. amyloliquefaciens, B. amyloliquefaciens* and *Achromobacter* sp., respectively. Their closest relatives were from Philippines and China.

Our strains were derived from the same genera as in previous reports. *Sphingobium, Sphingobium, Pseudomonas, Chryseobacterium, Pantoea, Acinetobacter* and *Sphingobacterium* were known to be nitrogen fixers found in the roots of potatoes plants (Marques *et al.*, 2014). *Pantoea* is widely discovered in animals and agricultural soil (Walterson & Stavrinides, 2015). This bacteria had been observed to exert anti-fungal effect towards *Candida albicans* (El Amraoui *et al.*, 2014) and *Phytophthora infestans* (a mold that causes potato blight) (Town *et al.*, 2016) due to anti-microbial metabolites such as pantocins, microcins and phenazines (Walterson *et al.*, 2014). Moreover, *Pantoea* and *Bacillus,* as endophytes found in roots of maize, had been used to promote growth by nitrogen fixation in combination with other species like *Azospirillum, Burkholderia, Brevundimonas, Klebsiella, Pseudomonas* and *Enterobacter* (Marag & Suman, 2018). This work was in agreement with the report of a *Pantoea* species isolated from sugarcane cultivation soil in Cuba that was capable of nitrogen fixation (Loiret *et al.*, 2004).

Our strains — *Achromobacter* sp. and *Bacillus* sp. — showed chitinase activity. In the previous studies, *B. thuringiensis* had been used as a bio-control agent in India (El-Ghany & El-Ghany, 2017). *Achromobacter* sp. was used to degrade heavy metal and hydrocarbon from oil spills in China (Deng *et al.*, 2014), and also atrazine (herbicide) contamination in Brazil (Fernandes *et al.*, 2018).

These 15 bacterial isolates with agricultural potential identified in this study could be used as bio-inoculants or bio-fertilizer to promote the growth of crops in Thailand. The use of indigenous bacterial culture in agriculture would help reduce the cost of importing bacterial stocks from overseas, and this might also be a safer option for the local ecosystem.

Conclusion

This is the first report to isolate and identify bacteria from an unexplored resource in Thailand with potential applications in agriculture. These bacteria included chitinase-producing, phytase-producing, phosphate-solubilizing and nitrogen-fixing bacteria. Some of the bacteria species were reported of their enzyme-producing attributes for the first time. Developing these bacteria as agricultural bio-inoculants could bring agronomical and ecological benefits to plants and crops in Thailand.

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Conflict of interest

All authors declare they have no conflict of interest in this paper.

Author contributions

Conceptualization: Luang-In V. Methodology: Luang-In V., Saengha W, Deeseenthum S, Maneewan K. Software: Udomwong P. Writing - original draft: Luang-In V. Writing - review & editing: Luang-In V, Deeseenthum S, Maneewan K.

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