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Unraveling growth-promoting potential of plant beneficial actinobacteria on tropical bryophytes

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ABSTRACT

Bryophytes are non-vascular plants with dominant gametophyte stage that play vital ecological roles in natural ecosystems. Unfortunately, their populations are currently in decline due to habitat destruction and various anthropogenic activities. The conservation efforts for bryophytes are hampered by their slow growth rates. This study aims to investigate the potential of actinobacteria to promote the growth of bryophytes. In this study, three plant growth-promoting actinobacteria, *Dermacoccus abyssi* MT1.1^{T}, *Micromonospora chalcea* CMU55-4 and *Streptomyces thermocarboxydus* S3 were cultured in International *Streptomyces* Project medium 2 (ISP2) broth to obtain culture filtrates containing bioactive compounds for enhancing the growth of two bryophyte species, *Physcomotrium sphaericum* (C. Ludw.) Fürnr and *Sphagnum cuspidatulum* C. Müll. Interestingly, the incorporation of actinobacterial culture filtrates into 1/16 Murashige and Skoog (MS) medium yielded superior growth performance of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll, as observed from the thallus height, fresh weight, total chlorophyll contents, and total carotenoid contents compared to control groups. In addition, the inoculation of *M. chalcea* CMU55-4 on *S. cuspidatulum* C. Müll grown in sterile peat moss demonstrated the highest values for thallus height, fresh weight, dry weight, total chlorophyll content, and total carotenoid content. All actinobacteria successfully colonized the moss seedlings without any observable negative impacts, indicating beneficial interactions between actinobacteria and bryophytes. This research sheds light on the potential of harnessing plant beneficial actinobacteria to enhance the growth of bryophytes for conservation purposes.

1. Introduction

Actinobacteria, a large group of Gram-positive bacteria, are well-known for their remarkable ability to produce bioactive compounds and are widely present in both terrestrial and aquatic environments (Barka et al., 2016). Actinobacteria have been demonstrated to establish beneficial associations with various plant species, including bryophytes, acting as endophytes within the plant tissues without causing any harmful effects to their host plants (Rosenblueth and Martínez-Romero, 2006; Insuk et al., 2020; 2022). These endophytic actinobacteria are prolific producers of a diverse range of secondary metabolites that find applications in agriculture, industrial processes, and pharmaceutical biotechnology (Ganapathy and Sivakumar, 2018; Singh et al., 2022). Members of the actinobacteria recognized as plant growth-promoting rhizobacteria (PGPR) confer advantages to plants by synthesizing phytohormones that promote plant growth and by protecting them against phytopathogens through the production of antibiotics and siderophores (Ayswaria et al., 2020; Shurigin et al., 2022; Al-Quwaie, 2023). Several publications demonstrate the potential of actinobacteria to stimulate the growth of plants (Lasudee et al., 2018; Rangseekaew et al., 2022; Nazari et al., 2023; Das et al., 2024). However, the majority of these studies primarily focused on higher plants. Our research group recently reported the isolation of actinobacteria associated with bryophytes and their surrounding environments (Insuk et al., 2020; 2022). These actinobacteria demonstrated the ability to promote moss growth and produce bioactive compounds that possess inhibitory properties against plant pathogens. This observation is attributed to the release of various bioactive metabolites, including indole-3-acetic acid (IAA), an auxin hormone that plays a pivotal role in the proliferation, morphogenesis, and cellular differentiation of plants (Emenecker and Strader, 2020).

Bryophytes, encompassing mosses, liverworts, and hornworts, represent non-vascular plants that thrive in diverse ecosystems such as

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forests, wetlands, and alpine regions (Sukkharak and Chantanaorrapint, 2014). These plants play critical ecological roles in soil stabilization, erosion prevention, water flow regulation, and maintenance of soil moisture (Rousk et al., 2017; Zuijlen et al., 2020; Yuqing et al., 2021). Despite their ecological significance, bryophytes have received comparatively less attention than vascular plants in terms of conservation efforts. Their populations are declining primarily due to habitat destruction (Pykälä, 2019). For example, most *Sphagnum* species are widespread and typically found in wetlands in Europe and North America, but they are less common in tropical regions (Rochefort, 2000; Sundberg, 2000). A checklist of bryophytes in Thailand documented eleven *Sphagnum* species from 1911 to 1977. In Thailand, *Sphagnum* is primarily confined to the highlands of the northern and northeastern areas. *Sphagnum cuspidatulum* C. Müll was only found in the Ang Ka area of Doi Inthanon National Park, Thailand. A significant decline in their local abundance has been reported (Sitthichoptham et al., 2023). Furthermore, bryophytes exhibit slow growth rates, and there is a lack of research focused on their growth enhancement. Consequently, this study aims to use plant growth-promoting actinobacteria to promote the growth of bryophytes.

2. Material and methods

2.1. Microorganisms and plant species

Three actinobacteria, namely *Streptomyces thermocarboxydus* S3, *Dermacoccus abyssi* MT1.1^T, and *Micromonospora chalcea* CMU55-4, were previously isolated from distinct sources: arbuscular mycorrhizal spore (Lasudee et al., 2018), the Mariana Trench sediment (Pathom-aree et al., 2006), and moss [*Campylopus involutus* (Müll. Hal.)] (Insuk et al., 2020). They have been demonstrated as potential plant growth-promoting actinobacteria and were selected for this study. *S. thermocarboxydus* S3 is capable of producing ACC deaminase, IAA, siderophores, solubilizing phosphate, and promoting rice seedling growth under drought conditions. *D. abyssi* MT1.1^T is a salt-tolerant strain that produces IAA, siderophores, and solubilizes phosphate. *M. chalcea* CMU55-4 produces IAA and siderophores and is a bryophyte growth-promoting strain. All actinobacteria were cultured in International *Streptomyces* Project medium 2 (ISP2) broth (Shirling and Gottlieb, 1966) supplemented with 2 mg/ml of L-tryptophan. The cultures were incubated in dark condition at room temperature on a shaker at 140 rpm for 7 days. Subsequently, the culture broths were centrifuged at 7000 rpm and 4 °C for 10 min to collect the supernatants.

Physcomitrium sphaericum (C. Ludw.) Fürnr and *Sphagnum cuspidatulum* C. Müll were obtained from Dr. Narin Printarakul, Department of Biology, Chiang Mai University. For *S. cuspidatulum* C. Müll, shoot tips (0.5 cm) were carefully excised and thoroughly washed with running tap water for 5 minutes. Subsequently, the shoot tips were surface sterilized using 1 % (v/v) sodium hypochlorite solution for 2 minutes. Following the sterilization process, the shoot tips were washed 4 times with sterile distilled water before being placed onto 1/16 Murashige and Skoog (MS) agar (Murashige and Skoog, 1962). The plant samples were incubated in an incubator under controlled temperature (22 ± 2 °C), with a light intensity of 35,000–40,000 lux (16 h light/8 h dark) for 3 months. For *P. sphaericum* (C. Ludw.) Fürnr, spores were aseptically transferred to 1/16 MS agar using sterile forceps and subsequently incubated in an incubator under controlled temperature (22 ± 2 °C), with a light intensity of 35,000–40,000 lux (16 h light/8 h dark) for 3 months to promote protonema formation. Each obtained protonema was transferred to a new tissue culture bottle containing 1/16 MS agar and incubated under the same conditions for 3 months, thereby facilitating the development of moss plantlets for further experiments.

2.2. Effect of 1-naphthaleneacetic acid (NAA) on growth of *Physcomitrium sphaericum* (C. Ludw.) Fürnr and *Sphagnum cuspidatulum* C. Müll

The 3-month-old plantlets of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll were aseptically transferred onto 30 ml of 1/16 MS agar in tissue culture bottles, each containing different concentrations of the chemical growth regulator NAA. The treatments comprised: (1) control (1/16 MS), (2) 0.5 mg/l NAA, and (3) 5 mg/l NAA. Subsequently, the plantlets were incubated in a controlled-temperature incubator (22 ± 2 °C), with a light intensity of 35,000–40,000 lux (16 h light/8 h dark) for 3 months. The experiment was conducted in three replicates. On Day 90, the following parameters were measured: thallus height, fresh weight, total chlorophyll content, and total carotenoid content (Arnon, 1949).

2.3. Effect of culture filtrate on growth of *Physcomitrium sphaericum* (C. ludw.) Fürnr and *Sphagnum cuspidatulum* C. müll

The 3-month-old plantlets of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll were aseptically transferred onto 30 ml of 1/16 MS agar in tissue culture bottles, with each bottle supplemented with 1 ml of cell free supernatant of each actinobacteria. The following treatments were prepared: (1) control 1 (1/16 MS), (2) control 2 (ISP2 broth), (3) supernatant of *S. thermocarboxydus S3*, (4) supernatant of *D. abyssi* MT1.1^T, and (5) supernatant of *M. chalcea* CMU55-4. Subsequently, the plantlets were incubated in a controlled-temperature incubator (22 ± 2 °C), with a light intensity of 35,000–40,000 lux (16 h light/8 h dark) for 3 months. On day 90, the following parameters were measured: thallus height, fresh weight, total chlorophyll content, and total carotenoid content (Arnon, 1949). The experiment was conducted in three replicates.

2.4. Effect of actinobacteria inoculation on growth of *Sphagnum cuspidatulum* C. müll

S. cuspidatulum C. Müll was selected as the representative bryophyte to investigate the impacts of actinobacteria inoculation on bryophyte growth. The three-month-old moss plantlets were transferred from an agar-based growth medium to sterile peat moss. Cell suspensions (10^7 to 10^8 CFU/ml) were prepared from 7-day-old cultures of actinobacteria in ISP2 broth using sterile distilled water. The experimental treatments consisted of: (1) control (Sterile distilled water), (2) cell suspension of *S. thermocarboxydus* isolate S3, (3) cell suspension of *D. abyssi* MT1.1^T, and (4) cell suspension of *M. chalcea* CMU55-4. Each treatment had three replicates. A 1 ml aliquot of each actinobacterial cell suspension was inoculated around the base of the plantlets. Subsequently, the plantlets were incubated in a controlled-temperature incubator (22 ± 2 °C), under a light intensity of 35,000–40,000 lux (16 h light/8 h dark) for 3 months. On day 90, the following parameters were measured: thallus height, fresh weight, dry weight, total chlorophyll content, and total carotenoid content (Arnon, 1949). Dry weight was determined after a 7-day drying period in a 60 °C oven.

2.5. Colonization study

At the end of experiment (3 months), the plantlets were carefully uprooted from the sterile peat moss and washed with sterile distilled water. The resulting wash water was serially diluted up to 10^{-6}, and 0.1 µl from the 10^{-4} to 10^{-6} dilution was spread onto ISP2 agar plates supplemented with 25 µg/ml each of nalidixic acid and ketoconazole. Simultaneously, one gram of plantlet was finely crushed in 1 ml of sterile distilled water. The suspension was subjected to a 10-fold serial dilution up to 10^{-6}, after which 0.1 µl from the 10^{-4} to 10^{-6} dilution was spread onto ISP2 agar plates supplemented with 25 µg/ml each of nalidixic acid and ketoconazole. The inoculated plates were incubated at room

temperature for 72 h. Colonies with morphology similar to the inoculated actinobacteria (*S. thermocarboxydus* isolate S3, *D. abyssi* MT1.1^T, and *M. chalcea* CMU55-4) were enumerated and isolated to obtain pure cultures. Confirmation of these actinobacteria was done by 16S rRNA gene sequence analysis using primers 27F and 1492R. The obtained sequences were compared with related sequences in the EzBioCloud database using the BLAST program (https://www.ezbiocloud.net/). A neighbor-joining phylogenetic tree was constructed using MEGA version 11 program (Tamura et al., 2021).

2.6. Statistical analysis

Analysis of variance (ANOVA) was conducted using SPSS 22.0 to evaluate the statistical significance of the data. Tukey's multiple range test was employed to detect any significant differences among the means, with a significance level of 0.05.

3. Results

3.1. Effect of 1-naphthaleneacetic acid (NAA) on growth of Physcomitrium sphaericum (C. Ludw.) Fürnr and Sphagnum cuspidatulum C. müll

The effect of NAA on the growth of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll is demonstrated in Table 1. *P. sphaericum* (C. Ludw.) Fürnr treated with 0.5 mg/l NAA exhibited significantly greater thallus height, fresh weight, total chlorophyll, and total carotenoid levels compared to the control. Conversely, *S. cuspidatulum* C. Müll treated with 0.5 mg/l NAA displayed significantly higher total chlorophyll levels than the control. Furthermore, both *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll treated with 5 mg/l NAA did not exhibit any significant differences in thallus height, fresh weight, total chlorophyll, and total carotenoid levels when compared to the control.

3.2. Effect of culture filtrate on growth of Physcomitrium sphaericum (C. ludw.) Fürnr and Sphagnum cuspidatulum C. Müll

The impact of the culture filtrate of actinobacteria on the growth of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll is presented in Table 2. Generally, the growth of both *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll improved when exposed to the culture filtrate from the three strains of actinobacteria compared to the non-treated controls. After a three-month experimental period, *P. sphaericum* (C. Ludw.) Fürnr treated with the culture filtrate of *M. chalcea* CMU55-4 and *D. abyssi* MT1.1^T exhibited significantly higher thallus height, fresh weight, total chlorophyll content, and total carotenoid content compared to the non-treated control. In contrast, *P. sphaericum* (C. Ludw.) Fürnr treated with the culture filtrate of *S. thermocarboxydus S3* did not exhibit any significant differences in fresh weight, total chlorophyll content, and total carotenoid content compared to the control group. Furthermore, *S. cuspidatulum* C. Müll treated with the culture filtrate of *D. abyssi* MT1.1^T demonstrated significantly higher thallus height, fresh weight, total chlorophyll, and total carotenoid levels compared to the non-treated group. However, *S. cuspidatulum* C. Müll treated with the culture filtrate of *S. thermocarboxydus* S3 and *M. chalcea* CMU55-4 did not exhibit any significant differences in thallus height compared to the non-treated group.

Table 1
Effect of NAA on growth of *Physcomitrium sphaericum* (C. Ludw.) Fürnr and *Sphagnum cuspidatulum* C. Müll.

Bryophyte species	Treatment	Thallus height (cm)	Fresh weight (g)	Total chlorophyll (mg/g)	Total carotenoid (mg/g)
P. sphaericum	Control	1.07 ^{a} ± 0.06	1.24 ^{a} ± 0.21	0.0404 ^{a} ± 0.01	0.0099 ^{a} ± 0.00
	NAA 0.5 mg/l	1.63 ^{b} ± 0.06	2.09 ^{b} ± 0.40	0.0651 ^{b} ± 0.01	0.0174 ^{b} ± 0.00
	NAA 5 mg/l	1.10 ^{a} ± 0.10	1.21 ^{a} ± 0.11	0.0300 ^{a} ± 0.00	0.0090 ^{a} ± 0.00
S. cuspidatulum	Control	1.30 ^{ab} ± 0.20	1.18 ^{a} ± 0.18	0.0511 ^{a} ± 0.01	0.0098 ^{a} ± 0.00
	NAA 0.5 mg/l	1.80 ^{b} ± 0.40	1.59 ^{a} ± 0.33	0.0749 ^{b} ± 0.01	0.0127 ^{a} ± 0.00
	NAA 5 mg/l	1.07 ^{a} ± 0.21	1.16 ^{a} ± 0.14	0.0367 ^{a} ± 0.00	0.0096 ^{a} ± 0.00

The data represent the mean values of three replicates ± SD. Different letters (a, ab, b) indicate a significant difference in NAA concentration according to Tukey at $p < 0.05$.

Table 2
Effect of culture filtrate on growth of *Physcomitrium sphaericum* (C. Ludw.) Fürnr and *Sphagnum cuspidatulum* C. Müll.

Bryophyte species	Treatment	Thallus height (cm)	Fresh weight (g)	Total chlorophyll (mg/g)	Total carotenoid (mg/g)
P. sphaericum	Control 1	1.07 ^{a} ± 0.06	1.24 ^{a} ± 0.21	0.0404 ^{a} ± 0.01	0.0099 ^{a} ± 0.00
	Control 2	1.67 ^{b} ± 0.29	1.76 ^{ab} ± 0.30	0.0553 ^{ab} ± 0.01	0.0143 ^{ab} ± 0.00
	S3	2.20 ^{c} ± 0.20	2.33 ^{bc} ± 0.35	0.0652 ^{ab} ± 0.02	0.0168 ^{bc} ± 0.00
	MT1.1	2.53 ^{c} ± 0.06	2.79 ^{c} ± 0.16	0.0801 ^{c} ± 0.01	0.0216 ^{c} ± 0.00
	CMU55-4	2.23 ^{c} ± 0.21	2.50 ^{c} ± 0.25	0.0761 ^{c} ± 0.01	0.0172 ^{bc} ± 0.00
S. cuspidatulum	Control 1	1.30 ^{a} ± 0.20	1.18 ^{a} ± 0.18	0.0511 ^{a} ± 0.01	0.0098 ^{a} ± 0.00
	Control 2	1.67 ^{ab} ± 0.21	1.41 ^{a} ± 0.28	0.0556 ^{ab} ± 0.00	0.0098 ^{a} ± 0.00
	S3	2.24 ^{abc} ± 0.25	1.73 ^{ab} ± 0.32	0.0733 ^{bc} ± 0.01	0.0152 ^{b} ± 0.00
	MT1.1	2.90 ^{c} ± 0.66	2.50 ^{b} ± 0.28	0.1114 ^{d} ± 0.01	0.0201 ^{b} ± 0.00
	CMU55-4	2.63 ^{bc} ± 0.32	2.32 ^{b} ± 0.43	0.0809 ^{c} ± 0.01	0.0153 ^{b} ± 0.00

The data represent the mean values of three replicates ± SD. Different letters (a, b, c, ab, abc, bc) indicate a significant difference in growth of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll according to Tukey at $p < 0.05$.

3.3. Effect of actinobacteria inoculation on growth of Sphagnum cuspidatulum C. Müll

Table 3 displays the results of *S. cuspidatulum* C. Müll plantlets inoculated with actinobacteria. In general, *S. cuspidatulum* C. Müll inoculated with actinobacteria exhibited higher values in all recorded parameters compared to the control (Table 3) and showed better growth appearance, as seen in Fig. 1. Notably, inoculation with *M. chalcea* CMU55-4 resulted in the highest values in thallus height, fresh weight,

Table 3
Effect of actinobacteria inoculation on growth of *Sphagnum cuspidatulum* C. Müll.

Treatment	Thallus height (cm)	Fresh weight (g)	Dry weight (g)	Total chlorophyll (mg/g)	Total carotenoid (mg/g)
Control	3.73 ^{a} ± 0.25	1.78 ^{a}±0.56	0.13 ^{a} ± 0.02	0.0669 ^{a} ± 0.01	0.0051 ^{a} ± 0.00
S3	4.17 ^{a} ± 0.29	2.03 ^{ab}±0.76	0.14 ^{a} ± 0.04	0.0751 ^{a} ± 0.01	0.0081 ^{b} ± 0.00
MT1.1	4.33 ^{a} ± 0.58	2.51 ^{ab}±0.58	0.17 ^{a} ± 0.02	0.1038 ^{a} ± 0.01	0.0106 ^{bc} ± 0.00
CMU55-4	4.50 ^{a} ± 0.50	3.53 ^{b}±0.72	0.17 ^{a} ± 0.04	0.1102 ^{a} ± 0.03	0.0109 ^{c} ± 0.00

The data represent the mean values of three replicates ± SD. Different letters (a, ab, b, bc, c) indicate a significant difference in growth *S. cuspidatulum* C. Müll according to Tukey at $p < 0.05$.

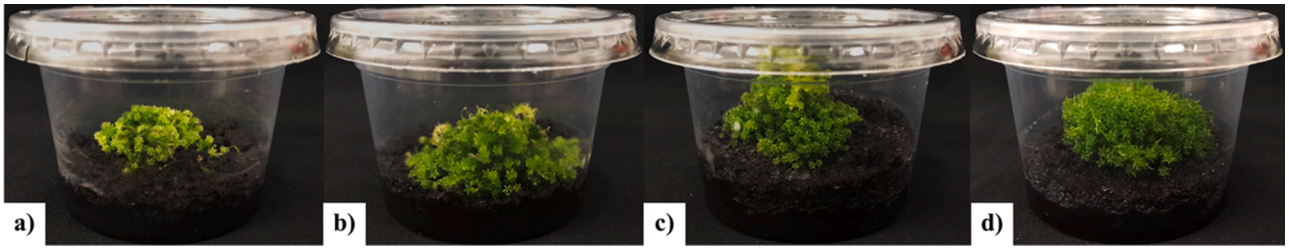

Fig. 1. Growth of *Sphagnum cuspidatulum* C. Müll inoculated with (a) Sterile distilled water (control) (b) *S. thermocarboxydus* isolate S3 (c) *D. abyssi* MT1.1^T (d) *M. chalcea* CMU55-4.

dry weight, total chlorophyll content, and total carotenoid content. However, these values were not statistically significant ($p < 0.05$), except for fresh weight and total carotenoid content.

3.4. Colonization study

All strains of actinobacteria were successfully re-isolated from inoculated *S. cuspidatulum* C. Müll plantlets. No actinobacterial colonies appeared on the control plate. Generally, the number of all actinobacteria decreased by approximately one log cycle from the initial cell count in both the plantlets and the washed water (Table 4). Analysis of 16S rRNA gene sequences of these re-isolated actinobacteria revealed that they were members of the genera *Dermacoccus, Micromonospora*, and *Streptomyces* (Table 5). Isolates M1 and S1 were identified as *D. abyssi* MT1.1^T with 100 % similarity. Isolates M2 and S2 shared 99.93 % similarity with *S. thermocarboxydus* DSM 44293^T. Isolate M3 and S3 exhibited a close relationship with *M. chalcea* DSM 43026^T (99.65 % similarity). Phylogenetic analysis based on nearly complete 16S rRNA gene sequence of the re-isolates is shown in Fig. 2. Isolates M1 and S1 formed a clade with *D. abyssi* MT1.1^T, with a bootstrap value of 62 %. Isolates M2 and S2 were placed in the *S. thermocarboxydus* clade, strongly supported by a bootstrap value of 87 %. Conversely, isolates M3 and S3 were positioned within the *M. chalcea* clade, supported by a 77 % bootstrap value.

4. Discussion

4.1. Effect of 1-naphthaleneacetic acid (NAA) on growth of Physcomitrium sphaericum (C. Ludw.) Fürnr and Sphagnum cuspidatulum C. Müll

This study aimed to examine the influence of NAA on the growth of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll. The results demonstrated that 0.5 mg/l NAA enhanced the thallus height, fresh weight, total chlorophyll content, and total carotenoid content of both bryophytes compared to the control. In contrast, 5 mg/l NAA did not improve the growth of the bryophytes (Table 1), indicating that this concentration exceeded the optimal level required for the growth of the tested bryophytes. Thelander et al. (2018) conducted a study to observe the influence of auxin on the development of *Funaria hygrometrica* moss in the gametophyte stage. Their findings indicated that lower concentrations of auxin stimulated shoot elongation and branching. Similarly, Klanrit et al. (2023) investigated the effects of NAA on the shoot proliferation of *Philodendron erubescens* (Pink Princess). The results revealed that 0.5 mg/l NAA stimulated shoot proliferation and increased the number of leaves compared to 1 mg/l NAA. In addition, Poniewozik et al. (2021) tested NAA at concentrations ranging from 0.5 to 5 mg/l and found that 0.5 mg/l NAA increased the width of rosettes and the length and width of leaves of *Paphiopedilum insigne*. Conversely, NAA at a concentration of 5 mg/l did not promote growth of *P. insigne*, similar to the control. It is evident from our findings and previous studies that NAA promotes the growth and development of various plant species, including bryophytes, particularly at low concentration (0.5 mg/l).

Table 4
A count of re-isolated actinobacteria from the *Sphagnum cuspidatulum* C. Müll.

Actinobacteria	Initial cell count (CFU/ml)	A count of re-isolated bacteria	
		Plantlets (CFU/g)	Washed water (CFU/ml)
S3	2.50×10^7	3.03×10^6	3.17×10^6
MT1.1	3.15×10^7	3.00×10^6	3.10×10^6
CMU55-4	3.50×10^7	3.30×10^6	3.50×10^5

4.2. Effect of culture filtrate on growth of Physcomitrium sphaericum (C. Ludw.) Fürnr and Sphagnum cuspidatulum C. Müll

The growth-promoting capacity of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll was assessed using the culture filtrate from three strains of actinobacteria. The results indicated that the culture filtrate of each actinobacterial strain promoted the growth of both bryophyte species compared to the control group (Table 2). Among them, the culture filtrate of *D. abyssi* MT1.1^T exhibited the highest efficacy in enhancing the thalli height, fresh weight, total chlorophyll content, and total carotenoid content of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll. A study by Rangseekaew et al. (2022) demonstrated the growth enhancement of tomato (*Solanum lycopersicum*) under salinity stress by *D. abyssi* MT1.1^T. Tomatoes inoculated with *D. abyssi* MT1.1^T displayed significantly greater shoot length and dry weight compared to the non-inoculated tomatoes subjected to salt stress. This observation showcases the positive impact of the culture filtrate of *D. abyssi* MT1.1^T on plant growth, which has not previously been documented in lower plants, though it is frequently observed in the context of higher plants.

The culture filtrate of *M. chalcea* CMU55-4 demonstrated increases in the thallus height, fresh weight, total chlorophyll content, and total carotenoid content of *P. sphaericum* (C. Ludw.) Fürnr and *S. cuspidatulum* C. Müll, ranking second only to *D. abyssi* MT1.1^T (Table 2). Della Mónica et al. (2018) examined the effects of endophytic actinobacteria isolated from ryegrass (*Lolium multiflorum*) by co-cultivating the ryegrass with three *Micromonospora* strains (SB3, TW2.1, and TW2.2). They observed that *Micromonospora* strain SB3 significantly increased ryegrass biomass, root length, and seedling survival rates upon transplantation to soil. These results indicate that *Micromonospora* is beneficial for plant growth, enhancing survival rates, and promoting growth under suitable conditions. *P. sphaericum* (C. Ludw.) Fürnr treated with the culture filtrate of *M. chalcea* CMU55-4 showed better growth than the control but less growth than *S. cuspidatulum* C. Müll treated with the same culture filtrate, particularly in terms of thallus height, total chlorophyll content, and total carotenoid content (Table 2). These findings align with research conducted by Insuk et al. (2020), where *P. sphaericum* (C. Ludw.) Fürnr inoculated with *M. chalcea* CMU55-4 for four weeks resulted in the production of new green leaves and the formation of reproductive structures known as capsules. Additionally, *M. chalcea* CMU55-4 increased the fresh weight, dry weight, and carotenoid content of *P. sphaericum* (C. Ludw.) Fürnr and helped the moss survive the transition from agar to soil.

Table 5
Identification of re-isolated actinobacteria from *Sphagnum cuspidatulum* C. Müll based on 16S rRNA gene sequence analysis.

Re isolates no.	Isolation media	Length (bp)	Completeness (%)	Similarity (%)	Closest phylogenetic neighbors
M1	ISP2	1467	100	100.00	*Dermacoccus abyssi* MT1.1^T
S1	ISP2	1467	100	100.00	*Dermacoccus abyssi* MT1.1^T
M2	ISP2	1469	100	99.93	*Streptomyces thermocarboxydus* DSM 44293^T
S2	ISP2	1472	100	99.93	*Streptomyces thermocarboxydus* DSM 44293^T
M3	ISP2	1453	100	99.65	*Micromonospora chalcea* DSM 43026^T
S3	ISP2	1453	100	99.65	*Micromonospora chalcea* DSM 43026^T

Note: M, isolate from *S. cuspidatulum* C. Müll plantlets; S, isolate from washed water of *S. cuspidatulum* C. Müll plantlets; ISP2, International *Streptomyces* Project mediu 2 agar

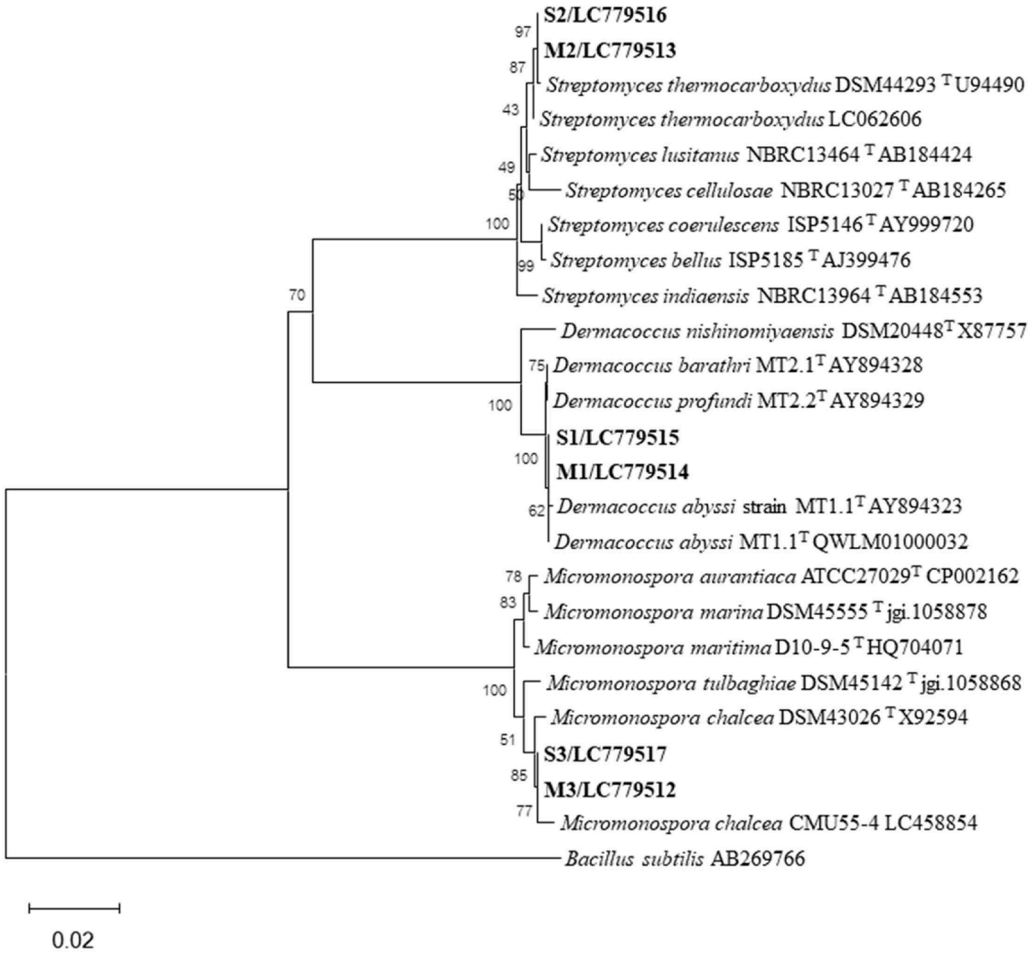

Fig. 2. Neighbor-joining phylogenetic tree based on an almost complete 16S rRNA gene sequence of re-isolated actinobacteria and their related taxa. Bootstrap values are based on 1000 re-samplings.

A comparison between the culture filtrate of three strains of actinobacteria and NAA (an auxin phytohormone) revealed that the culture filtrate of all actinobacteria exhibited superior growth-promoting effects on both moss species compared to NAA (Table 1 and Table 2). Previously, these three actinobacteria were demonstrated to generate plant growth-promoting substances, including IAA production, siderophore production, and phosphate solubilization, as evidenced in studies by Lasudee et al. (2018), Rangseekaew et al. (2022), and Insuk et al. (2020). Indole-3-acetic acid (IAA), an auxin phytohormone, plays a fundamental role in plant growth and development, regulating processes such as cell division, cell elongation, and various cellular transformations (Tsavkelova et al., 2006). These activities are involved in the initiation of root formation or seed germination (Davies, 2004; Glick, 2012; Bhatti et al., 2017). Since MS medium contains sufficient amounts of iron and phosphorus for plant growth, the observed enhancement in bryophyte growth may be due to the auxin hormone, particularly IAA, produced by inoculated actinobacteria. *D. abyssi* MT1.1^T produced the highest IAA at 37.50 μg/ml in vitro, followed by *M. chalcea* CMU55-4 (11.35 μg/ml) and *S. thermocarboxydus* S3 (11.12 μg/ml). These values correspond with the observed bryophyte growth parameters in Table 2, though the differences are not statistically significant ($p < 0.05$). The culture filtrate containing IAA produced by these actinobacteria yielded better results than the use of chemically synthesized NAA. Our finding marks an initial step towards utilizing culture filtrate of actinobacteria to promote moss growth, potentially substituting chemical agents. However, further investigations are imperative to ascertain whether this approach directly yields positive effects on plants.

4.3. Effect of actinobacteria inoculation on growth of Sphagnum cuspidatulum C. Müll

S. cuspidatulum C. Müll was utilized as a moss model to investigate the beneficial effects of selected actinobacteria in promoting growth, given its status as a rare moss species in Thailand facing the risk of extinction (Sitthichoptham et al., 2023). The inoculation of *M. chalcea* CMU55-4 on *S. cuspidatulum* C. Müll resulted in the most pronounced enhancements in thallus height, fresh weight, dry weight, total chlorophyll, and total carotenoid (Table 3) though these values were not significantly difference ($p < 0.05$) except for fresh weight and total carotenoid content. Similarly, Insuk et al. (2020) investigated the potential of *M. chalcea* CMU55-4 to promote the growth of another moss species, *P. sphaericum* (C. Ludw.) Fürnr., observing an increase in carotenoid content, dry weight, and fresh weight, as well as promoting capsule formation. This effect was partly attributed to auxin (IAA) production, aiding the moss during its transition from agar to soil. *M. chalcea* CMU55-4 produced lower IAA (11.35 μg/ml, Insuk et al., 2020) than *D. abyssi* MT1.1^T (37.50 μg/ml, Rangseekaew et al., 2022) and similar to *S. thermocarboxydus* S3 (11.12 μg/ml, Lasudee et al., 2018).

Despite not being originally isolated from *S. cuspidatulum* C. Müll and *P. sphaericum* (C. Ludw.) Fürnr, *M. chalcea* CMU55-4 demonstrated a capacity to enhance the growth of both moss species. The genome of *M. chalcea* CMU55-4 was reported to contain genes for various carbohydrate metabolic pathways, including genes for the utilization of saccharides found inside plant cells such as xylose, arabinose, mannose and D-galacturonate (Insuk et al., 2020). These observations strongly indicate that *M. chalcea* CMU55-4, originally isolated from the moss *Campylopus involutus* (Müll. Hal.), is well adapted to the bryophyte lifestyle better than *S. thermocarboxydus* S3 (from arbuscular mycorrhizal spore) and *D. abyssi* MT1.1^T (from marine environment). These findings demonstrate that plant-microbe interactions play an important role in bryophytes growth promotion.

The successful re-isolation of *S. thermocarboxydus* isolate S3, *D. abyssi* MT1.1^T, and *M. chalcea* CMU55-4 from *S. cuspidatulum* C. Müll plantlets demonstrates their ability to colonize and persist within plant tissues. *S. thermocarboxydus* isolate S3 has previously been reported by Lasudee et al. (2018) to colonize rice roots. Similarly, Rangseekaew et al. (2022) demonstrated the successful colonization of *D. abyssi* MT1.1^T in tomato roots. These current findings, along with previous studies, highlight the broad host range of these plant growth-promoting actinobacteria, as they can colonize both lower (*S. cuspidatulum* C. Müll) and higher plant species, including monocotyledons (rice) and dicotyledons (tomato). This supports the view that actinobacteria serve as beneficial plant growth-promoting agents.

5. Conclusion

The present study clearly demonstrated that the three selected plant growth-promoting actinobacteria, namely *S. thermocarboxydus* S3, *M. chalcea* CMU55-4, and *D. abyssi* MT1.1^T, positively promote the growth of bryophytes. This is evidenced by a significant increase in thallus height, fresh weight, total chlorophyll content, and carotenoid content of two moss species, *P. sphaericum* (C. Ludw.) Fürnr, and *S. cuspidatulum* C. Müll, compared to the control ($p < 0.05$). The culture filtrates of *D. abyssi* MT1.1^T and *S. thermocarboxydus* S3 were the most effective in promoting the growth of *P. sphaericum*. On the other hand, the culture filtrates of *D. abyssi* MT1.1^T and *M. chalcea* CMU55-4 proved to be the most potent promoters of *S. cuspidatulum* C. Müll growth, resulting in a substantial and statistically significant increase in fresh weight, total chlorophyll content, and carotenoid content compared to the control group ($p < 0.05$). Similarly, an increase in thallus height, fresh weight, dry weight, total chlorophyll content, and total carotenoid content was recorded in *M. chalcea* CMU55-4 inoculated *S. cuspidatulum* C. Müll plantlets in sterile peat moss. The successfully colonization of *M. chalcea* CMU55-4, as well as *D. abyssi* MT1.1^T and *S. thermocarboxydus* S3 within *S. cuspidatulum* C. Müll plantlets, positively affirm beneficial interactions between actinobacteria and the moss. Our results provide further evidence on the potential of actinobacteria as growth-promoting agent for lower plant, particularly bryophytes.

Declaration of generative AI in scientific writing

During the preparation of this work, the author(s) used ChatGPT-3.5 to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the content as necessary and take(s) full responsibility for the publication's content.

CRediT authorship contribution statement

Mathurin Meethangdee: Writing – original draft, Visualization. **Wasu Pathom-aree:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Funding acquisition, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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