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ABSTRACT

The evaluation of endophytic actinomycetes diversity, growth-promoting strain effects on cauliflower seeds germination, and *in vitro* organogenesis are the objectives in this study. Moreover, fifteen strains from 125 isolates were determined to produce Indole-3-Acetic Acid (IAA), where majority was obtained from roots (66.67%), followed by from branches (26.67%) and leaves (6.67%). Specifically, *Jatropha* sp. is a plant species with the most endophytic actinomycetes content compared to others. In addition, all endophytic streptomyces strains were screened based on IAA production ability *in vitro* on YMG broth medium. The results showed the tendency for one strain with code *Streptomyces* sp. KMR-1E to generate a maximum IAA isolate from *Cinnamomun* sp. plant. Furthermore, the molecular taxonomy and phylogenetic analysis were recreated from 16S rRNA gene sequences, which attributed the KMR-1E to genus Streptomyces. Meanwhile, plant growth promotion was evaluated under *in vitro* condition. This exposed the individual ability to enhance the shoot and root length of cauliflowers. The untreated cultures with a strain free agar block was used as control.

Key words: *Endophytic actinomycetes*, *Plant growth promoter*, *Indole-3-acetic acid*, , *Brassica oleracea* L.

INTRODUCTION

Actinomycetes symbolize a group of microbes instrumental in the performance of various beneficial functions, including antimicrobial actions, organic matter fragmentation, alongside mineral solubilizion. Also, these bacteria are recognized as producers of plant growth modulators, and have found use in agriculture (Suzuki *et al*., 2000; Illic *et al*., 2007). These organisms are recognized as intrinsic growth regulators, phytohormone synthesizers, as well as groundbreaking resources in plant diseases management (Zhao *et al*., 2018).

In previous decades, several genera, particularly the endophytic Actinobacteria were separated from the surface-sterilized roots of several plant types. These endophytes, in detail, are bacteria or fungi understood to establish habitats in intracellular, and often intercellular spaces of healthy plant tissue, devoid of the initiation of evident disease symptoms (Maela & Serepa-Dlamini, 2019). Subsequently, the advantageous protection of these organisms from the competitive and demanding soil environment exhibits positive effects on the hosts (Shutsrirung *et al*., 2014). Moreover, some of the microbe's genetic properties are also shared with the molecular machinery required for plant-specific compound synthesis (Jasim *et al*., 2014). Also, the existence of endophytic Actinobacteria in the basal organs of healthy crops, including sorghum (Araujo *et al*., 2000), banana (Cao *et al*., 2004), rice (Debananda *et al*., 2009), as well as the passion fruit (Ali *et al*., 2017) have been revealed in numerous research articles. Endophytic microbes are worthy of note as growth inducers in various stages of a plant's lifespan including during biological nitrogen fixation (BNF), to facilitate the reduction of N_2 to ammonia (NH_3), the mobilization of insoluble phosphates, in conjunction with growth enhancement potentials (Sharma *et al*., 2013; Costa *et al*., 2014).

Furthermore, *S. olivaceoviridis*, *S. rimosus*, and *S. viridis* were implicated as members of the unique Streptomyces genera of endophytic plants assumed to play a role in IAA synthesis, and subsequently advance plant expansion by enhanced propagation of seeds, alongside root and shoot extension (Tokala *et al*., 2002; El-Tarabily, 2008; Khamna *et al*., 2010). However, data on the yield generated by the *Cinnamomum* sp. and the resultant application in growth promotion is deficient.

Additionally, the cauliflower (*Brassica oleracea* L.) has been cultivated constantly in South Sulawesi for over five decades and is considered an important commercial vegetable. However, increased production indicates a higher use of agricultural chemicals, including manure and insecticides. These chemical applications for extended periods threaten local communities, as well as the environment. Therefore, government policies aim to practice organic agriculture through reduced agrochemical use, and a tactic used involved the application of beneficial microorganisms as inoculants for biocontrols or biofertilizers (Berg, 2009; Malus'a *et al*., 2012).

Also, several benefits to the bacteria are enhanced through the diversion of host physiological processes by the synthesis of auxins. Moreover, present records demonstrate the strenuous effects of IAA on seed germination, as well as the potential for induction of *Brassica oleracea* L. explant organogenesis by this compound in actinomycetes.

MATERIALS AND METHODS

Sample collection

All the specimens (roots, branches, and leaves) were collected from a variety plant species in South Sulawesi, Indonesia. Subsequently, the samples were chopped up using a cutter blade, reserved in sterilized plastic bags labelled according to species as well as site sampling, and subsequently returned to the laboratory where bacteria segregation was performed within 48 hours.

Isolation and selection of endophytic actinomycetes

Dirt, debris, as well as surface agents were eliminated from the plant tissues samples by the use of running water. Then, the bacteria were isolated by the reduction of the plant parts into smaller portions (0.2 × 4 cm^2), and consequently sterilized externally through serial treatment with 70%v/v ethanol for ten minutes. Subsequently, 1% sodium hypochlorite was added for five minutes then rinsed four times in sterilized water. The air-dried specimen were further excised into approximate sizes of 2×3 mm and transplanted onto Starch Casein (SC) agar enhanced with 100μg/mL Nystatin. Moreover, bacteria colonies surrounded the plant tissue at 30°C after an incubation period of 2–3 weeks. These were therefore isolated by the transfer of the clusters onto fresh SC agar plates until purity was indicated by an individual colony. The unadulterated culture was then placed in a 15% sterile glycerol suspension at −80°C to facilitate preservation through an extended period.

Authentication of the surface sterilization protocol

The test procedures were validated by the inoculation of 0.1 mL aliquots of the resultant specimen suspension on SC agar. Subsequently, the samples were incubated at 30°C for five days, before microbial growth analysis. The absence of bacterial proliferation confirmed the protocol as effective.

Evaluation for indole-3-acetic acid (IAA) production by endophytic isolates

The amount of synthesized hormones was estimated in correspondence with the technique proposed by Bano & Musarrat (2003). This was implemented by the cultivation of the agar block (Φ= 6 mm) of the bacterial colonies on yeast extract–malt extract broth (YMG) (g/L) (made up of yeast extract 16g, malt extract 10 g, and glucose 4 g), alongside a final incubation at 35°C for a period of seven days. Then, the culture was transferred into 5 mL YMG broth inclusive of 2 mg/mL L-tryptophan, before the final incubation and concurrent agitation at 35°C and 125 rpm for seven days respectively. The cells were consequently

harvested by using a centrifuge at 11,000 rpm for fifteen minutes, and 1mL of the upper layer of the product combined with 2 mL of Salkowski reagent (1 mL of 0.5 M $FeCl_3$, in 49 mL of 35 % w/v $HClO_4$), with the resulting solution incubated at 30°C for 25 minutes in an environment without light. Subsequently, conversion of the color to a pink hue was suggestive of a positive test. The optical density (OD) was then ascertained with the employment of a spectrophotometer with a wavelength of $\lambda_{535\ nm}$, and the resultant level of IAA produced appraised against the standard.

Preliminary phenotypic characterization of IAA producing strain

A phenotypic characterization was performed on all strains producing endophytic IAA using the International Streptomyces Project (ISP) medium. Therefore, the morphological and cultural properties, including the spore mass color, existence of aerial hypha, diffusible pigments color, unique reverse colony color, as well as spore chain morphology were evaluated (Cao *et al*., 2004).

Expulsion and identification of IAA

This detection process involved the use of isolates assumed to produce the highest IAA. The area above this culture was then obtained using a centrifuge at 11,000 rpm for 15 min after fermentation on YMG broth media supplemented with 2 mg/mL L-trytophan at 35°C and 125 rpm for 7 days. Also, IAA extraction was conducted based on the procedure from Ahmad *et al*., 2008. This required acidifying the culture supernatant to pH 2.5 with HCl, and the supernatant culture: ethylacetate (1:2 v/v) was subsequently adopted. The confirmation of isolates determined to produce this acid was conducted using thin layer chromatography (TLC) methods. This method was further executed with 25 μL ethylacetate macerate stained on TLC plates (silica gel GF_{254}, thickness 0.25 mm, Merck, Germany) and developed using ethylacetate:chloroform:formic acid (55:35:10 v/v) as the mobile phase. Furthermore, UV light (254 nm) was used to confirm the points with similar Rf values as the original pure IAA chemical after Ehmann's reagen was sprayed on the LC plates (Vikram, 2011).

Optimization of IAA production condition

This IAA production process was improved by evaluating the impact of incubation period on the culture temperature and pH condition. Therefore, similar medium and conditions as described above were used to culture the isolate. Furthermore, the incubation period effect was examined over a10 day period.

Effect of soaking on seed germination

The cauliflower seeds were subjected to surface sterilization for 10 min, using 10% (w/v) NaOCl. This was followed by a thorough wash with sterile aquadest, and air drying, before soaking in the KMR-1E strain culture filtrate (grown in YMG broth medium broth amended with L-tryptophan 2 mg/mL, incubated for 7 days at 30°C). Furthermore, the authentic IAA (Merck) was immersed in the same sterile aquadest (0.5 μg/mL) and applied as the study control. Therefore, a tentative therapy was conducted over the agar block of KMR1 strain (grown on YMG agar medium modified L-trp). The seeds were then cultured on the water agar media (5% w/v) placed within a Petri dish (Φ=9 cm), and arranged vertically in the growth chamber, in an attempt to facilitate seed maturation with no restrictions. Subsequently, the extent of growth was evaluated after 10 days of germination, based on number of roots, fresh weight of plant, primary root and shoot length.

Assessment of plant growth promotion in cauliflower tissue culture treated with specific endophytic actinomycetes

The KMR-1E strain growth promotion capacity was assessed *in vitro* in a bottle chamber where Φ=6cm, and h=20cm. This was followed by using tap water to wash the cauliflower stalk and remove particles as well as other agents. The representative cauliflower (2×2.5cm) obtained per explant was purified through 10 min of continuous submersion in NaOCl 10% (w/v) and consequently soaked in an autoclaved distilled water four times. Subsequently, the explants were finally air dried after cutting into thin blocks (2×10 mm), and placed without or with IAA on the basal MS medium. This vehicle had an adjusted pH of 5.8 prior to autoclaving. Meanwhile, the IAA stock solution was prepared with 100 mL purified water, and filtered with a 0.2 µm sterile membrane filter.

The control (K0) sample encompasses the flowers branches of cauliflower explant yield cultivated on Skoog (MS) primary medium with 1% sucrose and Murashige. Also, different treatments, including the explant grown in this agent, and free strain YMG agar block medium modified L-trp (NS) were evaluated, and consequently transferred at a ratio of 1 to 3 per explant. In addition, YMG medium agar block amended L-trp with KMR1 strain (ST) was used in one of the experimental treatments, while the other involved genuine IAA (PC) altered to 0.15, 0.2, and 0.25 μg/mL, respectively. Figure 1 shows the test reserved within the conditions of 24°C, 24 h light, and 60% mm for the cultivated plant part. Therefore,

measurements were obtained all parameter data after a 3-week culture period. The three treatments were conducted using a completely randomized design.

Molecular identification of selected endophytic actinomycetes by 16S rDNA sequence analysis

The selected KMR-1E strain cells were cultured in YMG for 4 days. Therefore, the DNA genome was prepared with minor modification, according to Badji *et al*., 2006. In addition, two primers were applied during the amplification of 16S rDNA, including: 27f (50-AGAGTTTGATCCTGGCTCAG-30) and 1492r (50-GGTTACCTTGTTACGACTT-30). Also, through an Invitrogen kit, PCR was adopted for gene sequence amplification, and the final reaction mixture volume (50 μL) comprised of 50 ng template DNA; 10 *p*mol primer concentration; PCR master mix containing Taq DNA polymerase, dNTPs, Tris-HCl, $MgCl_2$ stabilizer; as well as tracking dye applied based on the manufacturer's instructions. The amplification process was conducted on a thermal cycler (Gene Cycler, BioRad) by exposing sample to an initial denaturation step for 5 min, with temperature set at 96 °C. Subsequently, 30 amplification cycles of 95°C for 1.5 min, 55.5°C for 1 min, 72°C for 5 min, and 72°C for 5 min was conducted. Therefore, electrophoresis was adopted for gene detection using 1.5% (w/v) agarose gels stained with ethidium bromide. In addition, using BLAST NCBI search (http://blast.ncbi.nlm.gov/), the 16S rRNA gene sequence in KMR-1E variants was subjected to similarity analysis. Subsequently, an evolutionary tree was suggested based neighbor-joining (Saitou & Nei, 1987), while phylogenetic analysis and multiple alignments involved the clustal X program (Thompson *et al*., 1994). The tree topology was evaluated based on 1,000 replicates, and using the bootstrap analysis (Felsenstein, 1985).

RESULTS AND DISCUSSION

Diversity of the endophytic strain

The various plant tissue possessed a total of 125 endophytic actinomycetes strains, and 15 were selected for further studies. Table 1 illustrates the negative bacteria growth on SC agar medium in the representative samples from every crop treated with the surface sterilization protocol. This proves the elimination process for this organism was effective using this method. Furthermore, from the isolates screened for endophytic plant, the highest and lowest number of strains were obtained from the roots and leaves where n = 64.4% and 18.4% respectively. These were discovered to produce IAA. The majority of isolates were separated from roots (66.67%), branches (26.67%), and leaves (6.67%). In addition, most of

the bacteria were obtained from *Jatropha* sp. and the predominant plant had at least one isolate yield, therefore the organism is able to colonize different parts of various tissues. The microbes' isolation in a culture medium is a simple method for assessing the bacterial communities (Andreote *et al*., 2009), although this is an underestimation in terms of diversity relating to the microbial populations. This is because bacterial identification is significantly influenced by the growth of selected specific culture medium. Also, microbial populations vary for different vegetation and species inhabit. The populations of microbes differ depending on the locations or climatic conditions even in similar plant species (Nair & Padmavathy, 2014). Moreover, colony appearances, including substrate hyphae and spore mass color on ISP 3 medium displayed the isolates in form of two different groups. These included the streptomyces-like strain distinguished by an abundant aerial mycelium with powdery spores and the non-streptomyces variant marked by red, orange, and brown to black slimy colonies. The streptomyces like-strain represented 66.67% of the endophytic species, majorly the S of spore chain type. According to Sardi *et al*., 1992, 499 endophytic actinomycetes were seperated from various crops, with approximately 96% streptomyces and 0.2% streptosporangium. The non-streptomyces-like strain was discovered to colonize roots in this study as noted earlier by Goudjal *et al*., 2013.

Screening of IAA production of endophytic strain

The isolates screening for the presence of beneficial traits indicated fifteen strains were discovered to be the producers of IAA. This acid generation has a high variability range of 17.65 to 86.94 μg/mL for Streptomyces-like strain and a value of 21.41 to 68.12μg/mL for non-streptomyces-like strain. Furthermore, 8 plants of endophytic actinomycetes were discovered with the potential to generate this acid in the present study. Table 2 demonstrated the high production ability of two variants termed KMR-1E and KMR-11. This was the most significant amongst all other genera, with a value of 86.94 μg/mL. Also, IAA was determined to be the main auxin in plants, due to the vital function possessed by this acid in the first processes of lateral and adventitious root formation and elongation (Idris *et al*., 2007). The presence of microorganism colonies in plant tissue has a vital role in vegetation. According to Shutsrirung *et al*., 2014, IAA produced by Streptomyces spp. were ranged between 1.05 to 60.95 μg/mL and 11 to 54 μg/mL. This study discovered a high variation level in the auxin production by Streptomyces with the exception of *Streptomyces viridis* CMU-H009 at a value of 143.95μg/mL (Khamna *et al*., 2010).

Detection and Optimization of IAA production by KMR1 strain

The detection and comparison of IAA-producing strain with standard auxins was performed using TLC chromatograms. Furthermore, pink color spot of ethyl acetate extract at $R_{f\,0.6}$, correspond to the authentic compound, therefore IAA-producing organism is a potential source for the chemical. Meanwhile, this technique is important for the qualitative or semi-qualitative determination detection of the acid. This method assured the hormone presence in the unrefined top layers of these samples of bacterial cultures. The Salkowski reagent reacted with the acid but not with L-tryptophan and Na-acetyl-L-tryptophan (Vaghasiat *et al*., 2011). Figure 2 presented the effects of incubation periods toward the hormone production of KMR-1E strain**.** The results showed this process commenced after 24 h, and gradually increased after 7 days of incubation with a reduction on the 8th day. This impact was studied to acquire knowledge on the optimal culture condition for the highest producer termed KMR-1E. Also, adjustments were made to the culture media pH, temperature, and L-trp concentration with reference to previous studies. The maximum yield was obtained after a 7 day incubation period, as shown in the results time exponent. This reduction occurs as a result of oxidase and peroxidase activity as indicated in the *Rizobium* sp. from *Cajanus cajan* (Datta & Basu, 2000). This congruent with the agreement in a research on Streptomyces sp. PT2 (Goudjal *et al*., 2013).

Effect of presoaking periods and IAA treatments on seed germination and root elongation

Table 3 showed a significant improvement in the germination of inoculated cauliflower seeds induced by the growth modulator of the KMR-1E strain against an uninoculated control, examined over ten days according to the test conditions. Furthermore, an agar block of medium containing the strain alongside IAA standard exhibited greater limb lengths and biomass, unlike the pre-soaked seed untreated with the crude supernatant of the KMR-1E strain (control). Meanwhile, important variations were not noted between specimens processed with IAA and an agar block of YMG without L-trp, in terms of percentage root and shoot lengths, higher biomass, as well as the number of adventitious roots. The samples impregnated with IAA, YMG agar slab alongside the strain modified with L-trp demonstrated substantial distinctions when assessed against the control, as well as the aforementioned pre-soaked seed sample. Therefore, the seed treatment was revealed to have affected the root and shoot expansion as shown in Figure 3. Subsequently, the ensuing outcome proved the immersion of the seeds for 5 h in standard IAA and agar block YMG medium amended with

L-trp as the major responsive assay. This outcome approved a former research performed with sugar beets (Shi *et al*., 2009), where considerable growth of the seedlings was observed. Several researches corroborated the ability of endophytic actinomycetes to synthesize IAA, and consequently improve plant growth by intensifying plant cultivation. The improvement of tomato seeds with the culture top layer containing the unadulterated IAA was also observed. Also, previous documentation described the advanced seed germination and root extension produced in *Ochetophilia trinervis* by IAA-generating endophytic actinomycetes (Solans *et al*., 2011). Also, the *Streptomyces* sp. isolated from *Centella asiatica* possessed the potential to enhance the earlier stated characteristics (Dochhil *et al*., 2013).

The organogenesis of the cauliflower explants through in vitro procedures

The stalk explants with dimensions of 2 × 2.5cm were transferred to a basal MS medium, and the length of the plant limbs, alongside the induction of adventitious roots were observed across all the samples after fourteen days of culture. However, the optimal response was observed for the cultures comprising of the strain adjusted with L-trp and IAA, where sizeable variations in branches and roots sizes, as well as the absence of alterations in the number of leaf regeneration, in contrast to the control and the sample without strain (p <0.05), were displayed. Therefore, species producing IAA from media enriched with L-tryptophan displayed greater potentials for shoots and adventitious roots height extension, as well as biomass weight enhancement in contrast with the control (Figure 4-5), as indicated by the results. Consequently, the potential of IAA-producing endophytic actinomycetes to induce the organs of *B. oleracea* L. explants was demonstrated. Despite the tissue culture on MS basal medium and treatment with YMG agar block, the media provided insignificant responses compared with the control. The medium treated with strain and IAA exhibited better outcomes of explant regrowth. Furthermore, cauliflower regeneration was influenced by several factors, including the media components (Kaur *et al*., 2006; Ovesna *et al*., 1993), growth regulators (Hoque, 2010), culture conditions as well as explant types (Kerlley *et al*., 2012). In general, the addition of auxin and bacterial metabolites were observed to appreciably improve organogenesis, especially of the plant limbs and roots. Also, the utilization of actinobacterial cultures for inoculation also significantly improved growth and development, specifically of the plant limbs, and biomass (Jog *et al*., 2014).

This experiment has also demonstrated the comparable *in vitro* shoot and root induction potential of IAA-producing endopytic actinomycetes to the commercially available phytohormone. The results indicate the inability for minimum concentrations in YMG

medium agar block to demonstrate significant organogenesis response in contrast with IAA standards for all parameter except number of roots.

Characteristics of Streptomyces sp.KMR-1E strain

Figure 6 shows the reconstruction of molecular taxonomy and phylogenetic analysis based on 16S rRNA gene sequences. This phenomenon prompted the strain designation to genus Streptomyces. KMR-1E strain shared 97% similarity with *Streptomyces tendae* NBRC 12822, and thus assigned as a closely related species to *Streptomyces albogriseolus* DSM 40003 and *Streptomyces violaceolatus* NBRC 13101. In addition, there was a spiral chain spore formation with KMR-1E strain. Meanwhile, the *S. tendae* retinaculiaperti or spiral on salts starch agar. *S. tendae* demonstrated a massive gray aerial color on yeast lactose, salts-starch agar, and oatmeal, while KMR-1E strain showed a pure white coloration, as observed in Table 4. The reverse side of a colony is yellow, while the KMR-1E strain showed a sand yellow coloration. This particularly utilized glucose, raffinose, inositol, sucrose, and D-mannitol while the *S. tendae* showed no growth signal on raffinose (Whitman et al., 2012).

CONCLUSION

These results showed the indole-3-acetic acid produced by endophytic Actinomycetes isolates of *Cinnamomun* sp. plant has the potential to promote seedling germination as well as induce shoot and root *in vitro* organogenesis in cauliflower stalk explant.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article

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Table 1

The occurrences of endophytic actinomycetes strain from different plant species

Plant species	Plant tissue of endophytic strain sources			No. of endophytic strain
	roots	stem barks	leaves	
Cinnamomum sp.	12 (2)	1	1	14
Piper sp.	5 (1)	2 (1)	4	11
Ficus	14 (1)	4	5	23
Hibiscus sp.	6	2 (1)	1	9
Areca sp.	7 (1)	1	1	9
Unidentified fern	11 (1)	3	4	18
Manihot sp.	9 (1)	1	4	14
Jatropha sp.	17 (3)	7 (2)	3 (1)	27
Total	83 (10)	21 (4)	23 (1)	**125**
Persentage	64.4 % (66.67%)	16.8 % (26.67%)	18.4 % (6.67%)	

*Value in brackets indicates the number of endophytic actinomycetes strain IAA producing

Table 2
Morphological characteristic and estimation of IAA production of endophytic Actinomycetes

Groups	Plant sources	Diffusible pigment	Spore-chain type	Group of Genera	Concentration of IAA (µg/mL)
KMR-1E	*Cinnamomum* sp.	-	F	Streptomyces	86.94±0.71
KMR-2	*Cinnamomum* sp.	-	RF	Streptomyces	38.00±0.67
KMR-4a	*Piper* sp.	-	S	Streptomyces	64.59±0.69
KMR-4b	*Piper* sp.	-	R	Streptomycete	86.29±0.79
KMR-11	*Ficus* sp.	Yellow	RA	Streptomyces	86.92±0.77
KMR-12	*Hibiscus* sp.	-	RF	Streptomyces	65.18±0.42
KMR-15	*Areca* sp.	-	S	Streptomyces	36.59±0.57
KMR-22	Unidentified fern	-	-	Non-streptomyces	21.41±0.31
KMR-23	*Manihot* sp.	Brown	-	Non-streptomyces	68.12±0.78
KMR-26a	*Jatropha* sp.	-	S	Streptomyces	17.65±0.34
KMR-26b$_1$	*Jatropha* sp.	-	S	Streptomyces	19.06±0.21
KMR- 26b$_2$	*Jatropha* sp.	-	-	Non-streptomycete	61.06±0.59
KMR- 26c	*Jatropha* sp.	-	S	Streptomyces	44.00±0.62
KMR- 26d	*Jatropha* sp.	-	-	Non-streptomycete	27.53±0.33
KMR- 26e	*Jatropha* sp.	-	-	Non-streptomycete	66.94±0.58

): RA, rectiaculiaperti; RF, rectiflexibiles; S, spiral

IAA was estimated 7 days after the endophytic actinomycete growth in YMG broth medium supplemented 2 mg/mL of L-tryptophan with absorbance at 540 nm

Table 3
Effects of endophytic actinomycetes strain on the growth of cauliflower seedling

Treatment	Fresh primary root length (cm)*	Fresh shoot length (cm)*	No of adventitive roots*	Fresh plant biomass weight (g)*
(A) Control	2.025^{a}	2.550^{a}	2.750^{a}	0.046^{a}
(B) YMG broth medium with KMR-1E strain and amended L-trp	6.950^{b}	4.700^{b}	5.250^{b}	0.061^{ab}
(C) YMG solid medium	7.720^{b}	4.950^{ab}	5.750^{b}	0.079^{b}

with KMR-1E strain and supplied L-trp				
(D) IAA authentic standard	7.550[b]	4.900[ab]	6.100[b]	0.073[b]

* It was measured after 10 days of seedling

Means followed by the same lowercase letter within the same column were not significantly different based on LSD test ($p = 0.05$). There were three replications per treatment (4 plants per replication).

Table 4
Cultural characteristics of KMR-1E strain on different culture mediums

Agar Medium	Aerial mycelium	Substrate mycelium	Growth
ISP1	Pure white	Brown beige	good growth
ISP2	Pure white	Sand yellow	moderate growth
ISP3	no growth	no growth	no growth
ISP4	Ochre brown	Orange brown	good growth
ISP5	Cream	Cream	moderate growth
ISP6	Pure white	Sand yellow	good growth

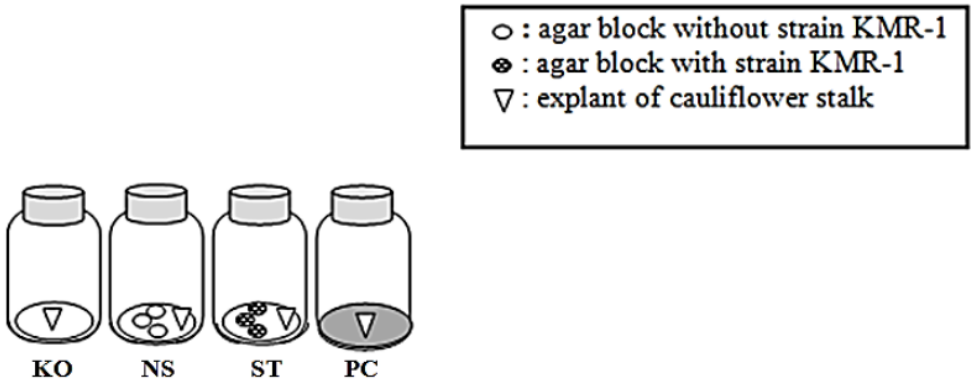


Figure 1. Schematic of *in vitro* evaluation of cauliflower stalk plant growth promotion

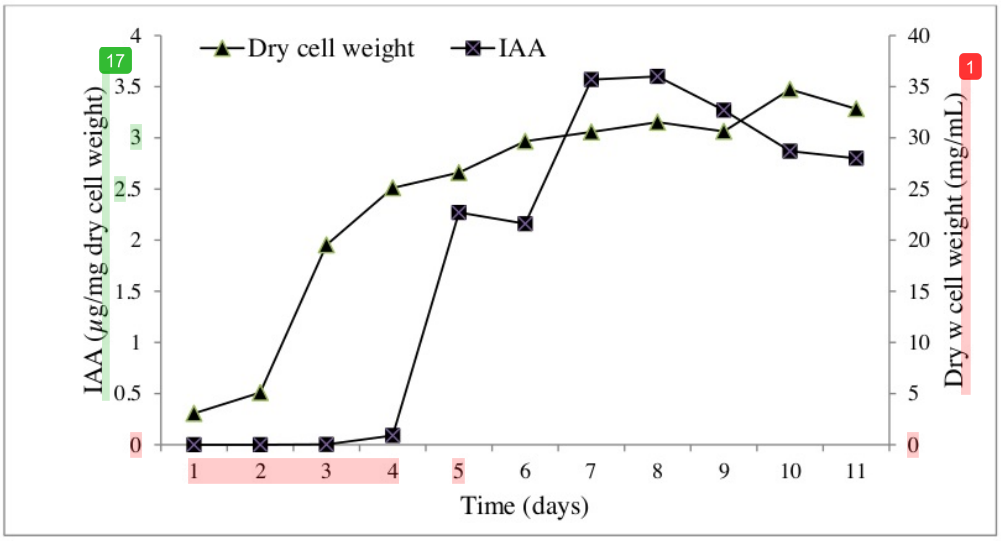
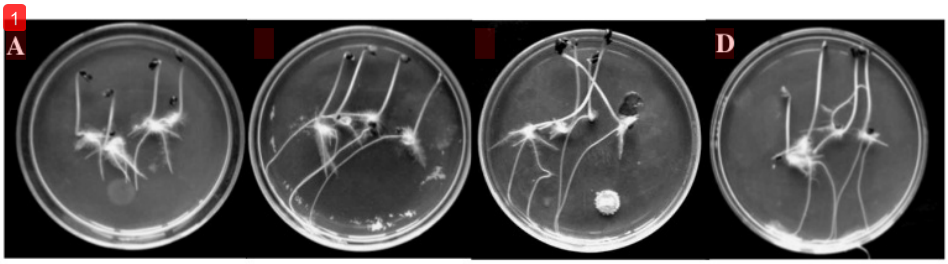


Figure 2. Time course of indole-3-acetic acid (IAA) production by strain Streptomyces KMR-1E on YMG broth (amended L-trp 2 mg/mL; pH 7±.05) at temperature 35°C for different incubation periods. In addition, each value was determined as the mean of three replicates.



A B C D

Note:

A. Brassica seed were applied with sterile aquadest as seed soaking treatments
B. Brassica seeds were soaked with YMG broth medium with KMR-1E strain
C. Media were applied as block agar YMG medium with KMR-1E strain placed on surface of growth media
D. Brassica seeds were soaked with IAA pure chemical (0.5 μg/mL)

Figure 3. Early development of cauliflower (*Brassica oleracea* L.) seedling. These were surface sterilized and placed on water agar (5%) for germination at 25°C in a plant growth chamber for 10 days. The images show representative photographs of cauliflower seedling, and each scale bar represents 2 cm.

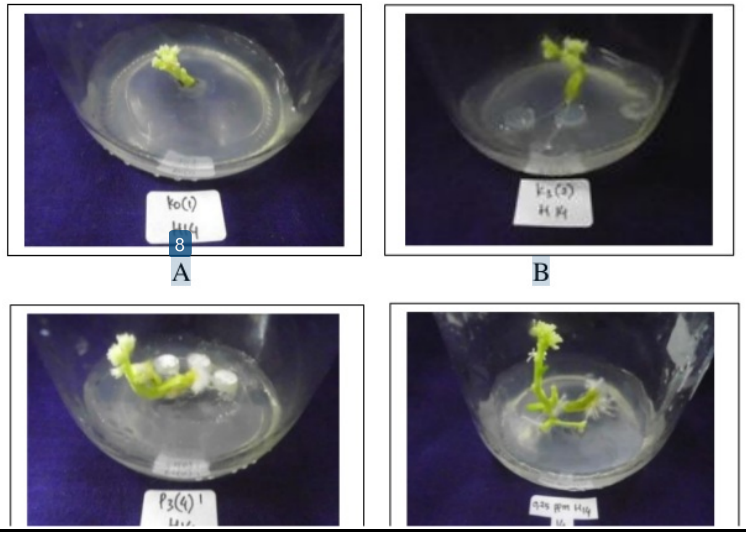


Figure 4. Effect of strain KMR-1E agar block on the organogenesis of cauliflower stalk after a 14 day culture period. The flower branches were cultured on Murashige and Skoog (MS) basal medium supplemented with 1% sucrose and consequently used as control, K0 then MS basal medium alongside agar block of YMG medium (A), without strain and amended L-trp, NS (B), characterized by amended L-trp with KMR-1E strain, ST (C), treated by autentic IAA, PC (D).

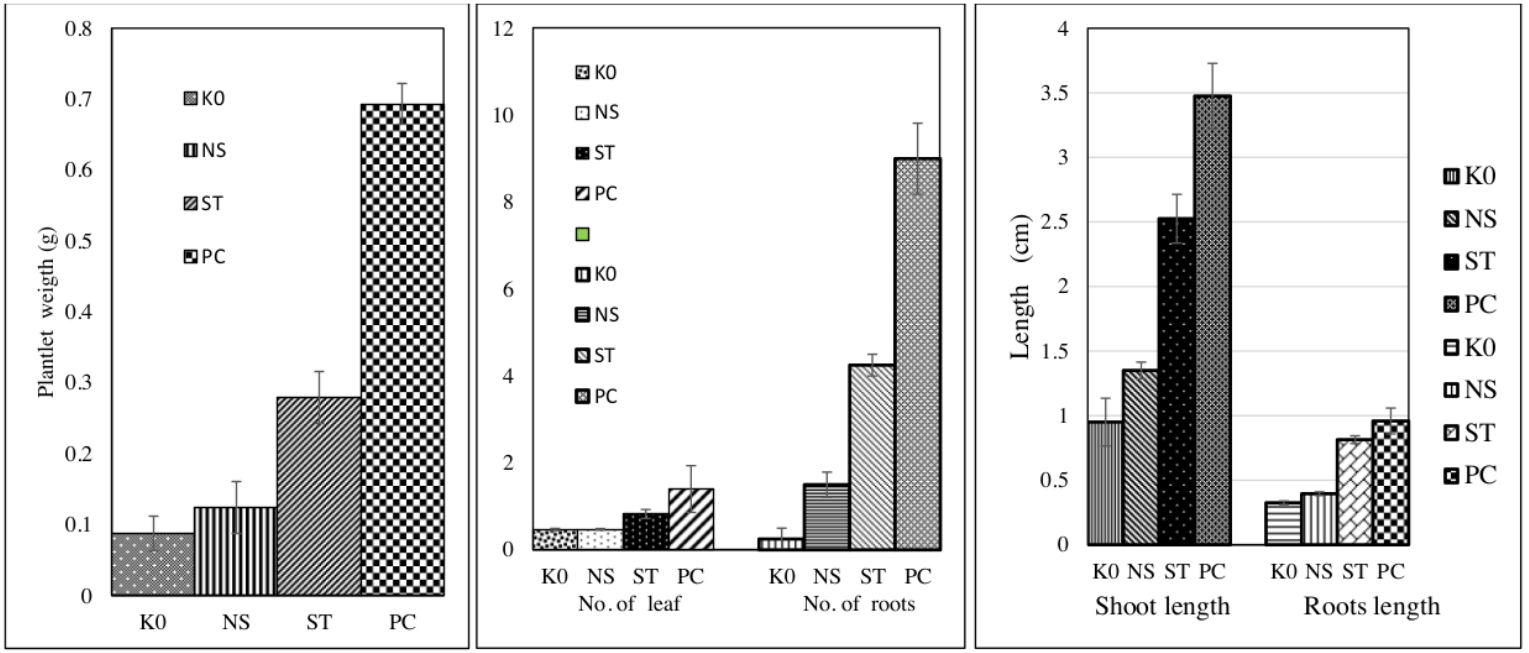


Figure 5. Effect of in vitro organogenesis of cauliflower stalk explant on planlet biomass (A), number of leaf and adventitive roots (B), and shoot and roots length

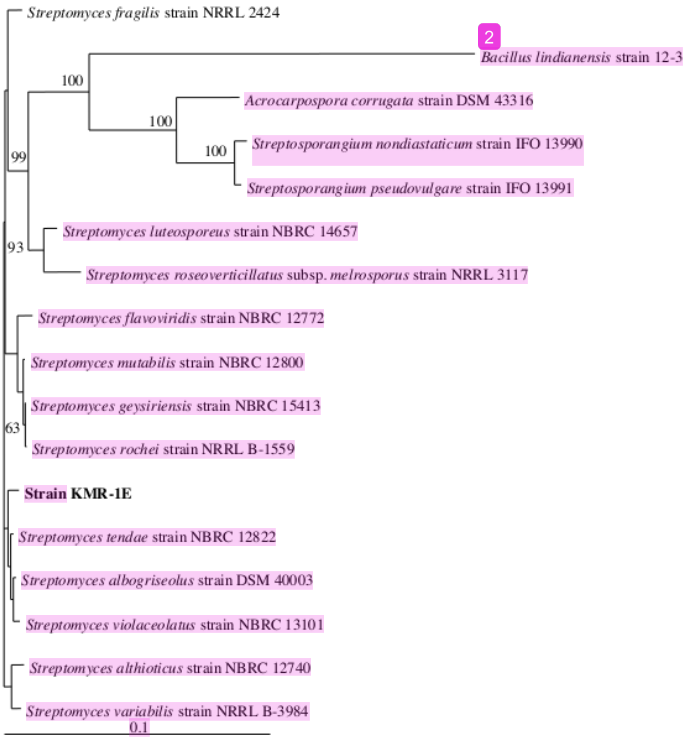


Figure 6. Neighbor-joining phylogenetic tree inferred from 16S rRNA gene sequences. This shows the relationship between endophytic bacteria KMR-1E strain and related genera. The bootstrap values were further expressed as percentages of 1000 replications, and those ≥50% are expressed at branch points. Furthermore, the score bar denotes 1 nucleotide substitution per 100 nucleotides.

Diversity of Endophytic Actinomycetes Producing Indole-3-Acetic Acid and In Vitro Evaluation of Plant Growth-Promoting Activity on Brassica oleracea L.

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