International Journal of Agricultural Technology 2025 Vol. 21(2):725-740
DOI: https://doi.org/10.63369/ijat.2025.21.2.725-740
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Characterization and biological screening of the culturable endophytic actinomycetes from *Garcinia cowa* Roxb.

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Tilarux, P., Suwanposri, A., Anartngam, P. and Supong, K. (2025). Characterization and biological screening of the culturable endophytic actinomycetes from *Garcinia cowa* Roxb. International Journal of Agricultural Technology 21(2):725-740.

Abstract Actinomycetes associated with plants are relatively interactive sources of potential natural products. Two culturable endophytic actinomycetes strains, GcL-T1-1 and GcLT2-2, were isolated from a leaf of cowa (*Garcinia cowa* Roxb.). Determination of morphological and physiological properties, strains GcL-T1-1 and GcLT2-2, formed filament cells and presented spores on the mycelia which were classified to actinomycete group. In addition, strain GcL-T1-1 belonged to *Streptomyces* based on morphological and chemotaxonomic characteristics, which contained *LL*- diaminopimelic acid in peptidoglycan of cell wall. The molecular identification using similarity value of 16S rRNA gene, a non-streptomycete GcL-T2-2 was classified within the family *Micromonosporaceae*, genus *Micromonospora* that presented the highest similarity with *M. schwarzwaldensis* HKI0641^T (99.93%), follow with *M. haikouensis* 232617^T (99.23%), *M. humi* DSM 45647^T (99.16%), *M. maritima* D10-9-5^T (99.16%), *M. sediminicola* DSM 45794^T (99.16%), *M. oryzae* CP2R9-1^T (99.16%) and *M. mangrove* 2803GPT1-18^T (99.16%). In addition, neighbour-joining phylogeny confirmed that strain GcL-T2-2 formed a phyletic line within *Micromonospora.* Biological screening for antimicrobial and cytotoxic activities, ethyl acetate crude extract of *Micromonospora* sp. GcL-T2-2 exhibited activity against *Bacillus cereus* and NCI-H187 tumor cells at MIC of 50 µg/mL and IC_{50} of 48.12 µg/mL, respectively. The *Garcinia cowa* associated *Streptomyces* strain GcLT1-1 exhibited cytotoxic activity with NCI-H187 tumor cells at the IC_{50} value of 43.20 µg/mL. However, antagonistic activity against *Alternaria brassicicola* and *Colletotrichum acutatum* was an inactive result that displayed MIC value of more than 50 µg/mL.

Keywords: Actinomycetes, Biological activity, Endophytes, *Garcinia cowa*

Introduction

Actinomycetes are filamentous bacterial group, that contain high G+C DNA content. These bacteria constitute one of the largest bacterial phylum (phylum *Actinomycetota*), which can be ubiquitously found in terrestrial soil, in addition they were isolated from other source such as marine organisms, animals, and plants (AbdElgawad *et al.,* 2020; Word and Bora, 2006; Supong *et al.,* 2023; Supong *et al.,*

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2024). Actinomycetes are an interesting microbial sources of antibiotics and biologically active compounds that are diverse immense chemical structure and biological activity, in addition, many of which have applications in biotechnology, medicine, and agriculture (Afzali *et al.,* 2021; Nazari *et al.,* 2022). Generally, endophytic actinomycetes are mostly classified as *Streptomyces* that are widely associated with hosts. Some *Streptomyces* are associated strains with specific plants. In addition, some actinomycete groups are mutualists and display properties for plant growth factors and phytohormone producers (Nair and Padmavathy, 2014). To date, more than 10 genera of filamentous actinobacteria have been isolated from various plants (Jiang *et al.,* 2018; Singh and Dubey, 2018) that comprised a major family *Streptomycetaceae* (genus *Streptomyces*) (Cheng *et al.,* 2014; Worsley *et al.,* 2020), along with other groups such as *Actinoplanes*, *Micromonospora* and *Mangrovihabitans* belong to *Micromonosporaceae* (Shen *et al.,* 2013; Ortúzar *et al.,* 2020; Liu *et al.,* 2017), family *Nocardioidaceae* comprised *Marmoricola*, *Nocardioides*, (Jiang *et al.,* 2017; Chen *et al.,* 2022), *Amycolatopsis* and *Pseudonocardia* (Boonsnongcheep *et al.,* 2017; Salam *et al.,* 2017), and a new genus *Wangella* within a family *Streptosporangiaceae* (Jia *et al.,* 2013). The diversity of medicinal plants-associated actinomycetes is an interesting source for their biotechnological applications, which has been the focus of several studies (Nalini and Prakash, 2017). Accumulating endophytic actinomycetes were especially isolated from medicinal plants, which had a promising new source of biologically active metabolites with antimicrobial, antiviral, anticancer, and anti-inflammatory properties (Viaene *et al.,* 2016). In addition, actinobacterial sources have also received much attention for agriculture technology, which used to control phytopathogens and used as plant-growth-promoting agents (Shimizu, 2011).

Garcinia cowa Roxb. belongs to the family *Clusiaceae*, and is widely distributed in Southeast Asia countries such as India, the Philippines, Japan, Indonesia, Vietnam, and Thailand (Delhi: CSIR, 1956; Lim, 2012; Atreya and Shrestha, 2020). *G. cowa* Roxb, commonly known as Cha-muang in Thai, presents thick dark green leaves with glossy leaf surfaces (Bewal *et al.,* 2023). In Thailand, this plant is used in Thai food (such as Moo Cha-muang, a common Thai curry from Chanthaburi province), ingredient, and traditional medicine. Cha-muang is a source of secondary metabolites that comprises flavonoid, steroid, terpenoid, alkaloid, and xanthone compounds, which exhibit activity against bacteria, antioxidant and anti-α- glucosidase activities (Tinrat, 2022; Sriyatep *et al*., 2015; Mahabusarakam *et al.,* 2005).

In this study, two endophytic actinomycete strains, GcL-T1-1 and GcL-T2-2, were isolated from *G. cowa* Roxb. The isolated strains were screened for biological activity and characterized morphological, physiological, partial chemotaxonomic, and molecular properties. Screening of biological activity, ethyl acetate crude extract of

the endophytic strains was assayed for anti-bacterial, anti-phytopathogenic and cytotoxic activities.

Materials and methods

Isolation and cultivation of endophytic actinomycetes

Actinomycetes were isolated from a leaf sample of *G. cowa* Roxb. collected in Chanthaburi province, Thailand (12.8377810, 102.1137192). For preparation and isolation methods, the left sample was washed with sterile distilled water three times and dried. Then, small pieces of the sample were prepared for surface-sterilized conditions according to the previously reported protocol (Supong *et al.* 2016). The crushed sample was diluted in 0.85% (w/v) NaCl solution, and the diluted suspension was spread on the isolation half-diluted ISP 2 agar medium (1 L of distilled water; 2 g glucose, 2 g yeast extract, 5 g malt extract, and 18 g agar, pH 7–8), the isolation medium supplemented with nalidixic acid and cycloheximide. After incubation at 30 °C for 21 days, the actinobacterial colonies were selected and sub-cultured onto ISP2 agar to give pure culture. The actinomycete strains (GcL-T1-1 and GcLT2-2) were preserved on ISP 2 slants and freeze-dried for long-term storage.

Morphological and physiological characterization

The substrate and aerial mycelia, and sporulation of the strains were characterized using light microscope, after activated on ISP2 agar medium for 14 days. The actinomycets were cultured on the International *Streptomyces* project ISP no. 1–7 and nutrient agar, incubated at 30 °C for 14 days to determine the cultural characteristics. The color of mycelia and the soluble pigment were compared with the ISCC–NBS Color Charts standard sample no. 2106 (Kelly, 1964). Determination of sugar degradation using the standard media was described by Shirling and Gottlieb (1966). Hydrolysis of starch and gelatin, peptonization, and reduction of potassium nitrate were determined using the methods described by Arai (1975) and Williams and Cross (1971). To determine the effect of temperature (10–50 ºC), pH (0–12), and NaCl concentration (w/v, 0–10) for growth were tested on ISP 2 agar for 2 weeks.

Chemotaxonomic characterization

Preparation of biomass for diaminopimelic acid analysis, the actinobacterial strain was inoculated into ISP 2 broth and incubated at 30 °C for 7 days with a rotary shaker (200 rpm.). The culture broth was centrifuged and washed three times with

distilled water, and then the isomer of diaminopimelic acid was extracted, separated, and determined using TLC plate (Staneck and Robert, 1974).

16S rRNA gene sequences and phylogenetic analysis

The selected actinomycete, strain GcL-T2-2, was subjected to genotypic characteristics based on 16S rRNA gene and phylogenetic analysis. The 16S rRNA gene of the strain was amplified to give the target PCR product using universal 27F and 1492R primers (Inahashi *et al.*, 2010). The PCR product of the 16S rRNA gene was sequenced using primers 785F (5′-GGATTAGATACCCTGGTA-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). BLAST sequences to determine the similarity values were compared and analyzed using GenBank and EzBioCloud DataBase (Yoon *et al.,* 2017). Then, the closely related sequences were selected and manually aligned using BioEdit (Ibis Biosciences). In addition, gaps and ambiguous nucleotides were eliminated before the data were used to analyze phylogenetic evolution. Phylogenetic tree based on the 16S rRNA gene was constructed with neighbor-joining (NJ) method (Saitou and Nei, 1987), and that analyzed using MEGA 11 (Tamura *et al.,* 2021). The bootstrap values at 1000 replicates were used as a stability of each tree topology (Felsenstein, 1985).

Screening of biological activity

The stock culture of actinomycete strains GcL-T1-1 and GcL-T2-2 was activated on ISP 2 agar and incubated at 30 °C for 4 days. Preparation of seed culture, the stock culture was inoculated into 250 mL Erlenmeyer flasks that contained 100 mL of ISP 2 broth. After incubation at 30 °C for 4 days with shaking conditions, the culture was transferred into 500 mL Erlenmeyer fermented flasks that contained 200 mL of broth medium/flask. After fermentation at 30 °C on a rotary shaker at the rate of 200 rpm. for 2 weeks, the whole culture was extracted three times with organic solvent (ethyl acetate, EtOAc) to give the EtOAc extract solution. The extract solution was dried over with sodium sulfate anhydrous and evaporated to the dryness of a crude extract. The crude extract was used to test with anti-bacterial, anti-phytopathogenic and cytotoxic activities. The maximum concentration of crude extract was 50 μg/mL, used to test biological activities.

Antagonistic activity against *Alternaria basilica* and *Colletotrichum acutatum* was assayed by carboxy-fluorescein diacetate (CFDA) fluorometric detection (Aremu *et al.,* 2023; Guarro *et al.,* 1998; Haugland *et al.,* 2002). Preparation and spore activation, the spore suspension of the fungus was added into a well plate and incubated at 30 °C for 3 hr. The activated spores were mixed with the crude sample and incubated at 30 °C for 18 hr. Then, the mixture was added with a

cocktail solution that comprised CFDA and glycerol, and the mixture solution was incubated for 5–10 min in the dark at room temperature. After that, the well plate was whashed and added sterile distilled water, and measured the absorption at 485 nm and 535 nm. Calculation of inhibited percentage was analyzed by a formular 1 - [(FUT / FUC)] × 100, where FUT and FUC are the fluorescent units from cells tested with the crude sample and solvent (0.5% DMSO), respectively. The inhibition values less than 90% and more than 90% were reported as inactive and positive results, respectively. In this test, MIC value was represented the lowest concentration of positive result.

Anti-bacterial activity (*Acinetobacter baumannii*, *Bacillus cereus*, and *Staphylococcus aureus*) was analysed by the standard broth microdilution assay (Clinical and Laboratory Standards Institute, 2006). The crude extract and bacterial suspension mixture were added to a 384-well plate and incubated at 37 °C for 5 hr. Then, the optical density (OD) of the preparated sample test was determined by using a microplate reader at 600 nm. Inhibition values (%) were calculated following the formula [1 - [(FUT / FUC)] × 100, where FUT and FUC are fluorescent units from bacterial cells and crude samples, respectively. Analysis of Inhibition results, the value less than 90% was reported as an inactive result. In addition, the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test sample with the inhibition values more than 90%.

The green fluorescent protein microplate assay (GFPMA) was used for assey anti-*Mycobacterium tuberculosis* strain H37Ra and cytotoxic activity against non-cancerous cells (African green monkey kidney fibroblasts, Vero cells) (Changsen *et al.,* 2003). In addition, cytotoxic activity against tumor cells that comprised KB (oral human epidermoid carcinoma), MCF-7 (human breast cancer), and NCI-H187 (human small cell lung cancer), which were investigated by resazurin microplate assay (REMA) (O'Brien *et al.*, 2000). For anti-*M. tuberculosis*, minimum inhibitory concentration (MIC) defied the lowest concentration of the crude sample and displayed more than 90% inhibition. The IC_{50} value was the concentration for inhibition of 50% with tested cells according to the dose-response curve, which plotted between the concentrations of the test crude and inhibition value (%) with the curve-fitting method.

Results

Isolation and characterization of the endophytic actinomycetes

Two endophytic actinomycetes, strains, designed GcL-T1-1 and GcL-T2-2, were isolated from the leaf of *G. cowa* Roxb., collected in Chanthaburi province, Thailand. Based on morphological characteristics, strain GcL-T1-1 produced

substrate mycelium, and aerial hyphae on ISP 2 agar that presented the spore chain on the mycelium. However, strain GcLT2-2 produced single spores on the substrate hyphae but did not show aerial hyphae on ISP 2 agar. Strain GcL-T2-2 presented only grayish brown substrate mycelium on ISP2 (Figure 1).

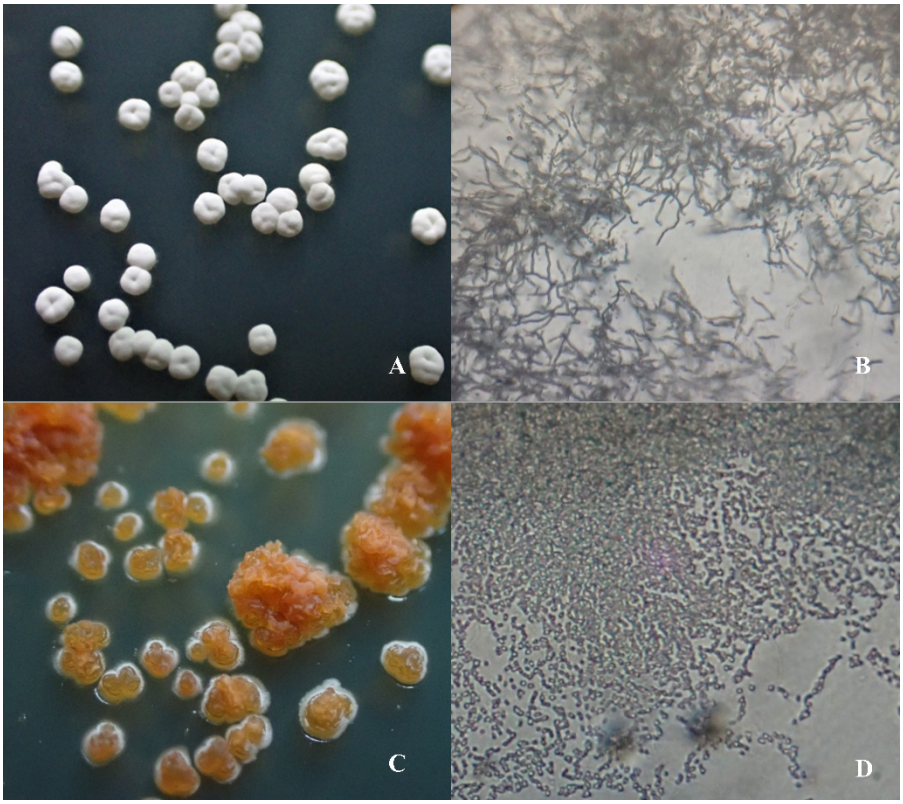

Figure 1. Characteristics of colony and hyphae of strains GcL-T1-1 (**A**, **B**) and GcL-T2-2 (**C**, **D**) grew on ISP2 agar

The cultural characteristics based on the ISP media revealed that strains GcL-T1-1 and GcL-T2-2 grew well on oatmeal agar (ISP 3) but poorly grew on ISP 1. An endophytic actinomycete strain GcLT1-1 grew moderately on ISP 2, ISP 4, ISP 5, ISP 6, and nutrient agars. Generally, strain GcLT2-2 was poor on ISP 5, ISP 6, ISP 7, and nutrient agar. This strain showed yellowish white aerial hyphae and pale yellow substrate hyphae that generally appeared white to pale yellow colonies on the standard ISP medium. The color of strain GcL-T2-2 colony formed brilliant orange-yellow to dark orange-yellow on ISP 1, ISP 2, ISP 3, ISP 4, ISP 6, and nutrient agar, but it showed white mycelium on ISP 5 and ISP 7. However, strains GcL-T1-1 and GcL-T2-2 could not produce the soluble pigment on the culture media (Table 1).

Table 1. Cultural characteristics of strains GcL-T1-1 and GcL-T2-2

Media	GcL-T1-1	GcL-T2-2
ISP 1		
Growth	poor	poor
Color of upper surface	white	pale orange yellow
Color of reverse surface	white	brilliant orange
ISP 2		
Growth	moderate	moderate
Color of upper surface	yellowish white	deep orange yellow
Color of reverse surface	pale yellow	deep orange yellow
ISP 3		
Growth	good	good
Color of upper surface	yellowish white	moderate orange yellow
Color of reverse surface	yellowish white	deep orange yellow
ISP 4		
Growth	moderate	moderate
Color of reverse surface	white	light orange yellow
Color of upper surface	white	brilliant orange yellow
ISP 5		
Growth	moderate	poor
Color of upper surface	pale yellowish pink	white
Color of reverse surface	brownish pink	white
ISP 6		
Growth	moderate	poor
Color of upper surface	white	dark orange yellow
Color of reverse surface	brilliant yellow	dark orange yellow
ISP 7		
Growth	good	poor
Color of upper surface	yellowish white	white
Color of reverse surface	pale yellow	white
Nutrient agar		
Growth	moderate	poor
Color of reverse surface	yellowish white	light orange yellow
Color of upper surface	pale yellow	light orange yellow

The effect of temperature for growth, strains GcL-T1-1 and GcL-T2-2 were mesophilic group that grew at 20–40 °C (optimum growth at 30 °C). The pH ranging of them was 4–10, and pH 7 was optimum condition for growth. The maximum NaCl concentration for growth of strains GcL-T1-1 was 4% (w/v) but strain GcL-T2-2 was 2% (w/v). Strain GcL-T1-1 could hydrolyze soluble starch, gelatin and reduce nitrate to nitrite. In addition, carbon utilization result showed that strain GcL-T1-1 utilized

monosaccharides D-fructode, D-galactose, D-glucose, D-xylose and L-arabinose, and disaccharides lactose and sucrose. Strain GcL-T2-2 showed positive results for carbon degradation only D-galactose, D-glucose, D-xylose, and L-arabinose (Table 2). Using morphological data revealed that these bacterial strains formed filamentous cells, strain GcLT2-2 presented only substrate mycelium but strain GcLT1-T produced both substrate and aerial mycelium.

Table 2. Biochemical and physiological characteristics of strains GcL-T1-1 and GcL-T2-2

Characteristics	**GcL-T1-1**	**GcL-T2-2**
Gelatin liquefaction	+	-
Growth temperature (°C)	20–40	20–40
Milk coagulation	+	-
Milk peptonization	-	-
NaCl tolerance (maximum, %)	4	2
Nitrate reduction	+	-
pH range	4–10	4–10
Starch hydrolysis	+	-
Utilization of:		
D-Fructose	+	-
D-Galactose	+	+
D-Glucose	+	+
D-Raffinose	-	-
D-Xylose	+	+
L-Arabinose	+	+
Lactose	+	-
Sucrose	+	-

+: Positive
w: weakly positive
⁻: Negative

Cell wall analysis based on diamino pimeric acid (DAP) isomer, strains GcL-T1-1 displayed *LL*-DAP, while GcL-T2-2 exhibited *meso*-DAP. On the basis of chemotaxonomic results, strain GcL-T1-1 was identified as *Streptomyces* that classified to the family *Streptomycetaceae*, but strain GcL-T2-2 was a non-streptomycete group. To evaluate the genus level, strain GcL-T2-2 was determined using molecular identification based on 16S rRNA gene sequence, which comprised 1437 bp. Comparison of 16S rRNA gene sequences between strain GcL-T2-2 and the selected closely related type strains of *Micromonospora* species indicated that strain GcL-T2-2 displayed the highest similarity value with *M. schwarzwaldensis* HKI0641^T at 99.93%, following *M. haikouensisother* 232617^T at 99.23% and related other species at 98.82–99.16% (Table 3).

Table 3. Similarity values (%) of strain GcLT2-2 and related *Micromonospora*

Rank	Closely related species	Strain	Similarity (%)
1	*Micromonospora schwarzwaldensis*	HKI0641^T	99.93
2	*Micromonospora haikouensis*	232617^T	99.23
3	*Micromonospora humi*	DSM 45647^T	99.16
4	*Micromonospora maritima*	D10-9-5^T	99.16
5	*Micromonospora sediminicola*	DSM 45794^T	99.16
6	*Micromonospora oryzae*	CP2R9-1^T	99.16
7	*Micromonospora mangrovi*	2803GPT1-18^T	99.16
8	*Micromonospora harpali*	NEAU-JC6^T	99.09
9	*Micromonospora siamensis*	DSM 45097^T	99.02
10	*Micromonospora zhangzhouensis*	HM134^T	99.02
11	*Micromonospora carbonacea*	DSM 43168^T	98.88
12	*Micromonospora marina*	DSM 45555^T	98.88
13	*Micromonospora wenchangensis*	2602GPT1-05^T	98.88
14	*Micromonospora chalcea*	DSM 43026^T	98.82
15	*Micromonospora halophytica*	DSM 43171^T	98.82

The neighbor-joining phylogenetic tree analysis revealed that GcL-T2-2 was classified to the genus *Micromonospora* and formed a cluster with *M. schwarzwaldensis* HKI0641^T (Figure 2). The results of a partial taxonomic study, an endophytic actinomycete strain GcL-T2-2 was identified as *M. schwarzwaldensis*. However, the basic taxonomy of species level should be compared to the polyphasic taxonomy based on properties between strain GcL-T2-2 and highly related species *M. schwarzwaldensis* by using phenotype, chemotaxonomy, and genotype using whole-genome analysis. For morphological results, strain GcL-T2-2 and *M. schwarzwaldensis* HKI0641^T formed orange colonies, and well-developed spores and branched substrate hyphae bear black on the ISP 2 agar.

Biological activity

To screen the biological activity of the endophytic actinomycetes, *Micromonospora* strain GcL-T2-2 exhibited activity against Gram-positive *B. cereus* and *C. acutatum* using the dual culture technique. This strain showed weak activity with the inhibition zone at 3 mm. Crude extract of strain GcL-T1-1 showed inactive activity (MIC values more than 50 µg/mL) with Gram-positive *B.* cereus, *S. aureus*, and Gram-negative *A. baumanniiwas* (Table 4). Anti-bacterial activity against *B.* cereus was found in the crude extract of an endophytic *Micromonospora* strain GcL-T2-2 that showed activity with the MIC value of 50 µg/mL. However, anti-fungal

activity against plant phytopathogens had negative results with a MIC value of more than 50 µg/mL (Table 4). On the other hand, crude EtOAc extract of GcL-T1-1 and GcL-T2-2 showed weak cytotoxicity against tumor cells (NCI-H187) with the IC_{50} of 43.20 and 48.12 µg/ml, respectively (Table 5).

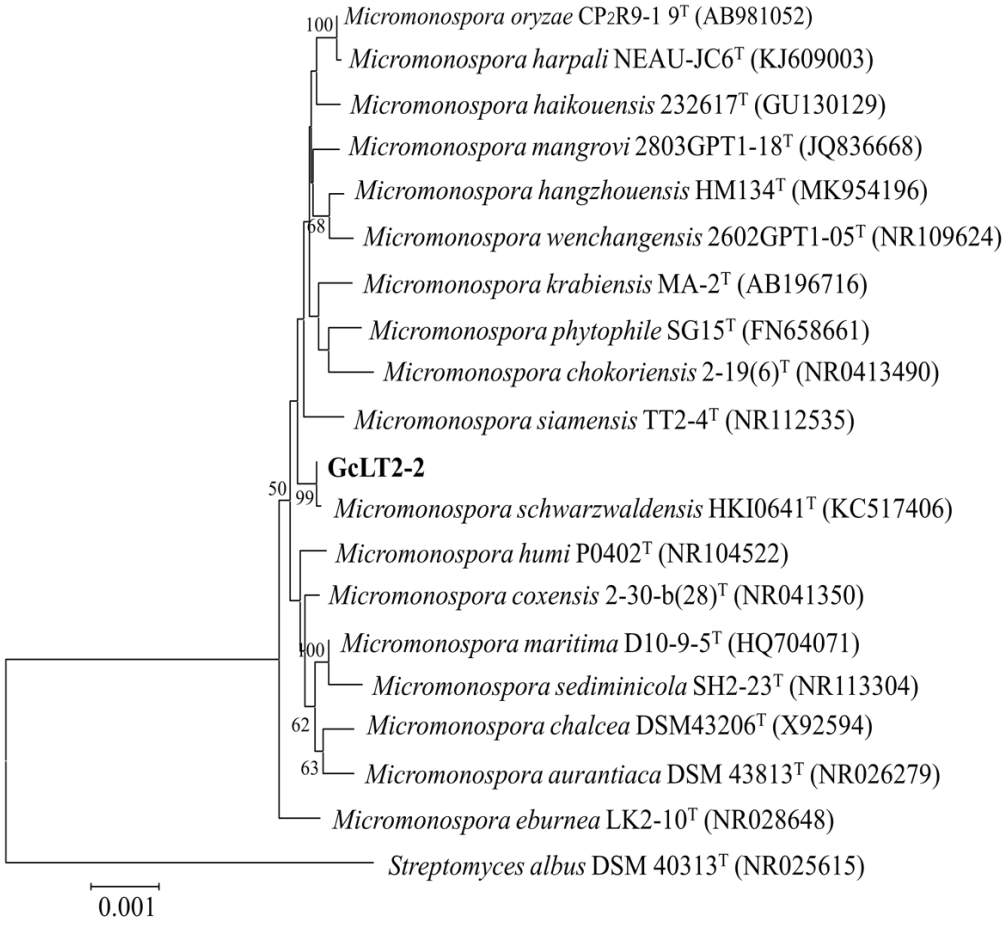

Figure 2. Neighbour-joining phyloginetic tree of strain GcL-T2-2 and closely related *Micromonospora* species based on 16S rRNA gene sequences. Bootstrap values are expressed as a percentage of 1,000 replications. Bar, 0.001 substitutions per nucleotide position

Table 4. Anti-bacterial activity

Compound	Anti-bacterial (MIC, μg/mL)			
	B. cereus	***S. aureus***	***A. baumannnii***	***M. tuberculosis***
Crude-GcL-T1-1	inactive	inactive	Inactive	
Crude-GcLT2-2	50.00	inactive	Inactive	
Rifampicin *	0.20	0.02	3.13	
Erythromycin*			25.00	
Vancomycin *	4.00	2.00		2.00
Ofloxacin*				0.39
Rifampicin*				6.25×10^{-3}
Isoniazid*				$9.38\ 10^{-2}$
Streptomycin*				0.63

*, positive controls
Inactive, MIC >50 μg/mL

Table 5. Anti-fungal and cytotoxic activities

Compound	Anti-fungal (MIC, µg/mL)		Cytotoxicity (IC_{50}, µg/mL)		
	A. brassicicola	***C. acutatum***	**McF7**	**NCI-H187**	**Vero**
Crude-GcLT2-2	inactive	inactive	inactive	43.20	inactive
Crude-GcLT1-1	inactive	inactive	inactive	48.12	inactive
Amphotericin B*	0.78	3.13			
Ellipticine*				3.19	1.33
Doxorubicin*			8.26	0.17	
Tamoxifen*			10.44		

*, positive control
Inactive, MIC >50 µg/mL

Discussion

In some cases, plants may be able to recruit beneficial microorganisms that could inhibit phytopathogens through a range of biological mechanisms, including competition for nutrients, production of bioactive secondary natural compounds, and induction of plant resistance (Wang *et al.,* 2023). In addition, the endophytic microorganisms can produce biological sources that are used for plant growth-promoting agents such as auxin phytohormones family (indole-3-acetic acid, IAA) and siderophores (Hernández *et al.,* 2023; Sitlaothaworn *et al.,* 2023). In this study, plant-associated actinomycete strains, GcLT1-1 and GcLT2-2, were isolated from a leaf of *G. cowa* Roxb., these strains were characterized and screened to determine the biological activity. Characteristics of the isolated strains based on phenotypic and partial chemotaxonomic with

diaminopimeric acid data presented that strain GcLT1-1 produced branched substrate hyphae and developed spores on the aerial hyphae. This strain belonged to the genus *Streptomyces* which contained *LL*-DAP in the peptidoglycan of the cell wall. This genus contained cell-wall type I with no diagnostic sugar of bacterial whole-cell hydrolysates (Nouioui *et al.,* 2018). Based on phenotypic and DAP isomer analysis, an endophytic strain GcLT2-2 could not identify to the genus level that it was confirmed by genotypic data using 16S rRNA gene sequence. BLAST and phylogenetic results indicated that strain GcLT2-2 classified to the genus *Micromonospora*, showed the highest similarity with *M. schwarzwaldensis* HKI0641^T at 99.93%. As previously reported, *M. schwarzwaldensis* was isolated from soil (Gurovic *et al.,* 2013). This species produced anti-*Staphylococcus aureus* telomycin antibiotic. To delineate the separated species level, strain GcLT2-2 should compare the genomic data with the closely related species and display the digital DNA–DNA Hybridization (dDDH) values significantly lower than the threshold value of 70 % (Wayne *et al.,* 1987). In this study, strain GcLT2-2 and closely related *M. schwarzwaldensis* showed inactive activity against *Bacillus cereu*s with a MIC value of more than 50 μg/mL. Investigation of Biological activity of *Streptomyces* sp. strain GcLT1-1, crude extract exhibited only cytotoxicity against NCI-H187 with IC_{50} values 43.20 μg/ml.

Acknowledgments

In this research for providing financial support (during the fiscal year of 2020), the authors would like to express their gratitude to Rajamangala University of Technology Tawan-ok.

References

AbdElgawad, H., Abuelsoud, W., Madany, M. M. Y., Selim, S., Zinta, G., Mousa, A. S. M. and Hozzeinm, W. N. (2020). Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomolecules, 10:1675.

Afzali, E., Eslaminejad, T., Amirheidari, B. and Ansari, M. (2021). Cell immobilization of *Streptomyces griseobrunneus* by microcrystalline cellulose for production of cyclodextrin glucanotransferase enzyme. Cogent Engineering, 8:1868145.

Arai, T. (1975). Culture media for actinomycetes. Tokyo: The Society for Actinomycetes, Japan.

Aremu, E. A., Furumai, T., Igarashi, Y., Sato, Y., Akamatsu, H., Kodama, M. and Otani, H. (2003). Specific inhibition of spore germination of *Alternaria brassicicola* by fistupyrone from *Streptomyces* sp. TP-A0569. The Journal of General Plant Pathology, 69:211-217.

Atreya, P. N. and Shrestha, J. (2020). Biodiversity of neglected and underutilized fruits of Nepal: a review. Fundamental and Applied Agriculture, 5:470-483.

Bewal, T., Bhatt, I. D. and Devkota, H. P. (2023). *Garcinia cowa* Roxb. ex Choisy. In: Himalayan fruits and berries: bioactive compounds, uses and nutraceutical potential, Elsevier, ACADEMIC PRESS, pp.197-202.

Boonsnongcheep, P., Nakashima, T., Takahashi, Y. and Prathanturarug, S. (2017). Diversity of endophytic actinomycetes isolated from roots and root nodules *of Pueraria candollei* grah. ex benth. and the analyses of their secondary metabolites. Chiang Mai Journal of Science, 44:1-14.

Changsen, C., Franzblau, S. G. and Palittapongarnpim, P. (2003). Improved green fluorescent protein reporter gene-based microplate screening for antituberculosis compounds by utilizing an acetamidase promoter. Antimicrobial Agents and Chemotherapy, 47:3682-3687.

Chen, M. S., Chen, F., Chen, X. H., Zheng, Z. Q., Ma, X. and Tuo, L. (2022). *Nocardioides mangrovi* sp. nov., a novel endophytic actinobacterium isolated from root of *Kandelia candel*. International Journal of Systematic and Evolutionary Microbiology, 72:5295.

Cheng, G., Huang, Y., Yang, H. and Liu, F. (2014). *Streptomyces felleus* YJ1: potential biocontrol agents against the sclerotinia stem rot (*Sclerotinia sclerotiorum*) of oilseed rape. Journal of Agricultural Science, 6:91-98.

Clinical and Laboratory Standards Institute (2006). M2-A9. Performance standards for antimicrobial disk susceptibility tests; approved standard, 9th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Council of Scientific and Industrial Research (CSIR) (1956). The wealth of India (raw materials), Vol. IV. New Delhi: CSIR; pp.99-108.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39:783-791.

Guarro, J., Pujol, I., Aguilar, C., Llop, C. and Fernandez-Ballart, J. (1998). Inoculum preparation for in-vitro susceptibility testing of filamentous fungi. The Journal of Antimicrobial Chemotherapy, 42:385-387.

Gurovic, M. S. V., Muller, S., Domin, N., Seccareccia, I., Nietzsche, S., Martin, K. and Nett, M. (2013). *Micromonospora schwarzwaldensis* sp. nov., a producer of telomycin, isolated from soil. International Journal of Systematic and Evolutionary Microbiology, 63:3812-3817.

Haugland, R. P. (2002). Handbook of Fluorescent Probes and Research Products. Molecular Probes. Eugene, O. R., USA.

Hernández, I., Taulé, C., Pérez-Pérez, R., Battistoni, F., Fabiano, E., Villanueva-Guerrero, A., Nápoles, M. C. and Herrera, H. (2023). Endophytic seed-associated bacteria as plant growth promoters of Cuban rice (*Oryza sativa* L.). Microoganisms, 11:2317.

Inahashi, Y., Matsumoto, A., Danbara, H., Omura, S. andTakahashi, Y. (2010). *Phytohabitans suffuscus* gen. nov., sp. nov., an actinomycete of the family *Micromonosporaceae* isolated from plant roots. International Joural of Systematic and Evolutionary Microbiology, 60:2652-2658.

Jia, F., Liu, C., Wang, X., Zhao, J., Liu, Q., Zhang, J., Gao, R. and Xiang, W. (2013). *Wangella harbinensis* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. Antonie van Leeuwenhoek, 103:399-408.

Jiang, Z. K., Pan, Z., Li, F. N., Li, X. J., Liu, S. W., Tuo, L., Jiang, M. G. and Sun, C. H. (2017). *Marmoricola endophyticus* sp. nov., an endophytic actinobacterium isolated from the *Spesia populnea*. International Journal of Systematic and Evolutionary Microbiology, 67:4379-4384.

Jiang, Z. K., Tuo, L., Huang, D. L., Osterman, I. A., Tyurin, A. P., Liu, S. W., Lukyanov, D. A., Sergiev, P. V., Dontsova, O. A., Korshun, V. A., Li, F. and Sun C. (2018). Diversity, novelty, and antimicrobial activity of endophytic actinobacteria from mangrove plants in Beilun Estuary National Nature Reserve of Guangxi, China. Frontiers in Microbiology, 9:868.

Kelly, K. L. (1964). Inter-Society Color Council–National Bureau of Standard Color Name Charts Illustrated with Centroid Colors, US Government Printing Office, Washington, DC.

Lim, T. K. (2012). *Garcinia cowa*. In: Edible Medicinal and Non-Medicinal Plants Volume 2 Fruits, Dordrecht Heidelberg London New York, Springer, pp.29-34.

Liu, S. W., Tuo, L., Li, X. J., Li, F. N., Li, J., Jiang, M. G., Chen, L., Hu, L. and Sun, C. H. (2017). *Mangrovihabitans endophyticus* gen. nov., sp. nov., a new member of the family *Micromonosporaceae* isolated from *Bruguiera sexangula*. International Journal of Systematic and Evolutionary Microbiology, 67:1629-1636.

Mahabusarakam, W., Chairerk, P. and Taylor, W. C. (2005). Xanthones *from Garcinia cowa* Roxb. Latex. Phytochemistry, 66:1148-1153.

Nair, D. N. and Padmavathy, S. (2014). Impact of endophytic Microorganisms on plants, environment and humans. Scientific World Journal, 2014:250693.

Nalini, M. S. and Prakash, H. S. (2017). Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Letter in Applied Microbiology, 64:261-270.

Nazari, M. T., Machado, B. S., Marchezi, G., Crestani, L., Ferrari, V., Colla, L. M. and Piccin, J. S. (2022). Use of soil actinomycetes for pharmaceutical, food, agricultural, and environmental purposes. 3Biotech, 12:232.

Nouioui, I., Carro, L. and García-López, M. (2018). Genome-based taxonomic classification of the phylum *Actinobacteria*. Frontiers in Microbiology, 9:2007.

O'Brien, J., Wilson, I., Orton, T. and Pognan, F. (2000). Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. European. Journal Biochemistry, 267:5421-5426.

Ortúzar, M., Trujillo, M. E., Román-Ponce, B. and Carro, L. (2020). *Micromonospora* metallophores: A plant growth promotion trait useful for bacterial-assisted phytoremediation. Science of the Total Environment, 739:139850.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology Evolution, 4:406-425.

Salam, N., Khieu, T. N., Liu, M. J., Vu, T. T., Ky, S. C., Quach, N. T., Phi, Q. T., Rao, M. P. N., Fontana, A., Sarter, S. and Li, W. J. (2017). Endophytic actinobacteria associated with

Dracaena cochinchinensis Lour: isolation, diversity, and their cytotoxic activities. Hindawi BioMed Research International, 2017:130856.

Shen, Y., Liu, C., Wang, X., Zhao, J., Jia, F. and Zhang, Y. (2013). *Actinoplanes hulinensis* sp. nov., a novel actinomycete isolated from soybean root (*Glycine max* (L.) Merr). Antonie van Leeuwenhoek, 103:293-298.

Shimizu, M. (2011). Endophytic actinomycetes: biocontrol agents and growth promoters. In: Maheshwari DK ed. Bacteria in agrobiology: plant growth responses. Elsevier Academic Press, San Diego, pp.201-220.

Shirling, E. B. and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology, 16:313-340.

Singh, R. and Dubey, A. K. (2018). Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. Frontiers in Microbiology, 9:1767.

Sitlaothaworn, K., Budsabun, T., Booncharoen, A., Panphut, W., Savarajara, A. and Tanasupawat, S. (2023). Diversity of plant growth-promoting endophytic bacteria, genome analysis of strain Sx8-8 and Its rice germination promoting activity. Microbiology, 92:269-283.

Sriyatep, T., Siridechakorn, I., Maneerat, W., Pansanit, A., Ritthiwigrom, T., Andersen, R. J. and Laphookhieo, S. (2015). Bioactive prenylated xanthones from the young fruits and flowers of *Garcinia cowa*. Journal of Natural Products, 78:265-271.

Staneck, J. L. and Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Applied Microbiology, 28:226-231.

Supong, K., Bunbamrung, N., Tanasupawat, S., Auncharoen, P., Nithithanasilp, S., Rachtawee, P. and Pittayakhajonwut, P. (2024). Frenolicins H and I from the caterpillar-associated *Streptomyces* sp. TBRC17107. Natural Product Research, 38:3773-3782.

Supong, K., Niemhom, N., Suriyachadkun, C., Phongsopitanun, W., Tanasupawat, S. and Pittayakhajonwut, P. (2024). *Actinomycetospora termitidis* sp. nov., an insect-derived actinomycete isolated from termite (*Odontotermes formosanus*). Journal of Antibiotics, 77:299-305.

Supong, K., Thawai, C., Choowong, W., Kittiwongwattana, C., Thanaboripat, D., Laosinwattana, C., Koohakan, P., Parinthawong, N. and Pittayakhajonwut, P. (2016). Antimicrobial compounds from endophytic *Streptomyces* sp. BCC72023 isolated from rice (*Oryza sativa* L.). Research in Microbiology, 167:290-298.

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology Evolution, 38:3022-3027.

Tinrat, S. (2022). Phytochemical screenings, antibacterial and anti-biofilm activities of *Garcinia cowa* Roxb. leaves extracts and its synergistic effect with antibiotic. Pharmaceutical Science Asia, 49:556-567.

Viaene, T., Langendries, S., Beirinckx, S., Maes, M. and Goormachtig, S. (2016). *Streptomyces* as a plant's best friend. FEMS Microbiology Ecology, 92: fiw119.

Wang, Z., Gao, C., Yang, J., Du, R., Zeng, F., Bing, H., Xia, B., Shen, Y. and Liu, C. (2023). Endophytic *Streptomyces* sp. NEAU-ZSY13 from the leaf of *Perilla frutescens*, as a

promising broad-spectrum biocontrol agent against soil-borne diseases. Frontiers in Microbiology, 14:1243610.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D. and Kadler, O. (1987). Report of the ad hoc committee on the reconciliation of approaches to bacterial systematics. International Journal of Systematic and Evolutionary Microbiology, 37:463-464.

Williams, S. T. and Cross, T. (1971). Actinomycetes. IN: Methods in Microbiology Volume4, London, Academic Press, pp.295-334.

Word, A. and Bora, N. (2006). Diversity and biogeography of marine actinobacteria. Current Opinion in Microbiology, 9:279-286.

Worsley, S. F., Newitt, J., Rassbach, J., Batey, S. F. D., Holmes, N. A., Murrell, J. C., Wilkinson, B. and Hutchings, M. I. (2020). *Streptomyces* endophytes oromote host health and enhance growth across plant species. Applied and Environmental Microbiology, 86:16e01053-20.

Yoon, S. H., Ha, S. M., Kwon, S., Lim, J. and Kim, Y. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology, 67:1613-1617.

(Received: 5 October 2024, Revised: 2 March 2025, Accepted: 7 March 2025)