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Antimicrobial Activities and Painting Application of Pigmented-Producing Actinobacteria Isolated from Rhizospheric Soils of Mosses (*Taxithelium nepalense* (Schwägr.) Broth. and *Barbula indica* (Hook.) Spreng.)

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ABSTRACT

In the survey of biodiversity of actinobacteria associated with mosses (*Taxithelium nepalense* (Schwägr.) Broth. and *Barbula indica* (Hook.) Spreng.), certain strains of pigment producing actinobacteria were isolated and purified on SCA and incubated at 30°C for 1 week. Based on deep-shade color of actinobacterial pigments, 4 strains were collected and used for painting color preparation. To evaluate the antimicrobial activities, the crude extracts were prepared from 4 actinobacterial strains and tested with *Escherichia coli* PSRU-01 and Staphylococcus aureus PSRU-01. The results indicated that the crude extracts of C7, C13, C15 and D13 could not inhibit growth of *E. coli* PSRU-01, but *S. aureus* PSRU-01 was inhibited. Two fungal testers, including *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01, were completely inhibited by the crude pigment extracts of C13, C15 and D13. Based on phylogenetic results, the actinobacterial strains were closely related to *Streptomyces californicus* (C7, 100% identity), *Streptomyces bungoensis* (C13, 99.8% similarity), *Streptomyces humi* (C15, 99.9% similarity), and *Streptomyces rectiverticillatus* (D13, 99.8% similarity). They also shared phenotypic characteristics with *Streptomyces*. The cultivated cells of actinobacteria on broken-milled rice were used for pigment extraction and followed by determination of the extracted pigments for mixing with acrylic color in the shade violet, green, orange and pink colors. Application of actinobacterial pigments in painting is the first report and it is an innovative utilization of actinobacterial pigments in non-scientific field in Thailand.

1. Introduction

Actinobacteria were found in rhizospheric soils of mosses distributed in natural and human-made habitats, such as on the ground, tree trunks, or concrete surfaces. They are gram-positive filamentous, free - living and saprophytic bacteria (Insuk *et al.* 2022). The beneficial roles of these actinobacteria were assumed that they play important roles in nutrient cycling, soil temperature and moisture controls, and biodegradation and decomposition (Insuk *et al.* 2020; Holland-Moritz *et al.* 2021). However, they produce many kinds of secondary metabolites, such as antimicrobials, antitumors, anticancers, antioxidants, immunostimulants, and plant-growth promoters (Andayani *et al.* 2015; Sreenivasa *et al.* 2020; Ibrahim *et al.* 2023; Rusyda *et al.* 2024). Certain taxa of pigmented-producing actinobacteria associated with mosses and isolated from rhizospheric soils of mosses, including *Streptomyces* and rare actinobacteria reported in biotechnological applications, including medicines, pharmaceuticals, agricultures, environments and industries (Ser *et al.* 2019; Goredema *et al.* 2020; Malisorn *et al.* 2020; Singh and Dubey 2020; Hamed *et al.* 2021; Sun *et al.* 2021; Elshamy 2022).

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Furthermore, actinobacteria isolated from rhizospheric soils of mosses were focused on painting application in this study. We have studied on application of pigmented-producing actinobacteria, especially *Streptomyces*, for natural fiber dyeing (Abou Elmaaty *et al.* 2020; Chen *et al.* 2021). In the last decades, actinobacterial dyes are the alternative dyes used in industrial fields, including textile and other industries (Chakraborty *et al.* 2015; Abraham and Chauhan 2018; Urtgam and Thurnkul 2021). However, cultural and fine arts are interested in application of actinobacterial pigments for created the painting via acrylic color ingredients. In the scientific databases, we could not only find the scientific publications detailed the application of actinobacterial pigments in cultural and fine arts. Use of Thai actinobacterial strains isolated from rhizospheric soils of mosses for production of painting colors and application of these colors in cultural and fine arts is the primitive and innovative research. We are the pioneers in the application fields for using the actinobacterial pigments in painting. In this study, antibacterial and antifungal properties were detected. Polyphasic identification of actinobacteria isolated from rhizospheric soils of mosses, and determination of the actinobacterial pigments extracted from pigmented-producing actinobacteria cultivated broken-milled rice for painting application were carried out.

2. Materials and Methods

2.1. Isolation of Pigmented-Producing Actinobacteria from Rhizospheric Soils of Mosses

We collected rhizospheric soils of mosses in Phitsanulok province, Thailand, for isolation of pigmented-producing actinobacteria. The actinobacterial strains were isolated from the rhizospheric soils of mosses collected previously by the protocol described as follows: 1 g of rhizospheric soil was prepared as soil suspension with 9 ml of sterile distilled water, and serially 10-fold diluted to 10^{-3}. 1 ml of soil suspension prepared previously was spread on sodium caseinate agar (SCA) composed of 2 g/L skimmed milk, 2 g/L glucose, 0.2 g/L K_2HPO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $FeSO_4 \cdot 7H_2O$, 0.1 g/L sodium propionate and 1 L distilled water. The experimental plates were incubated at 30°C for 5-7 days.

2.2. Pigment Extraction of Actinobacterial Cells

Pigmented-producing actinobacteria used in this study were cultivated on broken-milled rice prepared by the method of Abraham and Chauhan (2018) with minor modification by Urtgam and Thurnkul (2021) described as follows: 50 g of the broken-milled rice was used for carbon, energy, nitrogen, and growth factor sources for actinobacterial cultivation. After sterilization, pigmented-producing actinobacteria were inoculated and incubated at 30°C for 5-7 days. The cultivated and harvested cells of pigmented-producing actinobacteria were extracted and the pigments were recovered by the method indicated briefly: use of 100 ml of ethyl acetate for pigmented extraction, statically incubated for 48 h before solvent evaporation. The crude pigmented extracts obtained previous steps were continuously developed for antibacterial activity testing and used for ingredients of painting color mixed with acrylic color.

2.3. Determination Antibacterial and Antifungal Activity of Actinobacterial Crude Pigment Extracts

2.3.1. Testing of Antibacterial Activity of Actinobacterial Crude Pigment Extracts

The antibacterial activities of crude pigment extracts obtained from the actinobacterial strain were performed by agar-well method mentioned by Urtgam *et al.* (2023) The protocol was described as follows:

The bacterial testers were Escherichia coli PSRU-01 and Staphylococcus aureus PSRU-01. The cultures were cultivated on nutrient agar (NA) plates and incubated at 37°C for 24 h. The single colony of each bacterial tester was transferred into nutrient broth (NB), and incubated on rotary shaker at 120 rpm, 37°C for 24 h. The inoculant was evaluated, and equivalent as McFarland No.0.5 (1.5×10^8 CFU/ml) that was measured and had score between 0.08-0.1 by spectrophotometer at λ 625 nm before inoculation on the substrate prepared as mentioned above.

To test the antibacterial activities of the crude pigment extracts, the solution prepared mentioned above was swab on Muller Hinton Agar (MHA) and prepare the hole before the prepared crude pigments extract was fill into the hole at the final

concentration as 50 µg/ml that was diluted with 20 µL of Dimethyl sulfoxide (DMSO). The positive control was 50 µg/ml of chloramphenicol, and the negative control was DMSO. All experimental sets were incubated at 37°C for 24-48 h. The clear zone diameter was evaluated around the tested hole filled with the crude pigments extract, and the control sets.

2.3.2. Testing of Antifungal Activity of Actinobacterial Crude Pigment Extracts

To test antifungal activity of the actinobacterial crude pigments extract, the fungal testers namely *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01 were cultivated on PDA and incubated at room temperature for 3-5 days, the fungal growth on PDA was detected. Therefore, 5 mm of mycelial disc of these fungal testers were prepared by cork borrer and transferred onto PDA supplemented with 50 µg/ml the actinobacterial crude pigments extract incubated at room temperature for 1 week to check the antifungal activity. All treatments were done in triplicates. Percentage of fungal inhibition by the actinobacterial crude pigments extract was calculated according to the formula described by Zhang *et al.* (2020).

$$\% \text{ IRG} = \frac{R1 - R2}{R1} \times 100$$

%IRG = percentage of inhibition growth rate
R1 = diameter of mold colony in control plate
R2 = diameter of mold colony in test plate

Data analysis was done triplicate by analysis of variance (ANOVA). The means were compared by Duncan's new multiple range test (DMRT) at 95% of confidence with SPSS version 23 for statistical analysis.

2.4. Identification of Pigmented-Producing Actinobacterial Strains

Actinobacterial identification was carried out based on polyphasically taxonomic approach. Phylogenetic identification was firstly applied by comparative 16S rDNA sequences. The actinobacterial DNA was extracted with BioFactTM Genomic DNA Prep Kit (Biofactory, Korea). The extracted DNA was amplified by PCR protocol as described by Mullis *et al.* (1986) with the universal bacterial primers 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GTTACCTTGTTACGACTT-3') (Lane 1991). Purification of the PCR product was done by BioFact™ Gel and PCR Purification System (Biofactory, Korea). The partial 16S rDNA sequences of actinobacterial strains were analyzed by standard sequencing described by Bionics (Korea). Phylogenetic analysis was determined by Neighbor-joining method with MegaX software (Kumar *et al.* 2018).

2.5. Application of Acrylic Color Containing Actinobacterial Pigments as Color Ingredients

The acrylic color obtained from the actinobacteria used in this study was prepared with the white color-based ingredient as 95-99:1-5. This color was applied to the cultural and fine arts. Qualification of the painting colors was evaluated. The measurements of color perception were carried out using the CHORMA METER CR-400 from Konica Minolta. These are in accordance to the CIELAB color space system.

3. Results

3.1. Isolation of Pigmented-Producing Actinobacteria from Rhizospheric Soils of Mosses

A total of 18 actinobacterial strains were isolated from rhizospheric soils of *Taxithelium nepalense* (Schwägr.) Broth (C). Fifteen actinobacterial strains were isolated from rhizospheric soils of *Barbula indica* (Hook.) Spreng (D). All of 33 strains were pigmented-producing actinobacteria different from each other with color shades of pigments (Table 1). They were interested in pigment production using broken-milled rice as carbon, energy, nitrogen, and growth factor sources. On the basis of deep-shade colors presented, 4 actinobacterial strains, namely C7 (dark-violet), C13 (dark-blue), C15 (dark-brown) and D13 (dark-pink), were selected for actinobacterial pigment production in the next step. In the case of pigmented-producing actinobacterial strains that produced pale-yellow, pale-grey and pale-orange color shades, we did not choose these strains for the next studies because the color shades are not attractive after preparation of the crude pigment extracts when compared with the 4 chosen strains.

Table 1. Pigmented-producing actinobacteria on broken-milled rice

Strain names	Color shades
C7	dark-violet
C18	pale-violet
C13	dark- blue
C5, D2, D5	pale-blue
C15	dark-brown
C3, C14, D1, D4, D7, D9, D12	pale-brown
D13	dark-pink
C4	pale-pink
C6, C10, C12, D6, D10, D14	pale-yellow
C1, C2, C9, C16, C17, D3, D8, D11, D15	pale-grey
C8, C11	pale-orange

3.2. Pigment Extraction of Actinobacterial Cells

The actinobacterial strains, namely C7, C13, C15 (isolated from rhizospheric soils of *Taxithelium nepalense* (Schwägr.) Broth) and D13 (isolated from rhizospheric soils of *Barbula indica* (Hook.) Spreng.), were isolated previously by cross streaking method. The colony colors on SCA are diverse, including dark-violet, dark-blue, dark-brown and dark-orange (Figure 1). Pigment extraction by ethyl acetate was done with 4 strains of actinobacterial cells. The results obtained were crude extract shown to be the different colors, such as dark-violet, dark-green, dark-orange and dark-pink colors (Figure 2).

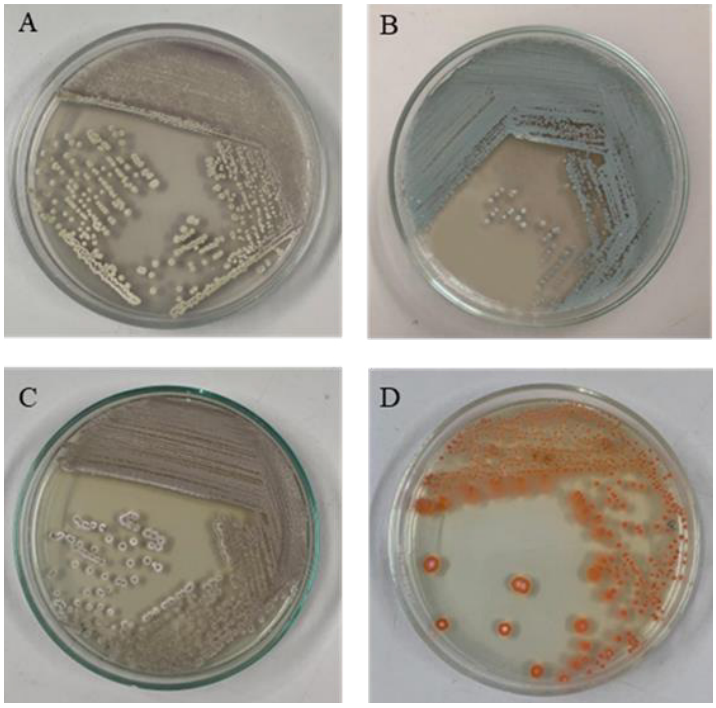


Figure 1. Colony characteristics of the actinobacterial strains on sodium caseinate agar (SCA): (A) C7, (B) C13, (C) C15, and (D) D13

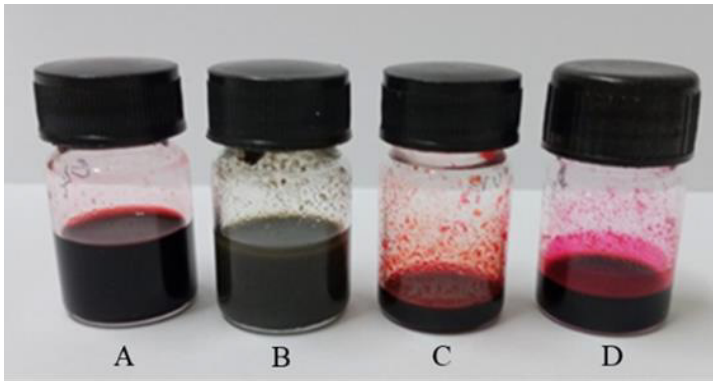


Figure 2. The extracted pigments of 4 actinobacterial strains: (A) C7, (B) C13, (C) C15, and (D) D13

3.3. Determination Antibacterial and Antifungal Activity of Actinobacterial Crude Pigment Extracts

3.3.1. Testing of Antibacterial Activity of Actinobacterial Crude Pigment Extracts

The crude pigment extracts obtained from 4 pigmented-producing actinobacteria, namely C7, C13, C15 and D13, were tested the antibacterial activities using *E. coli* PSRU-01 and *S. aureus* PSRU-01 as bacterial references. The crude pigment extracts obtained from 4 strains could not inhibit *E. coli* PSRU-01 growth, in contrast to the results of antibacterial inhibition tested with S. aureus PSRU-01 as shown on Table 2 and Figure 3.

Table 2. Antibacterial activity of the crude pigments extract of the actinobacterial strain C7, C13, C15 and D13

Actinobacteria	Clear Zone Diameter (mean ± SD) (mm.)	
	Escherichia coli PSRU-01	*Staphylococcus aureus* PSRU-01
Positive control	35.5±0.12	30.3±0.08[a]
Negative control	0	0
C7	0	26.7±0.31[a]
C13	0	10.0±0.11[c]
C15	0	15.8±0.15[b]
D13	0	16.2±0.10[b]

3.3.2. Testing of Antifungal Activity of Actinobacterial Crude Pigment Extracts

The results shown in Figure 4 and Table 3 indicated that two fungal testers, namely *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01, were strongly inhibited (100%) by the crude pigments extract of the bacterial strains, namely C13, C15 and D13. However, the crude pigments extract of strain C7 inhibited the growth of *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01 as 35.08 and 36.79 %, respectively.

3.4. Identification of Pigmented-Producing Actinobacterial Strains

The results of phylogenetic identification of pigmented-producing actinobacterial strains, including C7, C13, C15 and D13, were indicated that the strains used in this study belonged to genus Streptomyces. They shared the partial 16S rDNA sequences with the closest species in the different described species as followed: C7 (100% identity with *S. californicus*), C13 (99.8% similarity with *S. bungoensis*), C15 (99.9% similarity with *S. humi*) and D13 (99.8% similarity with *S. rectiverticillatus*). Phylogenetic tree was drawn and shown in Figure 5.

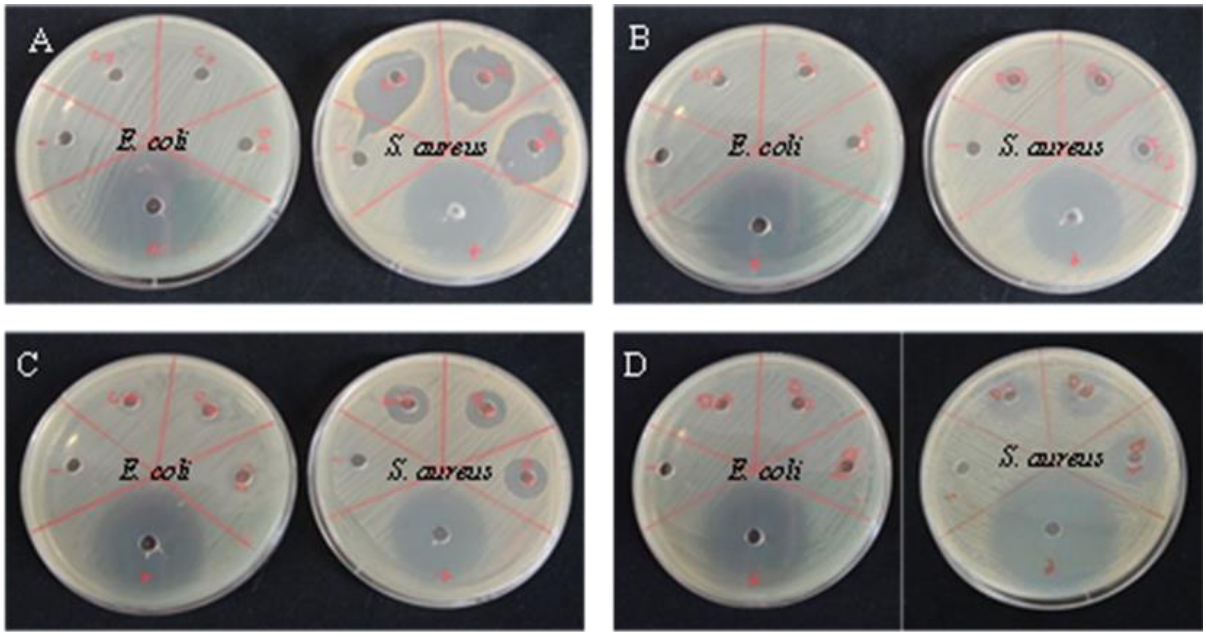
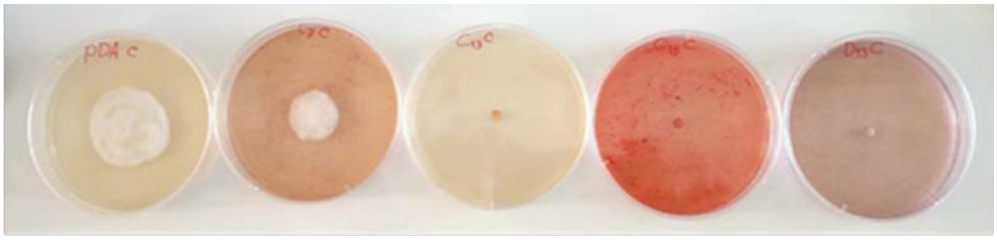
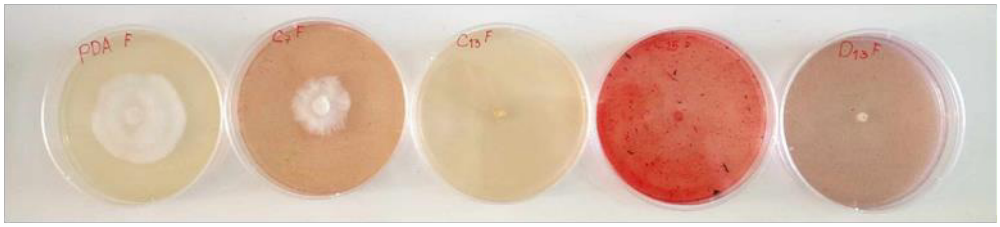


Figure 3. Inhibition of actinobacterial crude pigments extracts obtained from actinobacteria strain C7 (A), C13 (B), C15 (C) and D13 (D) against Escherichia coli PSRU-01 and Staphylococcus aureus PSRU-01



Colletotrichum PSRU-01 C7 C13 C15 D13



Fusarium PSRU-01 C7 C13 C15 D13

Figure 4. Inhibition of actinobacterial crude pigments extracts obtained from actinobacteria strain C7 (A), C13 (B), C15 (C) and D13 (D) against *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01

Table 3. Antifungal activity of the crude pigments extract of the actinobacterial strains

Strains	Percentage of inhibition rate (%IRG)	
	Colletotrichum sp. PSRU-01	*Fusarium* sp. PSRU-01
C7	35.08	36.79
C13	100	100
C15	100	100
D13	100	100

3.5. Application of Acrylic Color Containing Actinobacterial Pigments as Color Ingredients

Acrylic color was formerly prepared by co-mixtures with the white color-basal color with the proportion as 95-99:1-5. The results presented that the dark-violet, dark-orange and dark-pink colors were obtained as shown in Figure 6. However, the dark-green color could not use because its agglomeration was presented after mixing with the white color-basal color. The painting colors prepared with the extracted pigments of actinobacterial strains in this study were applied in fine arts as shown in Figure 6. After 30 days of painting at room temperature, L-value of acrylic color containing C7 (violet), C13 (orange), and D13 (pink) actinobacterial pigments was determined as shown on Table 4. In the case of acrylic color brightness, the results of painting on drawing papers presented (Figure 7) indicated that the brightness of pink acrylic color decreased much more when compared with other color tones, such as violet and orange (Table 4).

4. Discussion

Pigmented-producing actinobacteria belong to Domain Bacteria found in terrestrial and aquatic ecosystems, such as soils, freshwater and seawater, plants, animals and others (Hazarika and Thakur 2020). Rhizospheric soils are the terrestrial habitat discovered many kinds of actinobacterial taxa, especially the *Streptomyces* genus and phylogenetic relatives (Chen *et al.* 2018; Jin *et al.* 2019). *Streptomyces* was recovered from soils in different regions and described more than 1,000 species (Law *et al.* 2019; Rammali *et al.* 2022). In this decade, a variety of actinobacterial taxa were isolated from soils in Thailand (Sripreechasak *et al.* 2013; Klaysubun *et al.* 2022; Insuk *et al.* 2022). They were applied to biotechnological utilization included industrial, medical and others (El Othmany *et al.* 2021; Xie and Pathom-Aree 2021). In this study, a total of 4 strains of actinobacteria, namely C7, C13, C15 and D13, were isolated from rhizospheric soils found moss communities, and studied for application.

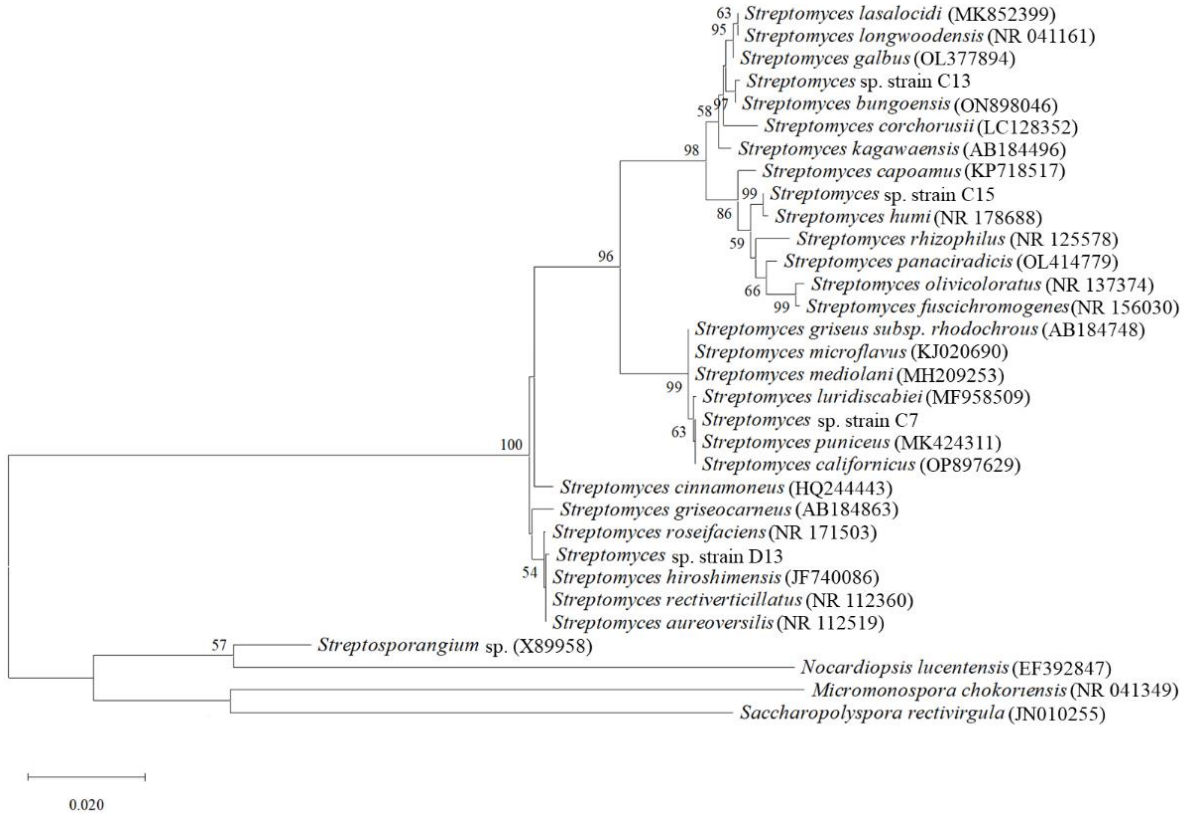


Figure 5. Phylogenetic tree of actinobacterial strains, namely C7, C13, C15 and D13, compared with *Streptomyces* spp. based on partial sequence of 16S rDNA gene analysis

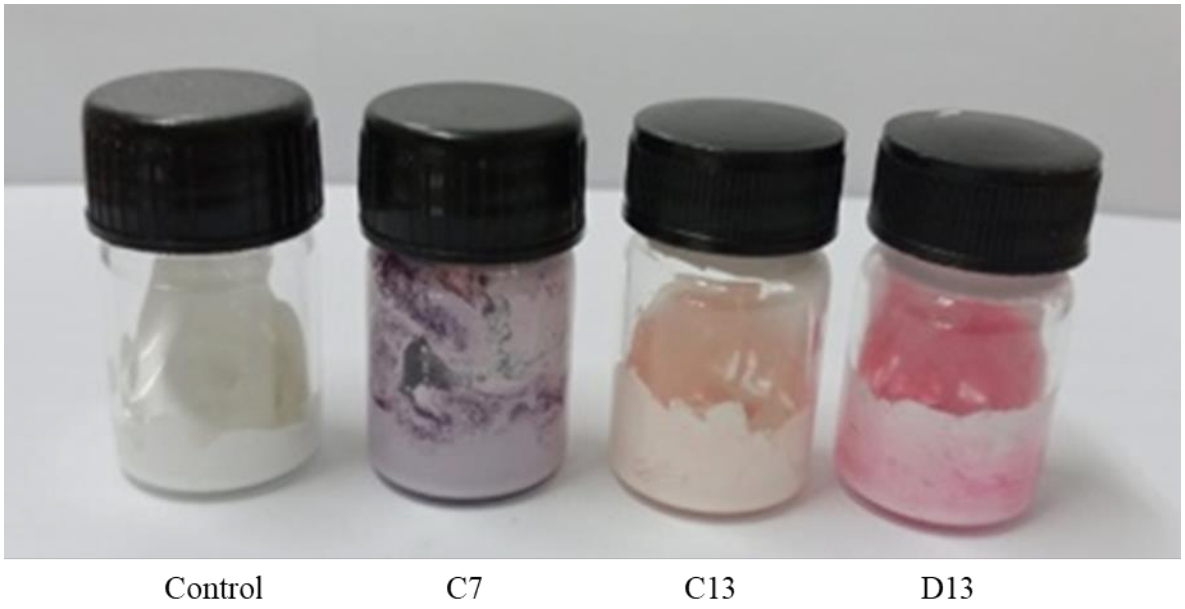


Figure 6. Acrylic colors containing the pigment extracted from the actinobacterial strains

Table 4. Determination of the brightness of the acrylic colors containing the crude pigments extract of actinobacteria strains C7, C13, and D13

Actinobacteria strains	Acrylic color	Time	Score compared with CIELAB system			CIELAB color difference		
			L	a*	b*	ΔL*	Δa*	Δb*
C7	Violet	1 day	64.97	10.58	-8.13	0.73	0.18	0.71
		30 days	65.70	10.76	-8.84			
		% color reduced	1.12	-	-	-	-	-
C13	orange	1 day	85.80	17.72	22.59	0.13	-1.56	-2.22
		30 days	85.93	16.16	20.23			
		% color reduced	0.15	-	-	-	-	-
D13	pink	1 day	84.69	20.36	-2.73	8.41	-18.70	1.67
		30 days	93.10	1.66	4.40			
		% color reduced	9.93	-	-	-	-	-

L* refers to lightness, with values ranging from 0 (black) to 100 (white); a* and b* are considered chromatic coordinates: a* for red (+), and green (−), and b* for yellow (+), and blue (−)

1 day 30 days

Figure 7. Application of acrylic colors containing the crude pigments extract of actinobacteria strains C7, C13, and D13 on painting

The crude pigment extracts obtained from 4 pigmented-producing actinobacteria, namely C7, C13, C15 and D13, were tested the antimicrobial activities, including antibacterial and antifungal activities, using *E. coli* PSRU-01, S. aureus PSRU-01, *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01 as bacterial and fungal references. The crude pigment extracts obtained from 4 strains could not inhibit *E. coli* PSRU-01 growth. In contrast to the results of antibacterial inhibition tested with *S. aureus* PSRU-0, inhibition of *S. aureus* PSRU-01 by the crude pigment extracts of C7, C13, C15 and D13 was found. It was indicated that all strains of pigmented-producing actinobacteria produced the anti-*S. aureus* compounds found in the crude pigment extracts prepared by ethyl acetate extraction. As we have known that *Streptomyces* spp. produce antibacterial compounds via secondary metabolism and their secondary metabolites presented antibacterial potentials to *S. aureus* (Al-Ansari *et al.* 2019; Gheni and Hasan 2022; Weslati *et al.* 2023). In the case of non-inhibitory activity to *E. coli*, the crude pigment extracts of 4 pigmented-producing actinobacteria could not penetrate to the cells of *E. coli* because *E. coli* cells had outer layer of membrane envelope covered peptidoglycan layer. However, the secondary metabolites found in the crude pigment extracts of 4 pigmented-producing actinobacteria may be degraded by some kinds of *E. coli* enzymes, then analysis of chemical components of the crude pigment extracts of 4 pigmented-producing actinobacteria should be analyzed in the future (Poirel *et al.* 2018). For antifungal inhibition test, the crude pigment extracts of C13, C15, and D13 completely inhibited the growth of *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01, however, the crude pigments extract obtained from C7 incompletely inhibited the growth of *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01.

The strains used in our study were identified by polyphasic approach based on phenotypic and phylogenetic results. They belonged to *Streptomyces* and shared 16S rDNA sequence similarity with 4 described species of *Streptomyces* as followed: C7 (100% identity with *S. californicus*), C13 (99.8% similarity with *S. bungoensis*), C15 (99.9% similarity with *S. humi*) and D13 (99.8% similarity with *S. rectiverticillatus*). Based on 98.5% 16S rDNA sequence similarities, two strains of bacteria are conspecific (Schleifer 2009). Therefore, 4 pigmented-producing bacteria were phylogenetically related to *S. californicus, S. bungoensis, S. humi,* and *S. rectiverticillatus*. In the case of phenotypic identification, all strains phenotypically shared the key characteristics of *Streptomyces*. In the survey of scientific publications in several databases and journal, the species were reported as soilborne actinobacteria (Malisorn *et al.* 2020; Singh and Dubey 2020; Sun *et al.* 2021). For application, *S. californicus* was applied for medical, agricultural, environmental and industrial applications (Chi *et al.* 1990; Singh and Dubey 2020; Hamed *et al.* 2021). *S. bungoensis* presented antimicrobial activities against *Fusarium oxysporum, Alternaria sesame, Rhizoctonia solani, Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231. (Goredema *et al.* 2020; Malisorn *et al.* 2020; Elshamy 2022). *S. humi* was found antioxidant activity (Zainal *et al.* 2016; Ser *et al.* 2019). *S. rectiverticillatus* was detected many kinds of bioactive compounds, including iso-coumarins showing antagonists against to phytopathogenic oomycete, *Plasmopara viticola*, and *Candida albicans* (Zinad *et al.* 2011), *Salmonella* sp., *Vibrio cholerae* Non-O1, *Mycobacterium phlei* DSM 750, *Micrococcus luteus* ATCC 10240, *B. subtilis* ATCC 9524, *B. cereus* ATCC 14579, *Arthrobacter aurescens* DSM200116 ATCC13344, *S. aureus* CCMM B3, *S. aureus* ATCC 25913 (Bensultana *et al.* 2010; Sun *et al.* 2021).

After phylogenetic identification, a total of 4 actinobacterial strains (C7, C13, C15 and D13) were applied for pigment extraction with ethyl acetate before production of painting colors. Use of actinobacterial pigments in painting, including cultural and fine arts, was not reported. Therefore, we interested in actinobacterial pigments application as painting colors because chemical painting colors commonly used were reported as toxic substances for human and other living organisms. It also effected on environments. Conversely, actinobacterial pigments had no toxic effects compared to chemical painting colors.

After production of acrylic color, and painting use, we found that these actinobacterial pigments extracted from C7, C15 and D13 cells were suitable and appropriate for painting when qualitative evaluation was carried out as mentioned previously. However, the solubility of actinobacterial C13 color was low and unsuitable for painting uses. Then, future research should be carried out for resolving the solubility problem. After 30 days of painting on drawing papers, L-value of acrylic colors containing with the crude pigments extract of C7 (violet), C13 (orange), and D13 (pink) increased to be 1.12, 0.15, and 9.93 % compared with the initial L-value. The results indicated that the acrylic color brightness was decreased by light or photooxidation, and the pink color obtained from D13 crude pigments extract was highest effected compared with those of C7 (violet) and C13 (orange), respectively. It was possible that the acrylic color structures obtained from the crude pigments extract of actinobacteria were sensitive to photooxidation (Takano *et al.* 2005; Pintus *et al.* 2012; Maresca *et al.* 2019). Therefore, the crude pigments extract from C7 and C13 could be developed in the future to produce the novel acrylic colors for painting and others. As mentioned above, application of actinobacterial pigments in painting was innovative uses in cultural and fine arts in Thailand. However, we attend to continue in this research due to the actinobacterial pigment extracted from C13 should be developed for resolving the solubility problem before painting uses as same as application of fungal pigments previously report (Robinson *et al.* 2018).

In conclusion, based on polyphasic approach, 4 strains of pigmented-producing actinobacteria isolated from rhizospheric soils of mosses (*Taxithelium nepalense* (Schwägr.) Broth. and *Barbula indica* (Hook.) Spreng.) belonged to the *Streptomyces* genus. They shared phylogenetically related to 4 species of *Streptomyces*, namely *S. californicus, S. bungoensis, S. humi* and *S. rectiverticillatus*. The crude pigment extracts of 4 strains of pigmented-producing actinobacteria presented antibacterial potentials to *S. aureus* PSRU-01. However, *E. coli* PSRU-01 was not inhibited by these extracts. The crude pigment extracts got from C13, C15 and D13 totally inhibited (100%) the growth of *Colletotrichum*

sp. PSRU-01 and *Fusarium* sp. PSRU-01. On the other hand, crude pigments extract presented partially inhibiton on the growth of *Colletotrichum* sp. PSRU-01 and *Fusarium* sp. PSRU-01. Actinobacterial pigments are the source of alternative painting colors. It had eco-friendly and non-toxic to human and other living organisms.

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