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Isolation, Identification, Antimicrobial Activities, and Application of the Crude Pigmented Extract of Soil Actinobacteria in Handicraft and Painting

Sittichai Urtgam[1], Chaowalit Puengtang[2], Muthita Nakthong[2], Pijittra Sa-ngat[2], Prattana Sirisan[3], Tawatchai Sumpradit[4], Naruemol Thurnkul[2]*

[1]*Biology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand*
[2]*Microbiology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand*
[3]*Art and Innovation Creative Design Program, Faculty of Industrial Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand*
[4]*Department of Microbiology and Parasitology, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000, Thailand*

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ABSTRACT

A total of 21 actinobacterial strains were isolated from soils in Thailand. The selected strains were applied for pigment extraction before handicraft and painting application. Based on the color tones, 4 actinobacterial strains, namely B3 (yellow), B8 (violet), F4 (brown), and F9 (pink), were selected. Their antimicrobial activities against pathogenic bacteria and molds, including *Escherichia coli* PSRU-01, *Staphylococcus aureus* PSRU-01, *Colletotrichum* sp. NPJM -01, and *Fusarium* sp. PSRU-01 were tested using the agar well and the poisoned food techniques. Phylogenetic identification was analyzed on the basis of partial 16S rDNA sequence comparison for species delineation. They closed *Streptomyces tendae* 99.73% (B3), *Streptomyces muensis* 99.52% (B8), *Streptomyces ardesiacus* 99.38% (F4), and *Streptomyces iakyrus* 99.59% (F9). These strains were evaluated for their potential as colorants in handicraft clay and painting colors. *Streptomyces* pigment may be a naturally produced and eco-friendly alternative for handicraft and painting applications.

1. Introduction

Actinobacteria are Gram-positive bacteria with high G+C DNA content that constitute one of the largest bacterial phyla, and are ubiquitously distributed in both aquatic and terrestrial ecosystems (Barka *et al.* 2016). They produce a diverse group of primary and secondary metabolites utilized for biotechnological applications, including agriculture (Zhang *et al.* 2021; Boubekri *et al.* 2022; Brito *et al.* 2022; Faddetta *et al.* 2023; Kaari *et al.* 2023), medicine (Cui *et al.* 2022; Kanchanasin *et al.* 2023), environment (Mawang *et al.* 2021; Boufercha *et al.* 2022), and cosmetics (Dahal *et al.* 2017; Law *et al.* 2020).

Pigments are the secondary metabolites produced by actinobacterial cells. Certain genera of actinobacteria were pigmented-producing taxa. However, the *Streptomyces* genus is commonly reported as the pigmented-producing actinobacteria that contain various color tones, including yellow, violet, brown, pink, red, orange, blue, green, and black (Qin *et al.* 2023). Due to its desirable properties, including non-toxic and biodegradable pigments, the Streptomyces pigments were biotechnologically applied in many industries, such as textiles, medicines, food, cosmetics, and others (Simon *et al.* 2017). The pigments extracted from Streptomyces were not only determined for application in food and cosmetics industries, but also

*Corresponding Author
E-mail Address: naruemol.t@psru.ac.th

to determine the potential for functional dyes indicated antibacterial activities (Al-Tekreeti *et al.* 2023; Ibrahim *et al.* 2023; El-Zawawy *et al.* 2024).

Phylogenetic identification based on 16S rDNA sequence analysis was firstly applied for species identification of actinobacteria, as well as other bacterial groups (Chen *et al.* 2016). Phenotypic and genetic characterization are also applied for actinobacterial identification in order to make precise data of taxonomic delineation of actinobacteria based on a polyphasic taxonomic approach, including phylogenetic, phenotypic, and genetic characterization (Van der Aart *et al.* 2019; Wang *et al.* 2023).

As mentioned above, actinobacteria had antibacterial and antifungal activity against pathogenic bacteria and fungi. Chanthasena *et al.* (2022) determined the antimicrobial activities of *Streptomyces actinomycinicus* against methicillin-resistant *Staphylococcus aureus*. Kadaikunnan *et al.* (2024) studied the antibacterial activity of *Streptomyces parvulus* against multidrug-resistant bacterial pathogens, including Gram-positive and Gram-negative bacteria. Chen *et al.* (2024) evaluated the determination of antifungal activities of the actinomycete *Streptomyces* sp. M5205 against *Fusarium* sp. LC8, *Colletotrichum gloeosporioides* BCRC 35178, and *Neopestalotiopsis* sp. BCRC 35002.

In our research, we focused on antibacterial, handicraft, and painting applications of the crude pigment extracts obtained from the selected actinobacterial strains after polyphasic identification. From a research article survey on handicraft and painting application of the actinobacterial pigments, the former two publications were reported by our research group (Urtgam *et al.* 2023, 2024). This research is the third report to indicate the biotechnological utilization of the actinobacterial pigment, especially the Streptomyces pigments, for handicraft and painting.

2. Materials and Methods

2.1. Isolation of Pigmented-Producing Actinobacteria from Soils

The pigmented-producing actinobacteria were isolated from soil samples collected in Phitsanulok and Phichit provinces, Thailand, followed by the protocol as described by Urtgam *et al.* (2024). Soil suspension was prepared by dissolving 10 g of soil in 90 mL of sterile distilled water. The 10-fold dilution of soil suspension was serially prepared and spread onto starch casein agar (SCA) [10 g/L soluble starch, 0.3 g/L casein, 2.0 g/L KNO_3, 2.0 g/L NaCl, 2.0 g/L K_2HPO_4, 0.05 g/L $MgSO_4 \cdot 7H_2O$, 0.02 g/L $CaCO_3$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 20 g/L agar, and 1 L distilled water]. The experimental plates were incubated at 30°C for 5-7 days. Colonies of actinobacteria found on the surface of SCA were selected and purified by the streaking technique.

2.2. Extraction of the Crude Pigments from Actinobacterial Cells

The solid-state fermentation processing was chosen for cultivation of the pigmented-producing actinobacteria, followed by the protocol of Urtgam *et al.* (2024). The actinobacterial strains were cultivated on sterile broken-milled rice and incubated at 30°C for 5-7 days before pigment extraction. The actinobacterial cells were extracted, and the crude pigment extracts were obtained by the method of Urtgam *et al.* (2024). The protocol was described briefly as follows: 100 mL of ethyl acetate was added to the pigmented-producing actinobacteria grown on the broken-milled rice, and statically incubated for 48 h before solvent evaporation. The crude pigmented extracts obtained from the previous steps were continuously developed for antibacterial activity testing and also used as ingredients of handicrafts and painting colors.

2.3. Determination of Antibacterial and Antifungal Activities of the Actinobacterial Crude Pigment Extracts

2.3.1. Testing of Antibacterial Activity of the Actinobacterial Crude Pigment Extracts

The antibacterial activities of the crude pigment extracts obtained from the actinobacterial strains were performed by the agar-well method mentioned by Urtgam *et al.* (2024). The bacterial testers were *Escherichia coli* PSRU-01 and *Staphylococcus aureus* PSRU-01. The tested 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 the nutrient broth (NB) and incubated on a rotary shaker at 120 rpm, 37 °C for 24 h. The bacterial cultures were measured to be equivalent to McFarland No.0.5 (1.5×10^8 CFU/mL). The score was 0.08-0.1, as determined by a spectrophotometer at 625 nm, before testing the antibacterial activities. The bacterial testers were swabbed on Muller-Hinton Agar (MHA). The crude pigment extract, previously prepared, was filled into the hole made on MHA at a final concentration of 50 mg/mL, diluted with Dimethyl Sulfoxide (DMSO) solution. The positive control was 50 mg/mL of ampicillin, 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 pigment extract and the control sets.

2.3.2. Testing of Antifungal Activity of Actinobacterial Crude Pigment Extracts

The poisoned food technique was applied to test the antifungal activities presented in the actinobacterial crude pigments extract. The fungal testers were *Colletotrichum* sp. NPJM -01 and *Fusarium* sp. PSRU-01. They were cultivated on PDA and incubated at room temperature for 3-5 days until the fungal growth was detected. Therefore, 5 mm of mycelial disc of the fungal testers was prepared by cork borer and transferred onto PDA supplemented with 50 mg/mL of the actinobacterial crude pigments extract. The experimental plates were incubated at room temperature for 5 days prior to determining the antifungal activity. All treatments were done in triplicate. The percentage of fungal inhibition by the actinobacterial crude pigments extract was calculated with the formula described by Urtgam *et al.* (2024).

2.4. Identification of Pigmented-Producing Actinobacterial Strains

Actinobacterial identification was carried out on the basis of a polyphasic taxonomic approach. Phylogenetic identification was firstly applied on the basis of partial 16S rDNA sequence comparison. The actinobacterial DNA was extracted with BioFact™ 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 & PCR Purification System (Biofactory, Korea). The partial 16S rDNA sequences of actinobacterial strains were analysed by standard sequencing described by Bionics (Korea). Phylogenetic analysis was determined by the Neighbor-joining method with MEGA11 software (Tamura *et al.* 2021). Morphological and cultural characteristics of the selected actinobacteria were studied by the standard method as described by Kämpfer (2012).

2.5. Application of the Crude Pigmented Extract of the Actinobacteria in Handicraft and Painting

The colors used in this study were acrylic, poster, and water colors obtained from the crude-pigmented extracts of the selected actinobacteria. The tested colors were prepared with a white color-based ingredient in a 95-99:1-5 ratio and applied to handicraft and painting. The qualification of these colors was evaluated. The measurements of color perception were evaluated using the Chromameter CR-400 from Konica Minolta in accordance with the CIELAB color space system.

3. Results

3.1. Isolation and Pigment Extraction of Pigmented-Producing Actinobacteria

A total of 21 actinobacterial strains were isolated from soil samples collected in Phitsanulok and Phichit provinces, North of Thailand. The Phitsanulok strains were named as B1-B12. The Phichit strains were coded to be F1-F9. The strains were pigment-producing actinobacteria cultivated on the broken-milled rice (Figure 1A). After pigment extraction by ethyl acetate, 4 strains, including B3 (yellow), B8 (violet), F4 (brown), and F9 (pink), were selected on the basis of the color tones (Figure 1B) that were possibly to be used in handicraft and painting applications.

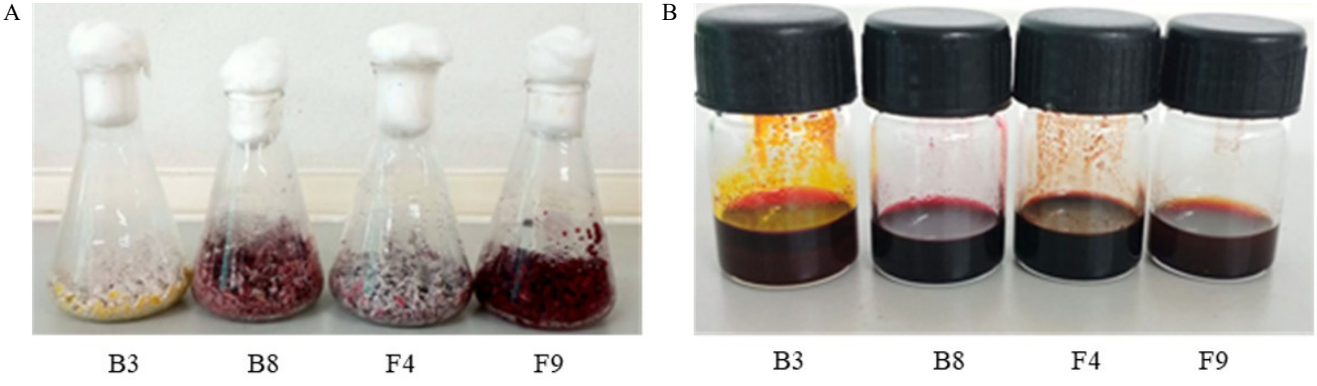

Figure 1. (A) The pigment-producing bacteria were cultivated on the broken-milled rice, and (B) the crude pigmented extract

3.2. Determination of the Antibacterial and Antifungal Activities of the Actinobacterial Crude Pigment Extracts

3.2.1. Testing of the Antibacterial Activity of the Actinobacterial Crude Pigment Extracts

The crude pigment extracts obtained from the 4 pigmented-producing actinobacterial strains, namely B3, B8, F4, and F9, were tested for antibacterial activities using *E. coli* PSRU-01 and *S. aureus* PSRU-01 as the bacterial testers. The crude pigment extracts of B3 and B8 could inhibit the growth of *E. coli* PSRU-01; however, the crude pigment extracts of F4 and F9 could not inhibit the growth of *E. coli* PSRU-01. The growth inhibition of *S. aureus* PSRU-01 was presented by the crude pigment extracts obtained from 4 strains. The results presented after 18 h of incubation are shown in Table 1 and Figure 2A.

Table 1. Antibacterial activity of the crude pigments extracts of the actinobacterial strain B3, B8, F4 and F9

Actinobacteria	Clear zone diameter (mean ± SD) (mm.)	
	Escherichia coli PSRU-01	*Staphylococcus aureus* PSRU-01
Positive control	29.53±0.47^{a}	43.53±1.43^{a}
Negative control	0	0
B3	2.7±0.62^{e}	21.20±0.20^{b}
B8	7.76±0.30^{b}	18.50±0.52^{cd}
F4	0	19.43±0.15^{c}
F9	0	17.63±0.50^{d}

Positive control: 50 mg/mL Ampicillin; negative control: DMSO, letter indicates statistical analysis value by Duncan's new multiple range test (DMRT) ($p<0.05$)

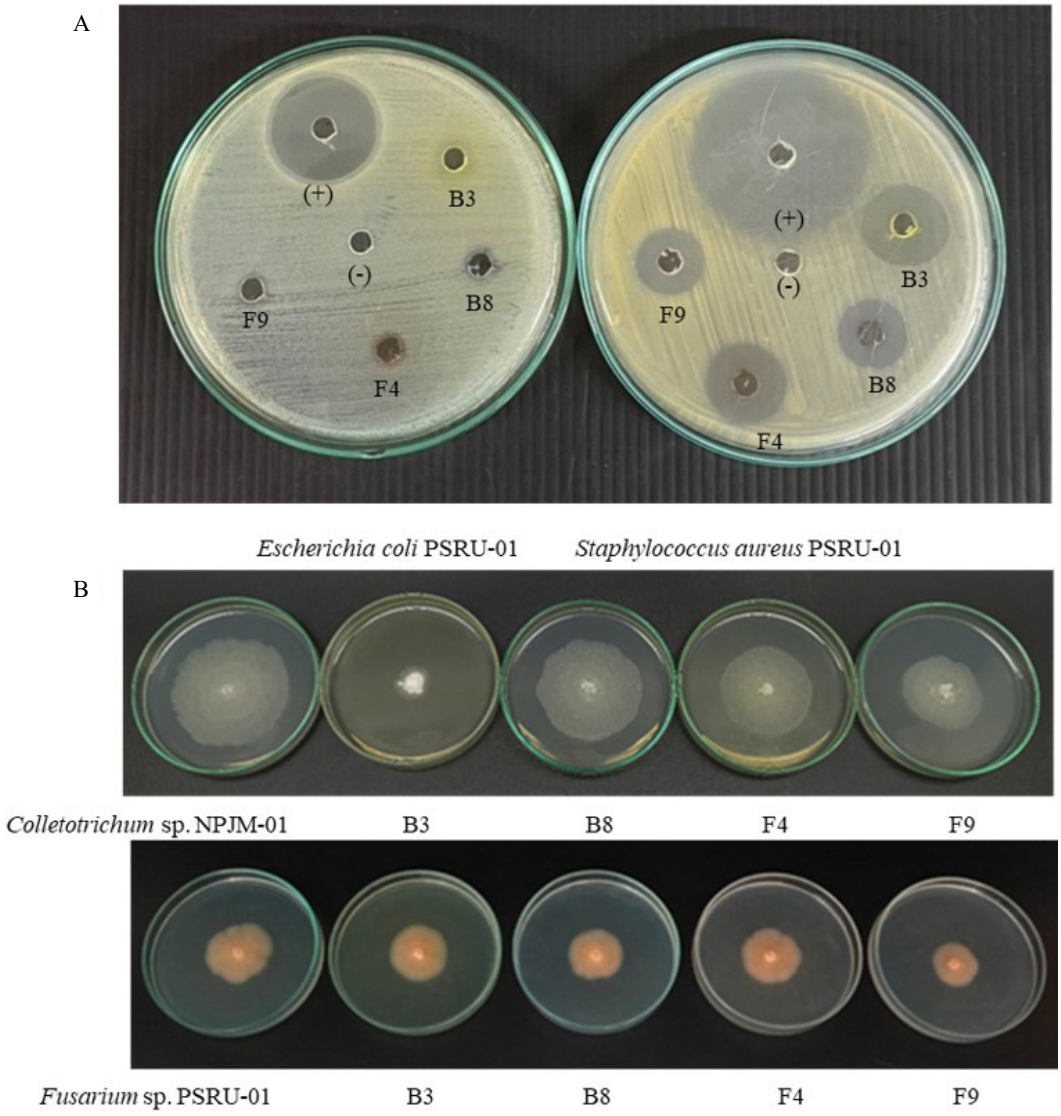

Figure 2. Inhibition activity of crude pigmented extracts of the 4 actinobacterial strains on (A) the bacterial growth and (B) the fungal growth of *Colletotrichum* sp. NPJM-01 and *Fusarium* sp. PSRU-01

3.2.2. Testing of Antifungal Activity of the Actinobacterial Crude Pigment Extracts

The crude pigment extracts of the 4 pigmented-producing actinobacterial strains, including B3, B8, F4, and F9, were determined for the antifungal activities using *Colletotrichum* sp. NPJM-01 and *Fusarium* sp. PSRU-01 as the fungal tester. The crude pigment extracts of could inhibit the growth of *Colletotrichum* sp. NPJM-01 and *Fusarium* sp. PSRU-01 after 5 days of incubation. These inhibitory effects depended on the actinobacterial strains used for the crude pigment extracts (Figure 2B and Table 2).

3.3. Identification of the Pigmented-Producing Actinobacterial Strains

Phylogenetic analysis was firstly applied for species identification on the basis of comparison of 16S rDNA sequences between the targeted genes of the actinobacterial strains, namely B3, B8, F4 and F9 and the same genes of all actinobacterial strains deposited in DNA Data Bank. The results indicated that 4 strains were placed into the Streptomyces genus supported with the similarity value of the phylogenetic genes used in this analysis that to be more than 98.65% (99.38-99.73%). They (B3, B8, F4 and F9) were identified to be *Streptomyces tendae* (accession no. NR025871.1) 99.73%, *Streptomyces muensis* (accession no. NR134820.1) 99.52%, *Streptomyces ardesiacus* (accession no. NR043486.1) 99.38% and *Streptomyces iakyrus* (accession no. NR041231.1) 99.59%, respectively. The phylogenetic tree shown in Figure 3 indicated a phylogenetic relationship between the actinobacterial strains (B3, B8, F4 and F9) and the closest actinobacterial species. The accession numbers of the partial 16S rDNA of B3, B8, F4 and F9 were PV867239, PV867246, PV872898 and PV873067, respectively. Morphological characteristics of 4 actinobacterial strains were determined for taxonomic delineation. The results presented that all strains were phenotypically shared with the Streptomyces genus. Cultural characteristics of actinobacterial strains were shown in the Figure 4.

Table 2. Antifungal activity of the crude pigment extract of the actinobacterial strains B3, B8, F4 and F9

Strains	Percentage of inhibition rate (%IRG) (mean ± SD)	
	Colletotrichum sp. NPJM-01	*Fusarium* sp. PSRU-01
B3	79.84±0.57^{a}	4.63±0.39^{c}
B8	14.17±0.15^{d}	9.04±0.08^{b}
F4	26.28±0.37^{c}	3.77±0.17^{c}
F9	36.68±1.27^{b}	23.03±0.25^{a}

Positive control: 50 mg/mL ampicillin; negative control: DMSO, letter indicates statistical analysis value by Duncan's new multiple range test (DMRT) ($p<0.05$)

3.4. Application of the Crude Pigmented Extract of Actinobacteria for Handicraft and Painting

A total of 4 actinobacterial strains were previously extracted to obtain crude pigment extracts for use as natural colors in handicraft and painting applications. The colors of crude pigment extracts of 4 strains were yellow (B3), violet (B8), brown (F4), and pink (F9).

For handicraft clay application, the increased color percentage of the crude pigment extracts was compared with the color tone of the crude pigment extracts at 1 day of extraction (Table 3, Figures 5 and 6). In the case of painting application, the increased color percentage of the crude pigment extracts was found after 30 days of extraction, depending on the actinobacterial strains used for preparation of the crude pigment extracts (Table 4, Figures 7 and 8).

The crude pigment extracts obtained from the selected actinobacteria strains had the potential for use as an alternative natural color in handicraft and painting (Tables 3 and 4 and Figures 5-8). The results indicated that most of the crude pigment extracts were not sensitive to light oxidation, as confirmed by the percentage of color reduction. It was noteworthy that the crude *Streptomyces* pigment extracts had the potential for handicraft and painting applications.

4. Discussion

Actinobacteria are a ubiquitous, heterogeneous group of Gram-positive members belonging to the class Actinobacteria that have distinct phenotypic and genotypic characteristics, especially filamentous and high GC content (Amsaveni *et al.* 2015; Udhayakumar *et al.* 2017; Tandale *et al.* 2018; Fernandes *et al.* 2021). They are distributed in many kinds of habitats, including terrestrial and aquatic ecosystems. However, soil is commonly found to have the diversity of actinobacteria, especially the pigmented-producing actinobacteria, which present antimicrobial activity (Selvameenal *et al.* 2009; Palanichamy *et al.* 2011;

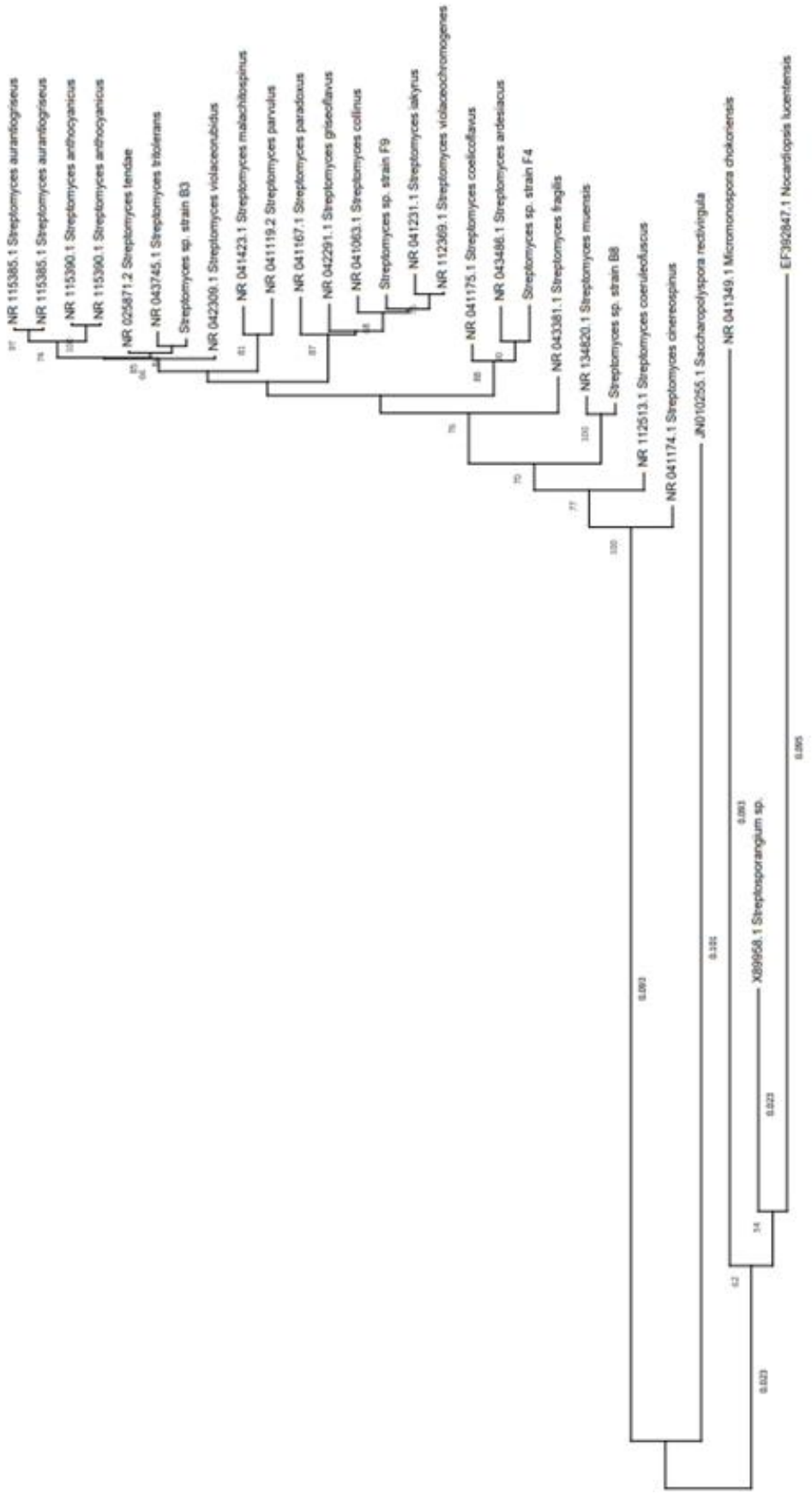

Figure 3. Phylogenetic tree indicated phylogenetic relationship between the actinobacterial strains (B3, B8, F4 and F9) and the closest actinobacterial species

Figure 4. Cultural characteristics of the actinobacterial strains, including B3, B8, F4 and F9

Table 3. Determination of the lightness and percentage of colour increase of the handicraft clays containing the crude pigments extract of actinobacterial B3, B8, F4 and F9 strains

Strains	Handicraft clay	Time	Score compared with CIELAB system			CIELAB color difference		
			L	a*	b*	ΔL	Δa*	Δb*
B3	yellow	1 day	79.60	7.86	42.41	-4.76	1.47	1.21
		30 days	74.84	9.33	43.62			
		% color increase	5.98	-	-	-	-	
B8	violet	1 day	80.84	10.42	4.13	-10.32	4.20	0.10
		30 days	70.52	14.62	4.23			
		% color increase	12.77	-	-	-	-	-
F4	brown	1 day	73.50	9.35	16.24	-5.49	0.70	-0.22
		30 days	68.01	10.05	16.02			
		% color increase	7.47	-	-	-	-	
F9	pink	1 day	79.70	8.09	12.10	-5.41	2.97	1.76
		30 days	74.29	11.06	13.86			
		% color increase	6.79	-	-	-	-	-

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 (−)

Radhakrishnan *et al.* 2016; Assia *et al.* 2018; Tang *et al.* 2019; Sapkota *et al.* 2020; Kazi *et al.* 2022; Wan *et al.* 2024). Similarly, certain strains of the pigmented-producing actinobacteria identified on the basis of polyphasic characteristics were collected from soil samples taken from many fields in Phitsanulok and Pichit provinces.

Based on the pigment color produced by the actinobacterial strains in this study, we selected four strains —B3 (yellow), B8 (violet), F4 (brown), and F9 (pink)— for the next step. To identify the selected strains, a comparative analysis of partial 16S rDNA sequences was applied. Their partial gene sequences shared a similarity to the same genes of several *Streptomyces* species deposited in the DNA Data Bank of Japan and GenBank. The results indicated that four strains were placed into the *Streptomyces* genus, supported by the similarity value of the phylogenetic genes used in this analysis, which was more than 98.65% (99.38-99.73%) (Nammali *et al.* 2021; Tunvongvinis *et al.* 2024). They (B3, B8, F4, and F9) were identified to be *Streptomyces tendae* 99.73%, *Streptomyces muensis* 99.52%, *Streptomyces ardesiacus* 99.38% and *Streptomyces iakyrus* 99.59%, respectively. The strains shared the similarity of 16S rDNA sequences, more than 98.7 % recognized to be conspecific (Schleifer 2009).

The research articles mentioned bioactivity of pigmented extracts from several species and strains of the pigmented-producing *Streptomyces*, especially antibacterial activity, were reported (Ibrahim *et al.* 2023; El-Zawawy *et al.* 2024). In this study, the crude pigment extracts of 4 selected *Streptomyces* strains exhibited antibacterial activity against *S. aureus* PSRU-01; however, these activities were less than those of the positive control. The crude pigment extracts obtained from B3 and B8 strains were tested for antibacterial

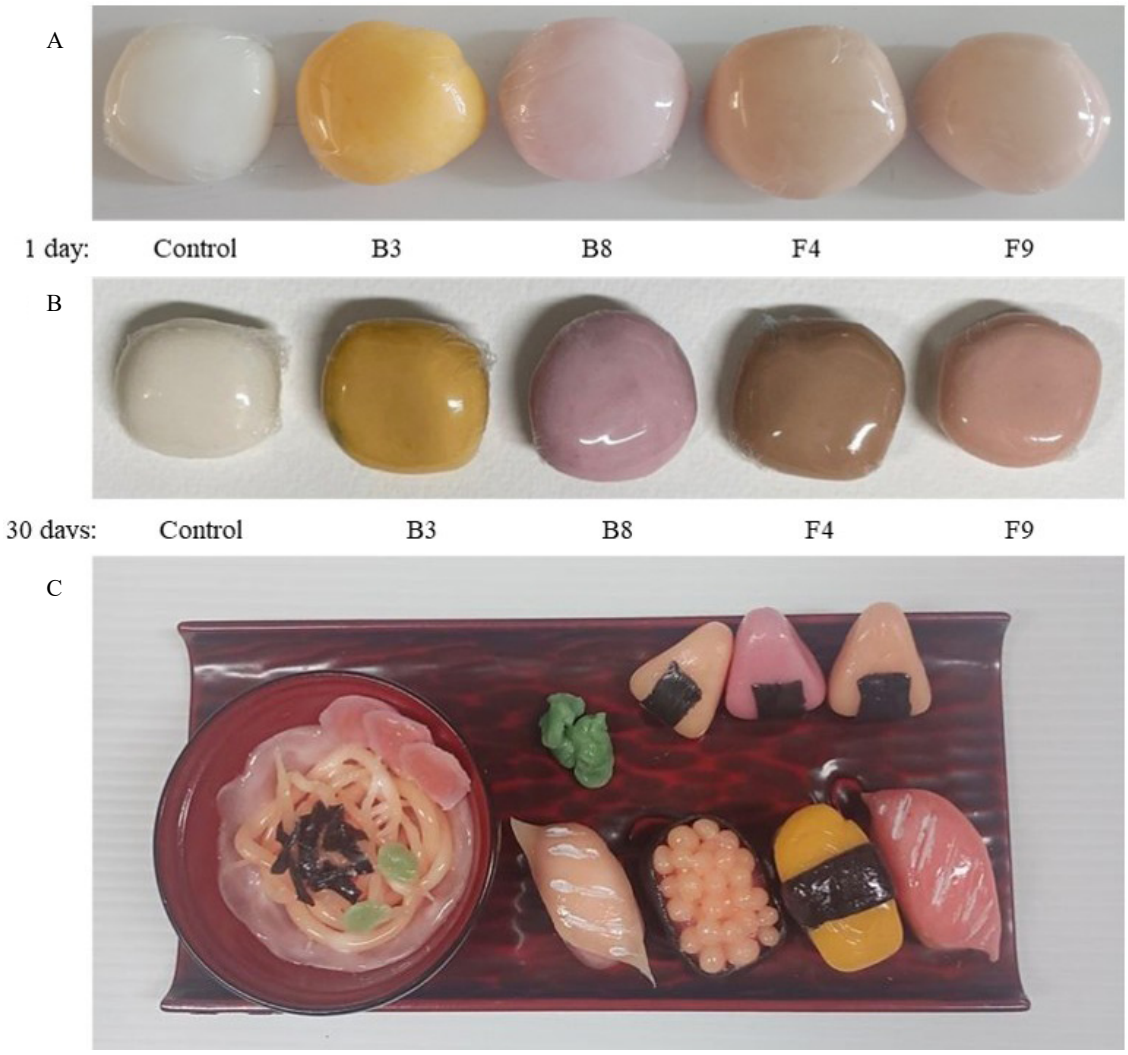

Figure 5. Handicraft clays containing the crude pigments extract of actinobacteria B3, B8, F4 and F9 strains; static condition for 1 day (A), and 30 days (B), and (C) the application of handicraft clays for model of traditional Japanese foods

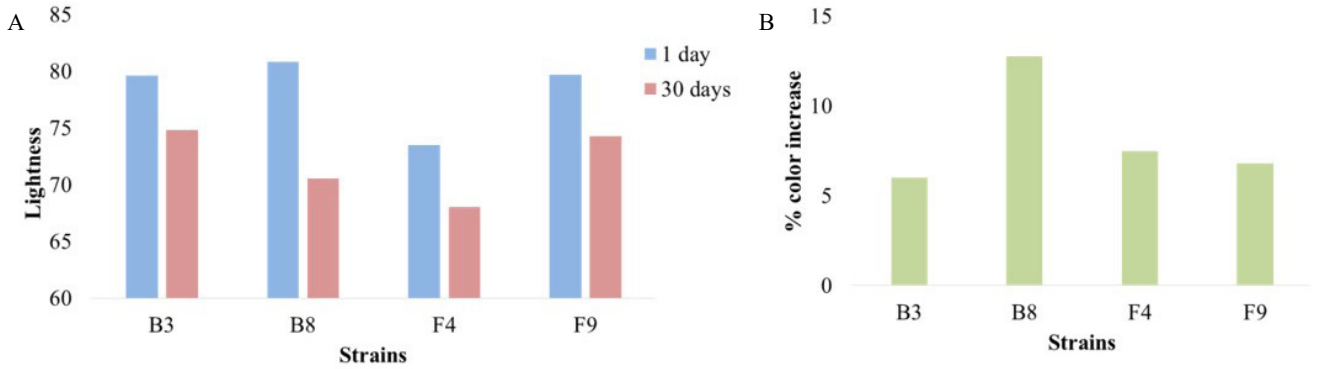

Figure 6. Lightness (A) and percentage of colour increase (B) of the handicraft clays containing the crude pigments extract of actinobacterial B3, B8, F4 and F9 strains

Table 4. Determination of the lightness and percentage of colour increase of the acrylic, poster and water colours containing the crude pigments extract of actinobacterial B3, B8, F4 and F9 strains

Color	Strains	Time	Score compared with CIELAB system			CIELAB color difference		
			L	a*	b*	ΔL	Δa*	Δb*
Acrylic	B3 (yellow)	1 day	85.65	-3.61	31.23	0.42	-1.25	-7.42
		30 days	86.07	-2.36	23.81			
		% color increase	0.49					
	B8 (violet)	1 day	82.61	-0.65	-6.44	-1.83	0.67	-1.56
		30 days	80.78	0.02	-4.88			
		% color increase	2.22					
	F4 (brown)	1 day	82.71	4.55	5.19	-2.11	-1.25	-1.50
		30 days	80.60	3.30	3.69			
		% color increase	2.65					
	F9 (pink)	1 day	85.57	6.26	4.38	-1.00	0.86	-1.05
		30 days	84.57	7.12	3.33			
		% color increase	1.27					
Poster	B3 (yellow)	1 day	84.41	-0.36	38.54	-0.18	-0.83	-11.41
		30 days	84.23	-1.19	27.13			
		% color increase	0.21					
	B8 (violet)	1 day	73.78	-1.56	-6.07	-1.02	-1.00	-0.56
		30 days	72.76	-2.56	-6.63			
		% color increase	1.48					
	F4 (brown)	1 day	73.44	5.06	4.47	0.98	-1.19	-1.26
		30 days	74.42	3.87	3.21			
		% color increase	1.33					
	F9 (pink)	1 day	82.12	5.98	5.35	-0.09	0.44	-0.77
		30 days	82.03	6.42	4.58			
		% color increase	0.11					
Water	B3 (yellow)	1 day	81.15	2.34	22.26	1.84	-1.40	0.16
		30 days	82.99	0.94	22.42			
		% color increase	2.37					
	B8 (violet)	1 day	77.02	6.25	1.76	-2.74	-1.87	-1.42
		30 days	74.28	4.38	0.34			
		% color increase	3.66					
	F4 (brown)	1 day	68.17	8.31	8.85	-5.95	0.28	0.29
		30 days	62.22	8.59	9.14			
		% color increase	8.73					
	F9 (pink)	1 day	79.22	6.76	5.80	0.12	-0.29	-0.01
		30 days	79.34	6.47	5.79			
		% color increase	0.15					

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 (−)

activity against *E. coli* PSRU-01, and the results were positive. Conversely, the crude pigment extracts obtained from F4 and F9 had negative results because the *E. coli* PSRU-01 growth was not inhibited. These results indicated that the cell wall structure between Gram-positive and Gram-negative bacteria, the chemical characteristics of the *Streptomyces* pigments, the methodology for crude pigment extraction and the chemical solvents used in the extraction process were the main factors affecting on the bacterial activity of the crude pigment extracts obtained from each strain of *Streptomyces* as same as the data reported by several research groups (Laidi *et al.* 2006; Ramesh *et al.* 2020; Meng-xi *et al.* 2021; Hemeda *et al.* 2022; Kazi *et al.* 2022; El-Zawawy *et al.* 2024; Sibero *et al.* 2024)

The crude pigment extract of 4 *Streptomyces* strains had antifungal activity against *Colletotrichum* sp. NPJM-01 and *Fusarium* sp. PSRU-01 was used

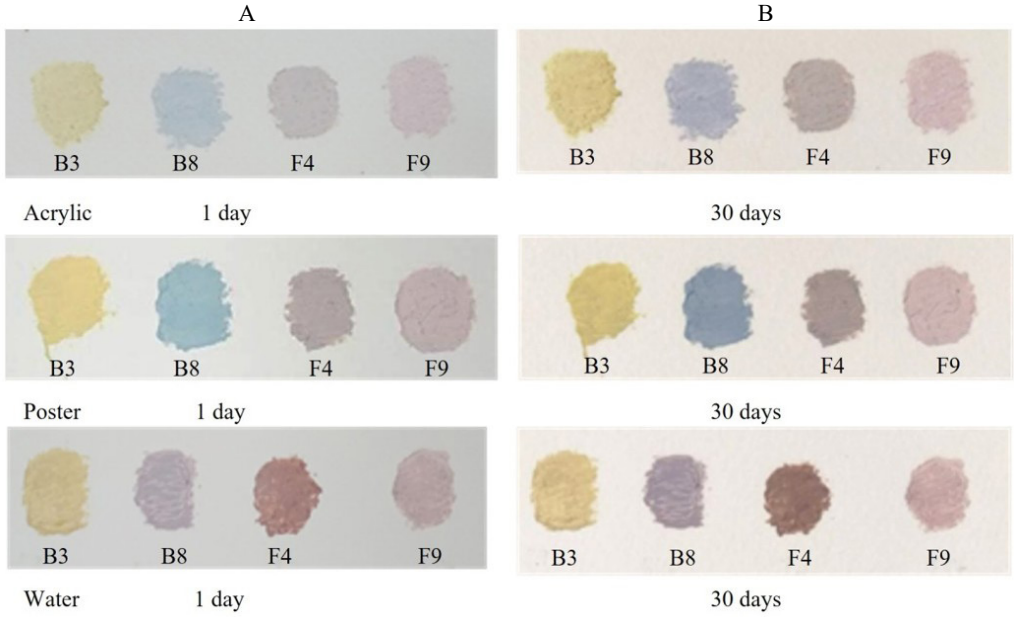

Figure 7. Application of acrylic, poster and water colours containing the crude pigments extract of actinobacterial B3, B8, F4 and F9 strains on paper; static condition for 1 day (A), and 30 days (B)

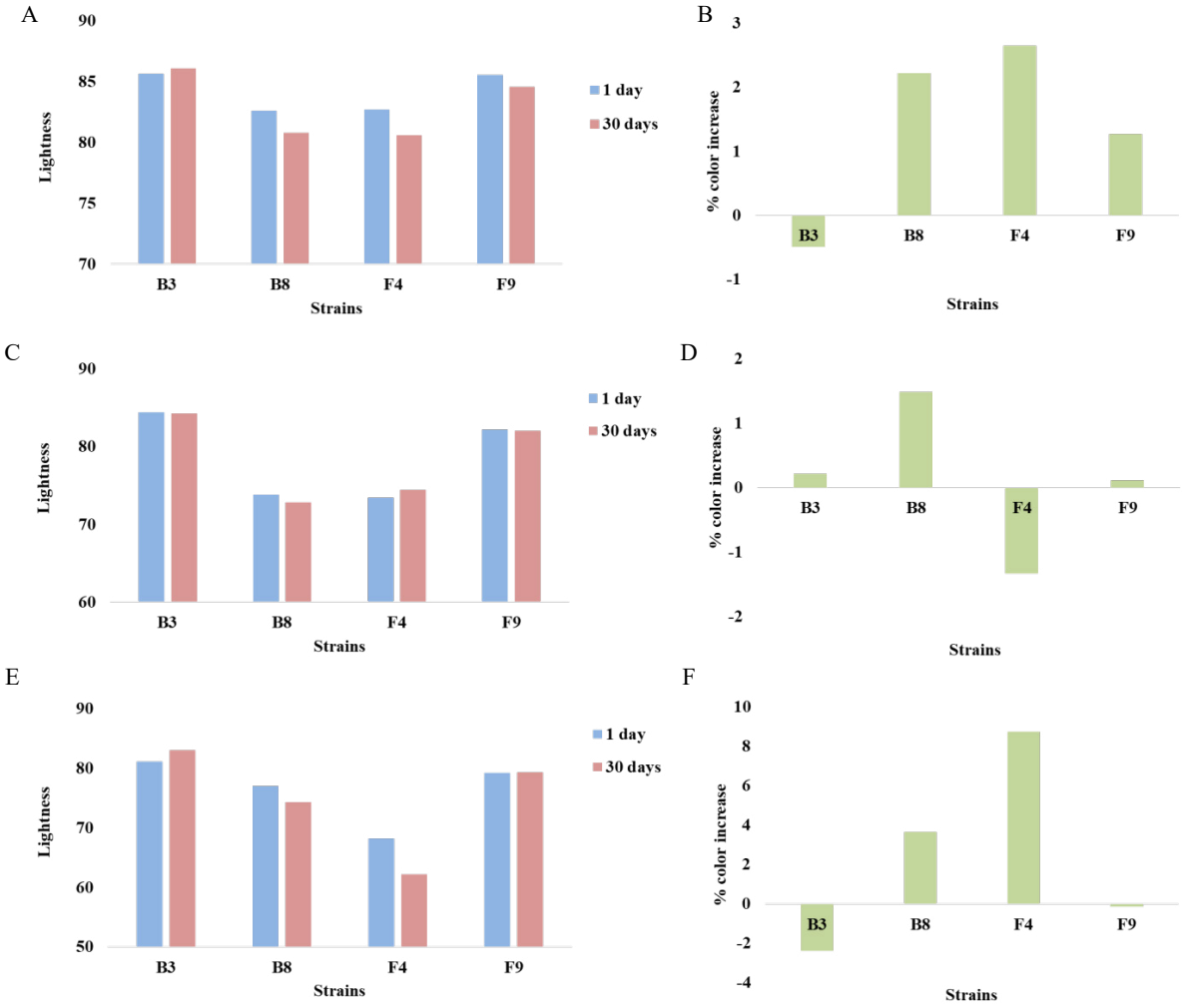

Figure 8. Lightness of the acrylic (A), poster (C) and water (E) colours and percentage of colour increase of the acrylic (B), poster (D) and water (F) colours containing the crude pigments extract of actinobacterial B3, B8, F4 and F9 strains

as the fungal tester. The growth of *Colletotrichum* sp. NPJM-01 was more inhibited than that of *Fusarium* sp. PSRU-01. As with the antibacterial activity, the cell wall structure of these fungal species, the chemical characteristics of the *Streptomyces* pigments, the methodology for crude pigment extraction, and the chemical solvents used in the extraction process were the main factors affecting the antifungal activity of the crude pigment extracts obtained from each strain of *Streptomyces*. Similar results were reported (Kavitha & Vijayalakshmi 2011; Al-Askar *et al.* 2013; Boukaew *et al.* 2021; Zou *et al.* 2021; Qi *et al.* 2022; Rejón-Martínez *et al.* 2022).

The crude pigment extracts obtained from strain B3, B8, F4, and F9 had the potential for use as an alternative natural color in handicraft and painting applications. The crude pigment extracts were resistant to light oxidation, as indicated by the percentage of color reduction. Controversy, the crude pigment extracts from the actinobacterial strain C7, C13, and D13 were sensitive to light oxidation (Urtgam *et al.* 2024). It was noteworthy that the crude Streptomyces pigment extracts had the potential for handicraft and painting applications.

As mentioned above, we are the pioneers for the application of actinobacterial pigments, especially Streptomyces pigments, in art and folk handicraft, and painting (Urtgam *et al.* 2023*,* 2024). Use of the *Streptomyces* pigments as natural dye was beneficial to solve the problems of application of chemical or synthetic dye in industrial and other fields, including toxicity to living organisms belonging to the Domain Archaea, Bacteria, and Eucarya, and environmental pollution (Konstadakopulos 2008; Ajò *et al.* 2019; Murcia Mesa *et al.* 2021; Yadav *et al.* 2022). Furthermore, the utilization of synthetic dyes may lead our country to spend a lot of money on these colours, contributing to a trade deficit (Gilbert & Cooke 2001; Ferreira *et al.* 2004; Hagan & Poulin 2021). The *Streptomyces* pigments are promising choices for handicraft SMEs in Thailand and other co-cultural countries, offering significant benefits for eco-friendliness and planet responsibility, as outlined in the BCG concept (Indrayani & Triwiswara 2020; Nurcahyanti *et al.* 2021; Yadav *et al.* 2022).

In conclusion, the soil actinobacteria isolated in this study belonged to the *Streptomyces* genus. They are phylogenetically close to certain species of *Streptomyces*, including *Streptomyces tendae* 99.73% (Strain B3), *Streptomyces muensis* 99.52% (Strain B8), *Streptomyces ardesiacus* 99.38% (Strain F4), and *Streptomyces iakyrus* 99.59% (Strain F9), on the basis of the partial 16S rDNA sequence comparison. Owing to their crude extracted pigments having antimicrobial activity, the selected actinobacteria could be used as sources of alternative and functional pigments that can be used as eco-friendly, biodegradable, and natural pigments for several biotechnological applications, including handicraft and painting. We intend to do further research focused on the enhancement of the stability of actinobacterial pigments applied for handicraft and painting, such as evaluation of mordants suitable for dyeing processing method incorporated with the crude pigment extracts for the development of alternative actinobacterial pigments used for the art subjects.

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