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Plant Growth-promoting Ability and Pathogen Inhibitory Effect of Actinomycetes Isolated from Fecal Pellets of the Giant Millipede *Thyropygus resimus* (Diplopoda)

Waraporn Sutthisa, Warisara Paraphong and Piyatida Pimvichai*

Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham Province, 44150 Thailand.

Abstract

The microbial properties of millipede fecal pellets have been studied mainly in Glomerida (pill millipedes), and much less in the significant majority of other millipede groups. Therefore, the present study examined actinomycetes isolated from the fecal pellets of the non-glomerid giant millipede *Thyropygus resimus* Attems, 1938 (Spirostreptida) to (1) test their plant growth-promoting ability, and (2) evaluate their potential to control and inhibit plant pathogenic microorganisms. Millipedes were collected from Phu Kum Khao, Kalasin Province, Thailand. A total of 59 actinomycete isolates were obtained and identified as belonging to the genus *Streptomyces* using 16S rRNA sequencing. The plant growth-promoting properties of the isolates were tested by screening four characteristics: nitrogen fixation, phosphate solubility, siderophore production, and indole-3-acetic acid (IAA) production. A nitrogen-fixation test on nitrogen-free solid malate media (NFM) showed that 54 isolates were capable of fixing nitrogen. Phosphate solubility was tested on double-layered glucose yeast extract agar (GYA) medium containing tricalcium phosphate. This showed that 42 isolates formed a clear zone around the colonies due to phosphate dissolution. Siderophore production was tested on chrome azurol sulfate (CAS) agar. This showed that 55 isolates could grow on this medium and form clear yellow to orange zones around their colonies. IAA production tests revealed that 41 isolates could produce IAA. Based on the combined results of these four tests, eight of the 59 isolates were the most effective in promoting plant growth: KLS-AC04, KLD-AC01, KLD-AC02-1, KLD-AC08, KLD-AC09, KLD-AC16, KLD-AC29-1, and KLD-AC30. Seventeen isolates inhibited the growth of *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight disease in rice, more effectively than rifampicin (100 ppm), with isolate KLS-AC02-1-1 being the most effective (inhibition zone, 58.25 mm in diameter). Therefore, these isolates can be used for growth promotion and rice disease control in the future.

Keywords: Millipede Faeces, Rice Bacterial Leaf Blight Disease, *Streptomyces*, Thailand, *Xanthomonas oryzae* pv. *oryzae*

***Correspondence:** piyatida.p@msu.ac.th

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INTRODUCTION

Millipedes (Diplopoda) are detritivorous soil-dwelling arthropods that consume leaf litter and dead plant material, through which they return nutrients to the soil and thus maintain soil quality.[1] As such, millipede feeding and excretion (through fecal pellets) increases the surface area of dead plant material available for the activity of the microorganisms responsible for soil nutrient recycling.[2] Therefore, the microbial and soil-fertilizing properties of millipede fecal pellets have mainly been studied in pill-millipedes (Glomerida), and far less so in the significant majority of other millipede groups. Nevertheless, several non-glomerid millipedes are promising model organisms in this field of research because they grow to large sizes and produce large amounts of fecal pellets, thus facilitating analytical and experimental studies. The giant harpagophorid millipede *T. resimus* Attems, 1938 (Spirostreptida), a species 12-20 cm long that is common in northeastern Thailand, was used in the present work. Millipedes play an important role in the ecosystem, helping to decompose waste and shovel soil, thereby enhancing its quality. This soil is rich in minerals and fertilizers including nitrogen (N), phosphorus (P), and potassium (K). In addition to millipede fecal matter, bacteria, fungi, and actinomycetes can be found in the soil, which are useful in increasing nutrients and promoting plant growth.

Plant growth-promoting rhizobacteria (PGPR) facilitate plant growth by enhancing beneficial nutrients or producing plant hormones such as indole-3-acetic acid (IAA).[3] PGPR also protect plants against pathogens either through pathogen antagonism or plant systemic resistance induction.[4,5] Therefore, PGPR may be employed as biofertilizers, phytostimulators, and antagonists in the rhizosphere.[6-9] Stimulation of plant growth and plant defense responses is achieved by actinomycetes, which have been found to have high potential in some strains.[10]

Actinomycetes are a major microbial component of the rhizosphere, where they, similar to PGPR, contribute to soil nutrient cycling,[11,12] and enhance plant growth.[13] The actinomycete genus *Streptomyces* comprises PGPR that occur in a broad diversity of habitats, including soils, composts, water, and plants. As such, *Streptomyces* strains may enhance the supply of plant nutrients, produce IAA, cytokinins, and siderophores, increase the solubility of phosphate, and control plant pathogens through antibiosis and the production of phenazines.[14-19] Horstmann et al.[20] isolated *Streptomyces* sp. from the Fabaceae rhizosphere and tested its plant growth-promoting properties, including siderophore production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, IAA, and phenazines. In Pakistan, Anwar and Sajid[8] isolated 98 rhizosphere actinomycetes from different fields of wheat and tomatoes. The isolates were studied morphologically, biochemically, and genetically, and tested for their growth-promoting properties in vitro. Thirty percent of the isolates were found to be PGPR, showing maximum genetic similarity (98–99%) to *Streptomyces* species using 16S rRNA gene sequencing. *Streptomyces nobilis* WA-3, *S. kunmingenesis* WC-3, and *S. enissocaesilis* TA-3 produced the highest amounts of IAA. *Streptomyces* sp. WA-1 and *S. djakartensis* TB-4 were the most phosphate-soluble. All rhizobacterial isolates produced siderophores, ammonia, and hydrogen cyanide.

Plant diseases caused by bacteria or fungi can be suppressed by actinomycetes.[21,22] Several strains of *Streptomyces* have been used as biocontrol agents because they can produce various antimicrobials, survive in extreme environments, and efficiently colonize the rhizosphere of various plants.[23,24] Similarly, *Streptomyces* can induce resistance, as described previously.[25,26] Based on these features, several *Streptomyces* strains have been investigating for the control of fungal and bacterial diseases in rice, such as bacterial late blight caused by *X. oryzae. Streptomyces toxytricini* VN08-A-12 was shown to control *X. oryzae* pv. *oryzae* (*Xoo*). The results showed that the growth and spread of ten major *Xoo* races were inhibited by 17 actinomycete strains.[27] In addition, *Streptomyces* sp. AB131-1 and AB131-2 (AB131-1 and LBR02, respectively) were found to have antagonistic properties because of their ability to produce chitinase, phosphatase, and siderophore.[28]

This study aimed to investigate the plant growth-promoting and plant pathogen-inhibitory properties of actinomycetes isolated from fecal pellets of the giant millipede *T. resimus* to explore their potential for further development and agricultural applications.

MATERIALS AND METHODS

Collection of fecal pellets of *T. resimus*

Fecal pellets of *T. resimus* were collected directly from Phu Kum Khao, Sahutsakhan district, Kalasin Province, Thailand, where *T. resimus* inhabits. Soil samples were collected from the surface of the soil at the same site for further analysis and isolation of actinomycetes in the laboratory.

Isolation of actinomycetes and plant nutrition analysis

Actinomycetes were isolated from 1 g of *T. resimus* fecal pellets or 1 g of soil sample that was transferred into sterile bottles, to which 9 mL of sterile distilled water was added. The bottles were then shaken at 120 rpm for 30 min at 25°C. Subsequently, the pH and amounts of organic matter in the samples were measured using a pH Test Kit and Fertilizer Test Kit KU 5 (developed at Kasetsart University). Finally, actinomycetes were isolated by applying a 10-fold serial dilution in the range 10^{-3}–10^{-5}, spread on actinomycete isolation agar (AIA) plates incubated at 30±2 °C for 48 h. The isolates were purified and stored at 4°C until use.[29]

Morphological actinomycete identification

Actinomycete colonies were morphologically characterized according to the method reported by Taddei et al.,[30] Intra et al.,[31] and Li et al.[29] Single colonies were cross-streaked on AIA plates and incubated at 30°C for 7–12 d. The colony characteristics included shape, margin, elevation, surface, and pigmentation. Gram staining was observed using a light microscope.

16S rRNA identification of the actinomycetes

Genomic DNA of actinomycetes was extracted using the TIANamp Bacteria DNA Kit (TIANGEN), according to the manufacturer's instructions. Genomic DNA was sequenced by Marcrogen, Inc. (Seoul, South Korea). The new sequences were compared with bacterial 16S rRNA sequences in the NCBI database using BLAST. The most similar sequences were downloaded and aligned with new sequences using CLUSTAL X software. Maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) methods were used for sequence analysis using MEGA 7.0 software.[32]

Assessment of plant growth promotion nitrogen fixation

To test nitrogen fixation, the actinomycete isolates were inoculated on nitrogen-free solid malate media (NFM) and then incubated at 37°C for 8 d. Nitrogen-fixing actinomycetes change the color of bromothymol blue media to blue.[33]

Phosphate solubility

Actinomycetes grown on AIA media for 7–12 d were transferred onto double-layered glucose yeast extract agar (GYA) medium containing tricalcium phosphate by inoculating four isolates per dish and incubating them at 30°C for 3–7 d. The formation of a clear blue zone around the colonies was a positive sign of phosphate solubility.[33]

Siderophore production

Actinomycete siderophore production was tested using chrome azurol sulfate (CAS) agar,[8] onto which actinomycete isolates were inoculated and incubated at 30°C for 7 d. The formation of a yellow-orange zone around the colonies was a positive sign of siderophore production.

IAA production

Actinomycete isolates were cultivated in 5 mL of tryptic soy broth with and without 0.5 mg/mL of L-tryptophan. The tubes were shaken for 7 d at 200 rpm and 30°C. Subsequently, the cultures were centrifuged for 10 min at 12,000 rpm, after which 1 mL of the supernatant was transferred to a microfuge tube. Salkowski's reagent (2 mL; 0.5 M $FeCl_3$ in 35% perchloric acid) was added to the supernatant, and the mixture was incubated at room temperature for 30 min in the dark. The pink color indicated the production of indole compounds in the supernatant.

Ability of actinomycetes to inhibit the growth of plant pathogenic bacteria

The agar disc method was used to examine the ability of the actinomycete isolates to inhibit *X. oryzae* pv. *oryzae (Xoo)*. *Xoo* was cultured in nutrient broth (NB) and incubated at 37°C for 16-18 h. The turbidity of the *Xoo* suspension was adjusted to McFarland Standards No. 0.5, which resulted in a concentration of approximately 1.5×10^8 culture-forming units (CFU)/mL. A sterile cotton swab was dipped in the *Xoo* suspension and smeared over the surface of a nutrient agar (NA) plate,[32] which was allowed to dry. Thereafter, 5-mm diameter discs of each actinomycete isolate were placed at four points in a cross pattern. There were three replicates per treatment; rifampicin was used as a positive control, and sterilized distilled water was used as a negative control. The isolates were incubated at 37°C for 48 h. The formation of a clear zone around the actinomycete colonies indicated inhibition of *Xoo* growth.[32]

RESULTS

Isolation of actinomycetes and plant nutrition analysis

The AIA medium yielded actinomycetes at a rate of 4.57×10^6 CFU/g for the millipede fecal pellets and 1.34×10^7 CFU/g for the soil samples. The average pH of the millipede fecal pellets was 7.76, and that of the soil samples was 8.57. The Fertilizer Test Kit KU 5 showed that both millipede fecal pellets and soil samples contained low to moderate levels of plant nutrients and low amounts of organic matter (Table 1).

Table 1 . Plant nutrient analysis, pH and organic matter of fecal pellets from *Thyropygus resimus* and soil using pH Test Kit and Fertilizer Test Kit KU 5 (developed at Kasetsart University)

Component	fecal pellets of *T. resimus*	soil
pH	8.0	7.0
Ammonia	moderate	moderate
Nitrate	moderate	moderate
Potassium	low	low
Phosphorus	low- moderate	moderate - high
Organic matter	low	low

Morphological actinomycete identification

In total, 59 actinomycete isolates were obtained, which were assigned to six morphological groups:

Group 1 Gram-positive filamentous rods. Aerial mycelia on AIA medium were gray, substrate mycelia were brown, brown pigment was produced, and spira-type aerial mycelia were observed. Eight isolates were identified: KLD-AC02, KLS-AC02-1-1, KLS-AC02-1-3, KLS-AC02-1-4, KLS-AC02-2, KLS-AC02-2-1, KLS-AC02-2-2, and KLS-AC02-2-3 (Figure 1).

Group 2 Gram-positive filamentous rods. Aerial mycelia on AIA medium were gray, substrate mycelia were pale to orange yellow, brown pigment was produced, rectiflexibile-type structures were observed, and fragmented branched aerial mycelia were observed. Forty-three isolates were identified: KLD-AC01, KLD-AC02-1, KLD-AC02-2, KLD-AC03, KLD-AC04-1, KLD-AC04-2, KLD-AC08, KLD-AC09, KLD-AC10, KLD-AC11, KLD-AC12, KLD-AC13, KLD-AC16, KLD-AC17, KLD-AC18, KLD-AC19-1, KLD-AC19-2, KLD-AC19-3, KLD-AC20, KLD-AC21-1, KLD-AC21-2, KLD-AC22, KLD-AC25, KLD-AC26, KLD-AC27, KLD-AC28, KLD-AC29, KLD-AC29-1, KLD-AC30, KLD-AC31, KLD-AC32, KLD-AC33, KLD-AC36, KLD-AC36-1-1, KLD-AC36-1-2, KLD-AC37, KLD-AC38, KLS-AC09, KLS-AC02-1-2, KLS-AC02-1-5, KLS-AC11-1, KLS-AC11-2, and KLS-AC12 (Figure 1).

Group 3 Gram-positive filamentous rods. Aerial mycelia on AIA medium was grayish blue, substrate mycelia were green, brown pigment was produced, and spira-type aerial mycelia were observed. Four isolates were identified: KLS-AC03, KLS-AC04, KLS-AC06, and KLD-AC06 (Figure 1).

Group 4 Gram-positive filamentous rods. Aerial mycelia on AIA medium were gray, substrate mycelia were white, no pigment formation was observed, and rectiflexibile-type aerial mycelia were observed. One isolate was identified: KLD-AC07 (Figure 1).

Group 5 Gram-positive filamentous rods. Aerial mycelia on AIA medium were white, substrate mycelium was reddish orange, pale brown pigment was produced, and retinaculiaperti-type aerial mycelia were observed. Two isolates were identified: KLS-AC08-1-1 and KLS-AC08-1-2 (Figure 1).

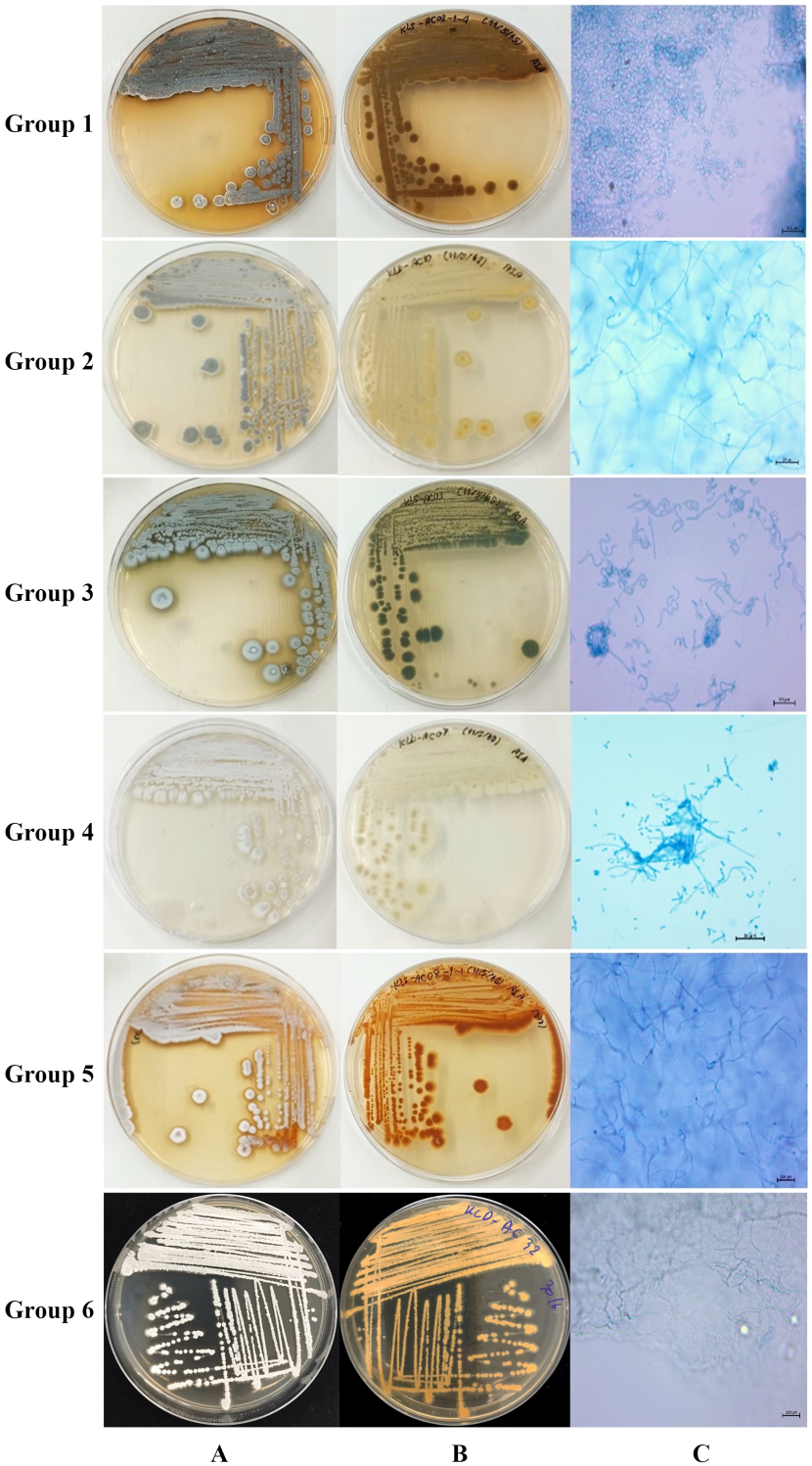

Figure 1. Colony morphology of *Streptomyces* spp. isolated from fecal pellets of *Thyropygus resimus* using actinomycetes isolation agar (AIA). A) Aerial mycelium, B) Substrate mycelium, C) spore chains

Table 2. Efficacy of actinomycetes isolated from fecal pellets of *Thyropygus resimus* for plant growth promotion and inhibition of rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*)

Isolates	Nitrogen fixation[1/]	Siderophore production[1/]	Phosphate solubility[1/]	IAA production[1/]	*Xoo* inhibition zone (mm) [1/]
KLS-AC02-1	+	+	-	+	20.25 ± 1.50 ^{ijklmnopq}
KLS-AC02-1-1	+	+	-	+	58.25 ± 4.86 ^{a}
KLS-AC02-1-2	-	+	-	-	28.75 ± 0.96 ^{fghi}
KLS-AC02-1-3	+	+	-	++	0.00 ± 0.00 ^{r}
KLS-AC02-1-4	+	+	-	++	47.75 ± 2.22 ^{abcd}
KLS-AC02-1-5	+	+	-	+	0.00 ± 0.00 ^{r}
KLS-AC02-2	+	++	-	+	43.50 ± 9.98 ^{cde}
KLS-AC02-2-1	+	++	-	+	51.50 ± 2.38 ^{abc}
KLS-AC02-2-2	+	+	-	++	47.50 ± 4.20 ^{abcd}
KLS-AC02-2-3	-	+	-	+	50.25 ± 1.71 ^{abcd}
KLS-AC03	++	+	++	-	20.25 ± 1.50 ^{ijklmnopq}
KLS-AC04	++	+++	++	+++	18.75 ± 0.96 ^{jklmnopq}
KLS-AC06	++	++	+++	++	15.75 ± 2.25 ^{mnopq}
KLS-AC08-1-1	++	-	+++	-	12.25 ± 0.96 ^{pq}
KLS-AC08-1-2	++	+	++	-	0.00 ± 0.00 ^{r}
KLS-AC09	+	+	+	++	45.00 ± 2.45 ^{bcde}
KLS-AC11-1	+	++	++	+	43.50 ± 1.50 ^{cde}
KLS-AC11-2	+	++	++	+++	55.00 ± 2.16 ^{ab}
KLS-AC12	++	+	++	-	0.00 ± 0.00 ^{r}
KLD-AC01	++	+++	+	++	23.50 ± 2.38 ^{ijklmno}
KLD-AC02	-	+	+	+	39.50 ± 5.26 ^{def}
KLD-AC02-1	+++	+++	++	+	23.25 ± 2.06 ^{ijklmnop}
KLD-AC02-2	+	++	+++	-	24.50 ± 1.29 ^{ghijklmn}
KLD-AC03	+	++	++	+	24.25 ± 0.50 ^{ghijklmn}
KLD-AC04-1	++	++	+++	-	18.75 ± 0.96 ^{jklmnopq}
KLD-AC04-2	++	++	-	-	35.25 ± 5.68 ^{efg}
KLD-AC06-1-2	-	-	++	-	26.25 ± 1.26 ^{ghijklm}
KLD-AC07	+++	++	+	+	22.50 ± 1.00 ^{ijklmnop}
KLD-AC08	+	++	+++	+++	30.50 ± 1.73 ^{fghi}
KLD-AC09	++	+++	++	+	0.00 ± 0.00 ^{r}
KLD-AC10	++	++	++	+	17.25 ± 1.26 ^{klmnopq}
KLD-AC11	++	++	+	+	26.50 ± 1.29 ^{ghijklm}
KLD-AC12	++	++	-	+	0.00 ± 0.00 ^{r}
KLD-AC13	++	+++	++	-	24.50 ± 2.38 ^{ghijklmn}
KLD-AC16	++	++	+++	+	23.25 ± 2.06 ^{ijklmnop}
KLD-AC17	++	+++	+	+	25.50 ± 2.38 ^{ghijklmn}
KLD-AC18	++	++	++	-	15.75 ± 10.24 ^{mnopq}
KLD-AC19-1	+	++	++	-	22.50 ± 1.29 ^{ijklmnop}
KLD-AC19-2	+	++	-	+	34.75 ± 1.26 ^{efgh}
KLD-AC19-3	+	++	++	++	11.00 ± 0.82 ^{qr}
KLD-AC20	+	+	-	+	25.75 ± 1.50 ^{ghijklm}
KLD-AC21-1	+	++	+	+	43.75 ± 17.11 ^{cde}
KLD-AC21-2	+	++	+	+	0.00 ± 0.00 ^{r}
KLD-AC22	+	+	-	+++	20.75 ± 0.96 ^{ijklmnopq}
KLD-AC25	+	++	+	++	12.50 ± 6.58 ^{opq}
KLD-AC26	+	++	+	-	14.50 ± 1.29 ^{nopq}
KLD-AC27	++	++	-	-	0.00 ± 0.00 ^{r}
KLD-AC28	++	+++	++	-	21.50 ± 0.58 ^{ijklmnopq}
KLD-AC29	+	++	++	+	28.25 ± 1.26 ^{ghijk}

Table 2. Cont...

Isolates	Nitrogen fixation[1/]	Siderophore production[1/]	Phosphate solubility[1/]	IAA production[1/]	*Xoo* inhibition zone (mm) [1/]
KLD-AC29-1	++	+++	++	+	27.50 ± 2.08 [ghijkl]
KLD-AC30	++	+++	++	+	26.00 ± 0.82 [ghijklm]
KLD-AC31	+	++	-	+	26.00 ± 5.23 [ghijklm]
KLD-AC32	+	++	++	+	25.50 ± 0.58 [ghijklmn]
KLD-AC33	++	+++	++	-	16.50 ± 19.06 [lmnopq]
KLD-AC36	+	++	+	+	15.75 ± 18.19 [mnopq]
KLD-AC36-1-1	+	++	++	++	3.00 ± 1.41 [fghi]
KLD-AC36-1-2	+	++	+	-	26.00 ± 1.83 [ghijklmn]
KLD-AC37	+	+	++	+++	24.00 ± 3.65 [ijklmn]
KLD-AC38	+	++	++	++	0.00 ± 0.00 [r]
Positive Control*	nd	nd	nd	nd	27.50 ± 1.29 [ghijkl]
Negative Control**	nd	nd	nd	nd	0.00 ± 0.00 [r]

1/ + = slight, ++ = moderate, +++ = high; 2/ Means followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test, p = 0.05; nd = not determined, * = 100 ppm rifampicin, ** = dH_2O

Group 6 Gram-positive filamentous rods. Aerial mycelia on AIA medium were white to pinkish white, substrate mycelia were pale pink, no pigment formation was observed, and retinaculiaperti-type aerial mycelia were observed. One isolate was identified: KLD-AC06-1-2 (Figure 1).

16S rRNA identification of actinomycetes

Seventeen isolates from each actinomycete group were selected for molecular identification. All 17 isolates were assigned to the genus *Streptomyces* because they showed a 16S rRNA sequence similarity of 99–100% with *Streptomyces* reference sequences from the NCBI database and were positioned with strong bootstrap support within a cluster of *Streptomyces* sequences in the NJ tree (Figure 2). Two clusters were obtained from these isolates. The first cluster consisted of 12 isolates, which included three subgroups (Ia, Ib, and Ic). Subgroup Ia consisted of nine isolates of *S. zaomyceticus*. Sub-group Ib consisted of two isolates, *S. griseorubiginosus* (KLS-AC08-1-1) and *S. phaeopurpureus* (KLS-AC08-1-2). Subgroup Ic consisted of one *Streptomyces* sp. The second cluster consisted of five isolates with two subgroups, IIa and IIb. Subgroup IIa consisted of two isolates, *S. pilosus* and *Streptomyces* sp. Subgroup IIb consisted of two isolates, *S. wuyuanensis* and *Streptomyces* sp.

Assessment of plant growth promotion nitrogen fixation

Fifty-four isolates (93.10%) grew in NFM medium, indicating that they can grow in conditions with fixed atmospheric nitrogen (Figure 3A). Two isolates exhibited the highest nitrogen fixation ability, namely KLD-AC02-1 and KLD-AC07 (Table 2).

Phosphate solubility

Forty-two isolates (72.41%) were able to solubilize phosphate in double-layered GYA medium, as indicated by the clear zones around actinomycete colonies 7 d after incubation (Figure 3B). The highest levels were observed for KLS-AC06, KLS-AC08-1-1, KLD-AC02-2, KLD-AC04-1, and KLD-AC08 (Table 2).

Siderophore production

Fifty-five isolates (94.83%) produced siderophores on CAS medium, forming a clear orange zone around the colonies after 5 d of incubation (Figure 3C). Nine isolates produced high levels of siderophores, namely KLD-AC01, KLD-AC02-1, KLD-AC09, KLD-AC13, KLD-AC17, KLD-AC28, and KLD. AC29-1, KLD-AC30, and KLD-AC33 (Table 2).

IAA production

Forty-one isolates (70.69%) produced IAA. Five of these isolates did so in a higher proportion

than the others (that is, their supernatants acquired a reddish color), namely KLS-AC04, KLS-AC11-2, KLD-AC08, KLD-AC22, and KLD-AC37 (Table 2).

Ability of actinomycetes to inhibit the growth of plant pathogenic bacteria

Seventeen isolates inhibited *Xoo* more effectively than 100 ppm rifampicin and produced

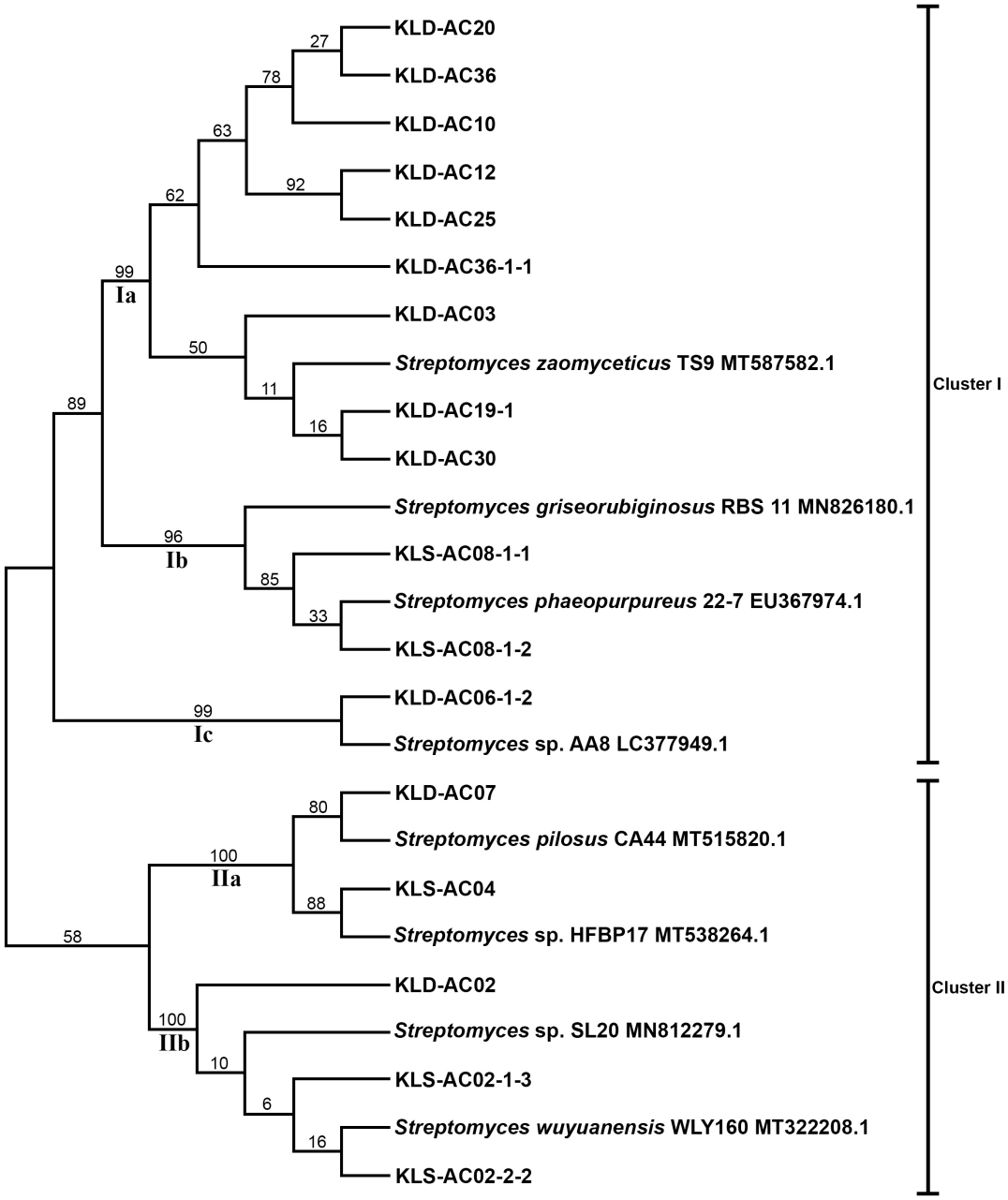

Figure 2. Neighbor-Joining tree of 17 selected actinomycete isolates from fecal pellets of *Thyropygus resimus* and their most closely related *Streptomyces* strains based on 16S-rRNA sequences. Arabic figures at the nodes are bootstrap values based on 1000 replicates.

inhibition zones with diameters between 28.75 and 58.25 mm (Table 2). The latter maximum value was observed for KLS-AC02-1-1.

DISCUSSION

Millipedes are detritivorous soil dwellers that play an important role in soil nutrient recycling by decomposing plant materials. This process occurs when the millipede gut microbiota breaks down plant chemical compounds into humus.[34,35] *Streptomyces* is the largest genus of Actinobacteria (actinomycetes) and is found in a variety of habitats, but is particularly abundant in soil. Over 500 species of *Streptomyces* have been identified to date. Colonies grow slowly and often have a soil odor owing to the formation of the volatile metabolite geosmin. They produce a wide range of pigments responsible for the coloration of vegetative and aerial mycelia.

In the present study, actinomyces were isolated from millipede fecal pellets and soil on AIA medium and 4.57×10^6 and 1.34×10^7 CFU/g were obtained, respectively. Morphological characterization of actinomycetes and molecular classification using 16S-rRNA sequences resulted in the classification of six groups, namely *S. zaomyceticus*, *S. griseorubiginosus*, *S. phaeopurpureus*, *S. pilosus, S. wuyuanensis,* and *Streptomyces* sp.

In the present study, all 59 actinomycetes isolates were tested for plant growth-promoting properties. The results showed that 54 isolates (93.10%) were able to fix nitrogen, 42 isolates (72.41%) were able to solubilize phosphate, 55 isolates (94.83%) were able to produce siderophores, and 41 isolates (70.69%) were able to produce IAA. Eight isolates were found to be the most effective in promoting plant growth, namely KLS-AC04, KLD-AC01, KLD-AC02-1, KLD-AC08, KLD-AC09, KLD-AC16, KLD-AC29-1, and KLD-AC30.

Streptomyces species are widely used in medicine and agriculture to produce commercially important secondary metabolites and enzymes in medicine and agriculture.[36] *Streptomyces* offers a wider range of new antibiotics than other genera. Therefore, they are of paramount importance for both industrial and medical applications.[37] They produce numerous secondary metabolites, especially antibiotics, that are currently beneficial to pharmaceutical companies, leading to large-scale research into new antibiotic discovery.[38]

In the present study, the efficacy of actinomycetes in inhibiting the growth of *Xoo* using the paper disc method showed that 17 isolates inhibited *Xoo* more effectively than 100 ppm rifampicin, with an inhibition zone diameter between 28.75 and 58.25 mm. The most effective inhibitor was KLS-AC02-1-1 with a 58.25 mm clear zone diameter. Consistent with the research of Van et al.,[27] 2690 actinomycete strains were tested as potential biological control agents against rice blight (BB) in Vietnam. Of these microorganisms, 17 actinomycete strains were able to inhibit all ten major *Xoo* races. One strain, VN08-A-12, exhibited effective selective inhibitory properties against all ten races in vitro but did

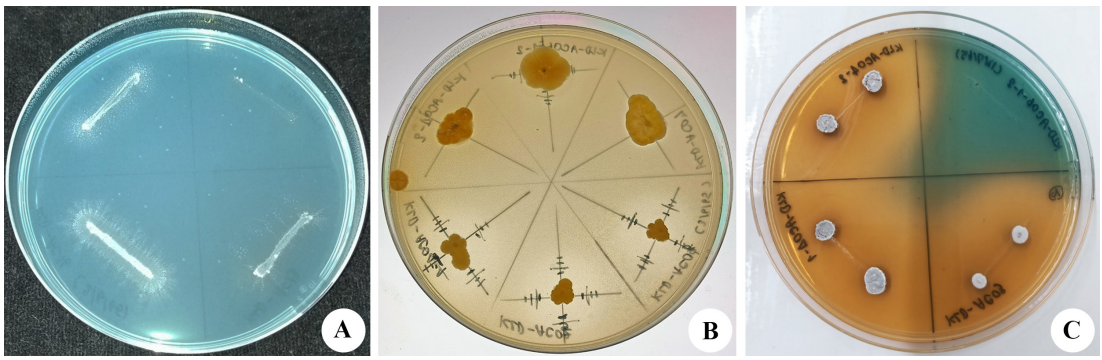

Figure 3. Evaluation of *Streptomyces* spp. isolated from fecal pellets of *Thyropygus resimus* for plant growth promotion. A) Nitrogen fixation, B) Phosphate solubility, C) Siderophore production

not inhibit most of the other microorganisms tested. Therefore, VN08-A-12 was selected for a two-season field trial with two rice cultivars, SS1 and KD18. The results showed that VN08-A-12 reduced the length of *Xoo* lesions in both rice cultivars and reduced *Xoo*-related crop losses in infected rice cultivars. Strain VN08-A-12 was found to be identical to *S. toxytricini*. Ham and Kim[39] studied the ability of *Streptomyces* spp. extracts to inhibit *X. oryzae* biofilm formation, and found that four extracts showed strong inhibitory activity. Isolation and purification of the extracts of strains 0320 and 4359 indicated that anthranilamide was responsible for this effect. Anthranilamide inhibits biofilm formation without affecting cell growth of *X. oryzae*. Therefore, it is a promising chemical candidate for the treatment of BB because it does not cause the emergence of resistant bacterial strains. The four selected *Streptomyces* strains are also feasible candidates for biological treatment of BB.

CONCLUSION

The most effective plant growth promoting bacterial isolates were KLS-AC04, KLD-AC01, KLD-AC02-1, KLD-AC08, KLD-AC09, KLD-AC16, KLD-AC29-1, and KLD-AC30. KLS-AC02-1-1 was the most effective isolate for inhibiting the growth of the rice bacterial leaf blight pathogen. However, further studies are required on the use of *Streptomyces* isolates as plant growth-promoting bacteria and for rice disease control.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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