BIODIVERSITAS
Volume 26, Number 1, January 2025
Pages: 326-334

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260132

Peat-derived *Streptomyces* spp. isolated from edamame rhizosphere with plant growth-promoting properties

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Manuscript received: 6 November 2024. Revision accepted: 19 January 2025.

Abstract. *Erdiandini I, Tjahjoleksono A, Astuti RI, Husen E, Wahyudi AT. 2025. Peat-derived* Streptomyces *spp. isolated from edamame rhizosphere with plant growth-promoting properties. Biodiversitas 26: 326-334.* Peatlands, which are known for their high organic matter content, are common habitats for actinomycetes. These microorganisms have been recognized for their potential as plant growth promoters. However, there have been limited reports on peat-derived actinomycetes with plant growth-promoting properties, especially in edamame-cultivated peatlands. This study aimed to isolate and investigate plant growth-promoting actinomycetes in the rhizosphere of edamame-cultivated peatlands. The results showed that a total of 46 strains were isolated from the edamame rhizosphere during the anthesis and reproductive phase. Importantly, 36 of these strains were found to be biologically safe, as showed by the negative hemolysis and hypersensitivity test. All 36 strains produced indole-3-acetic acid in the range of 2.42 to 50.07 µg/mL. Based on the in vivo plant growth-promoting activity assay, strains RT34, AR26, AR39, and BT59 promoted the highest primary root growth of edamame sprouts. Interestingly, the acetylene reduction assay revealed that only RT34 and AR39 strains exhibited nitrogenase activity as high as that of *Azotobacter* sp. as a positive control. The nitrogenase activity of these strains was up to 27.42 nmol C_2H_4 $h^{-1}tube^{-1}$ and 28.10 nmol C_2H_4 $h^{-1}tube^{-1}$. Furthermore, quantitative phosphate solubilization assay showed phosphate solubilization up to 527.76 µg/mL in the range of 258.31 to 527.76 µg/mL. Based on 16S rRNA sequencing, these strains were closely related to the genus *Streptomyces*. The finding of present results strongly indicates that these *Streptomyces* strains have plant growth-promoting properties and can be proposed as biostimulants to enhance edamame growth in peatlands.

Keywords: Biostimulant, edamame, peatland, plant growth promoting rhizobacteria, *Streptomyces*

INTRODUCTION

Edamame (*Glycine max* (L.) Merr) is a soybean cultivar with a high economic value in Indonesia. The increasing demand for edamame has led to a significant expansion of edamame cultivation areas in Indonesia over the past 20 years, growing from 30.5 to 1417 hectares (Nair et al. 2023). Nevertheless, this cultivation level must still be substantially below export demand. China dominates the market with a cultivation area of 400,000 ha, followed by Japan and Taiwan with 13,000 and 9,180 ha, respectively (Nair et al. 2023). One strategy to fulfill this demand involves agricultural extensification using peatlands. Indonesia has approximately 13.34 million hectares of peatlands spread across Sumatra, Kalimantan, Papua, and Sulawesi (Anda et al. 2021). Approximately 35.17% of these peatlands consist of shallow tropical peatlands, 50-100 cm thick, which holds promise for agricultural purposes (Masganti et al. 2020). Some farmers have used these shallow tropical peatlands for edamame cultivation. However, acidic conditions and poor soil fertility necessitate heavy reliance on chemical fertilizers (Sari et al. 2021). Chemical fertilizers may have negative impacts on the environment for long-term use, including soil acidification and degradation, accumulation of chemical residues, air pollution, and water eutrophication (Abebe et al. 2022). Adopting sustainable farming practices to mitigate the negative environmental impacts of chemical fertilizers is imperative.

Plant Growth-Promoting Rhizobacteria (PGPR) are commonly recognized as eco-friendly biofertilizers that can stimulate plant growth through direct mechanisms by producing phytohormones and supplying nutrients that are unavailable to plants (Adedeji et al. 2020). PGPR can produce the growth hormone indole-3-acetic acid, which is an important member of the auxin family of plant hormones that play a role in stimulating plant growth (Pantoja-Guerra et al. 2023). It also plays a role in increasing the availability of phosphate and nitrogen in a form that plants can directly use. Phosphate and nitrogen are essential plant nutrients that naturally occur in forms that plants cannot directly absorb. Therefore, the role of phosphate-solubilizing and nitrogen-fixing bacteria is important. The characteristics of dissolving phosphate and fixing N are both possessed by the PGPR. The inoculants have been introduced to cultivate edamame in peatlands (Sari et al. 2021). However, non-peat-derived inoculants such as exogenous inoculants, can be eliminated under harsh conditions in peatlands (Adedeji et al. 2020). Selecting peat-derived inoculants with plant

growth promoter traits provides valuable insights into establishing sustainable agriculture for cultivating edamame in peatlands.

Actinomycetes are Gram-positive bacteria with high GC content that contribute to their resilience in extreme habitats (Aly et al. 2019). These microorganisms are recognized as decomposers that inhabit environments rich in organic matter for decomposition, such as peatlands, increasing organic matter content (Abdelgawad et al. 2020; Heng et al. 2024). Additionally, actinomycetes have been widely reported for their ability to promote plant growth, particularly within the genus *Streptomyces. Streptomyces* spp. have been reported to produce indole-3-acetic acid, solubilize phosphates, and fix nitrogen (Fatmawati et al. 2019). It has significant potential as a group of microorganisms for use as biostimulant inoculants for edamame cultivation in peatlands. However, the isolation and characterization of *Streptomyces* strains with potential as plant growth promoters in edamame-cultivated peatlands still need to be reported. To the best of our knowledge, there is no published study on the development of peat-derived *Streptomyces* plant growth promoter inoculants specifically designed for cultivated edamame in peatlands. The aim of this study was to isolate and characterize *Streptomyces* sp. from edamame-cultivated peatlands and evaluate their potential as plant growth promoters.

MATERIALS AND METHODS

Sample collection

Actinomycetes were isolated from edamame (*Glycine max*) cultivated peatlands in Arang Limbung Village (0°07'47.38"S 109°22'38.84"E), Kubu Raya District, West Kalimantan, Indonesia. The samples used for isolation, included edamame rhizospheres from 20 plants in the anthesis stage and 16 plants from the reproductive stage (code BT and RT). The samples were then composited separately. The agricultural soil samples were then air-dried at room temperature for seven days before being used for further study.

Isolation of actinomycetes

Actinomycetes were isolated from peat soil using a modified *Streptomyces* isolation method (Tanasupawat et al. 2016). The modification involved adding a wet-heat pretreatment of the samples at 55°C for 10 min. Isolation was performed using a Humid acid - Vitamin (HV) medium supplemented with nalidixic acid (25 µg/mL) and cycloheximide (50 µg/mL). One gram of rhizosphere sample was preheated at 55°C for 10 min and diluted in 9 mL of 0.85% NaCl solution. The soil suspension was then transferred to a sterile tube to obtain 10^{-5} serial dilutions. A total of 100 µL of the 10^{-3} to 10^{-5} of serial dilution suspensions were plated on HV medium. The actinomycete isolates obtained from the isolation process were then purified on The International *Streptomyces* Project 4 (ISP4) medium and incubated at 28°C for further analysis. Isolates were identified based on spore chain morphology using the slide culture method (Akshatha and Kalyani 2022). For long-term preservation, actinomycetes were stored in 20% (v/v) glycerol at -80°C.

Hypersensitivity and hemolytic assays

A hypersensitivity assay was done on 2-month-old tobacco plants, with *Xanthomonas oryzae* as a positive control and *Escherichia coli* DH5α as a negative control. A hemolytic assay was performed by inoculating 7-day-old actinomycetes into blood agar (supplemented with 5% sheep blood). *Staphylococcus aureus* was used as a positive control, and *Escherichia coli* DH5α was used as a negative control (Fatmawati et al. 2019).

Indole acetic acid production

Indole acetic acid production was determined based on a colorimetric assay in ISP4 supplemented with 0.2% L-Tryptophan (L-Trp), according to Wahyudi et al. (2019). Measurement was conducted from a ten-day-old isolate, agitated at 150 rpm in a rotary shaker (Fatmawati et al. 2019). Subsequently, the cultures were centrifuged for 15 minutes at 11,000 rpm. A supernatant of 0.5 mL was mixed with 2 mL of Salkowski reagent (composition: 150 mL H_2SO_4, 7,5 mL $FeCl_3.6H_2O$ 0,5 M, and 250 mL distilled water). Positive test results were indicated by a color change to pink. The concentration of IAA was measured using a spectrophotometer at a wavelength of 535 nm and calculated using a standard curve. *Streptomyces* sp. ARJ11 was used as a positive control (Wahyudi et al. 2019), and uninoculated ISP4 was used as a negative control.

In vivo assay of plant growth-promoting activity

International Rules for Seed Testing was used for the seedling's growth promotion assay (ISTA 2018). Each actinomycete isolate was cultured on ISP4 medium supplemented with 0.02% L-trp for ten days and agitated at 150 rpm. Surface sterilization of edamame seeds (cv. Biomax 1) was conducted by 5 min immersion in 96% alcohol, followed by 30 sec in 2.5% sodium hypochlorite, and rinsed with sterile distilled water. The sterilized seeds were then immersed in the actinomycete culture for 30 min. The uninoculated ISP4 was used as a negative control. Germination tests were conducted in triplicate, each replicating nine seeds. Shoot length, primary root length, lateral root length, and dry weight were observed as growth parameters. The value of growth promoters was calculated by the percentage of delta (Δ) growth parameter value using the formula below (1). The delta (Δ) growth parameter value of each isolate was obtained by measuring the difference between the growth parameter value of each isolate and the negative control.

$$\text{Value of Growth Promoters (\%)} = \frac{\Delta \text{ Growth parameter value's isolate}}{\text{Growth parameter value's negative control}} \times 100$$

Molecular identification based on 16S rRNA gene analysis

Four selected strains were identified molecularly using 16S rRNA genes. The entire genome of actinomycetes was extracted using the Quick-DNA™ Fungal/Bacterial Miniprep Kit, according to the manufacturer's instructions. PCR was conducted for 35 cycles using 27F and 1492R primers

(Majer et al. 2021) under the following conditions: 95°C for 5 min, 95°C for 30 s, 55°C for 30 s, 72°C for 1 min 30 s, and 72°C for 10 min. The amplified fragments were sequenced by First Base Sequencing Services (Malaysia). The 16S rRNA gene sequences were aligned using the BlastN program from NCBI, and the phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with 1000x bootstrap in MEGA 11.0 software. The DNA sequences were deposited in GenBank http://www.ncbi.nlm.nih.gov under the accession numbers: PQ147047 (strain RT34), PQ147048 (strain AR26), PQ147049 (strain AR39) and PQ147050 (strain BT59).

Phosphate solubilization activity

The quantitative measurement of phosphate solubilization was conducted using a spectrophotometer (Amri et al. 2022). One plug of 7-days-old isolates on ISP4 medium was inoculated into 100 mL of Pikovskaya broth supplemented with $Ca_3(PO_4)_2$ and incubated on a shaker (DLAB orbital shaker) at 150 rpm at room temperature for 7 days. After incubation, 1.5 mL of culture was centrifuged at 10,000 g for 5 min. A 600 µL aliquot of supernatant was then reacted with 1,400 µL of reagent (10% ascorbic acid and 0.42% ammonium molybdate in 1N H_2SO_4) and incubated at 45°C for 20 min. Phosphate solubilization activity was measured using a spectrophotometer (DLABSP-UV) at a wavelength of 827 nm. The soluble phosphate concentration was determined using a standard curve of K_2HPO_4. *Streptomyces collinus* ARJ 38 strain was used as a positive control (Wahyudi et al. 2019; Amri et al. 2022).

Nitrogen fixation

The nitrogen fixation trait was determined quantitatively by measuring the nitrogenase activity. The nitrogenase activity of isolate was determined by acetylene reduction assay (ARA) (Suárez-Moreno et al. 2019). The seven-day-old isolate was used as a sample on nitrogen-free bromothymol blue (NFB) semisolid medium in sealed tubes. One mL of acetylene gas (C_2H_2) was injected into the sealed tubes and substituted with one mL of air from the tube, followed by two hours of incubation. Ethylene (C_2H_4) concentration was measured using gas chromatography (GC). Positive control of nitrogenase activity was conducted using *Azotobacter* sp.

Statistical analysis

All experiments were conducted in triplicate and were represented as mean ± SD. The mean data were analyzed using a one-way analysis of variance (ANOVA). Significant differences with a 99.00% confidence level were followed by Tukey's test and analyzed using R software. The P-value was considered statistically significant if it was less than 0.01.

RESULTS AND DISCUSSION

Isolation of actinomycetes from edamame-cultivated peatland

The results showed that a total of 46 isolates were successfully isolated from edamame-cultivated peatlands. Of these, 28 isolates were from the rhizosphere of edamame-cultivated peatland at the anthesis phase, and 18 strains were from the reproductive phase. The hemolysis assay test revealed that 10 actinomycete colonies grown on blood agar produced clear zones, indicating potential pathogenicity toward animals. Additionally, five of these actinomycetes showed necrosis in tobacco leaves in hypersensitivity test. A total of 36 actinomycete isolates that tested negative in both assays were selected for further study. These isolates exhibited spore chain morphology, including rectiflexibiles, spira, and verticillate (Figures 1.A-Figure 1.D). The colony shape varied from smooth to filled with the spores. Colony colors varied, including white, brown, cream-orange and grey-green.

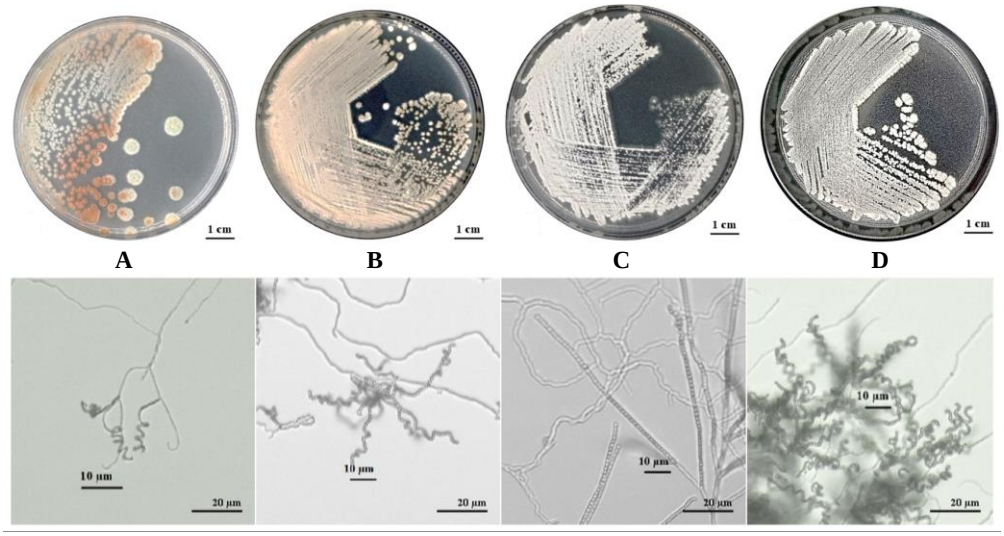

Figure 1. Colony (upper figure) and spore chain type (lower figure) morphologies of the four selected isolates. A. RT34 strain, smooth cream-orange colony with *spira* spore chain; B. AR26 strain, colony with brown spores and *verticillate* spore chain; C. AR39 strain, colony with white spores and *rectiflexibiles* spore chain; D. BT59 strain, colony with grey-green spores and *verticillate* spore chain

Screening based on plant growth-promoting activity

Result showed that all strains produced IAA and were categorized into four groups: low, moderate, high, and very high (Figure 2). Approximately 38.89% of the strains fell into the low category, suggesting that most actinomycetes isolated from the edamame rhizosphere have low IAA production. In this study, actinomycetes from the anthesis phase rhizosphere of edamame produce higher levels of IAA than those from the reproductive phase. Changes in the growth phases of plants result in the secretion of different root exudates, which in turn influence the microbial communities in the rhizosphere (Steinauer et al. 2023). During the anthesis phase, plants exhibit increased metabolic activity and produce specific root exudates, which trigger the chemotaxis mobility of microorganisms in the rhizosphere. This increased availability of nutrients can lead to greater microbial diversity and potentially enable plant growth-promoting bacteria to dominate the microbial community in the rhizosphere (Wagner et al. 2020; Upadhyay et al. 2022). Such bacteria contribute to more favorable plant conditions by mobilizing bound nutrients and interacting with other community members, thereby enhancing the overall microbial diversity (Li et al. 2024).

Isolates with plant growth-promoting capabilities were tested in vivo for edamame germination. In this study, strains that produced IAA generally exhibited improved growth parameters, both significantly and non-significantly (Figure 3). These findings revealed that actinomycetes from edamame-cultivated peatlands can act as plant growth promoters, primarily by enhancing the growth of edamame sprouts. Nonetheless, strains with lower IAA production also showed enhanced growth, suggesting the involvement of other mechanisms. Dicotyledonous plants are more sensitive to exogenous auxin and can respond to hormones at lower concentrations than monocotyledonous plants (Pantoja-Guerra et al. 2023). As a dicotyledonous plant, edamame has a root architecture comprising primary and lateral roots that are more sensitive to exogenous auxin. However, the enhancement of edamame sprout growth appears to be influenced by multiple factors beyond IAA production.

Another factor contributing to this variability is the complex interaction between IAA production and plant growth promotion. Factors, such as plant species, development stage, endogenous auxin production, and ACC deaminase activity might also play significant roles (Pantoja-Guerra et al. 2023). Plants produce growth hormones through complex physiological processes, with variations in quantity and sensitivity. Inoculating plants with auxin-producing inoculants can result in varying responses, from effective growth promotion to a less effective response, depending on the auxin concentration within the plant tissues. Thus, although IAA production is a key factor, it is not the sole determinant of plant growth promotion.

This study evaluated the effectiveness of IAA-producing actinomycetes as plant growth promoters based on their ability to enhance primary root length in seedlings. The selection of the most effective strains was determined by the highest percentage increase in primary root length (Table 1). The results showed that RT34 strain achieved the greatest increase in primary root length, indicating its superior potential for promoting root growth. This was followed by AR26, AR39, and BT59 strains, which also significantly enhanced root length but to a lesser extent (Figure 4).

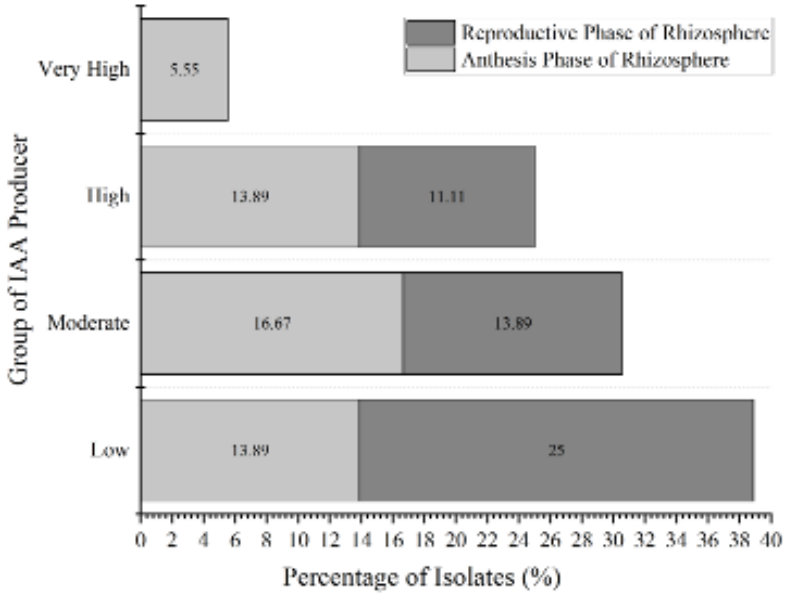

Figure 2. Groups of IAA producers identified based on total IAA production (low < 5 µg/mL; moderate 5-9 µg/mL; high 10-20 µg/mL; very high 30 µg/mL) and the origin of the samples used for isolation (anthesis phase of rhizosphere and reproductive phase of rhizosphere)

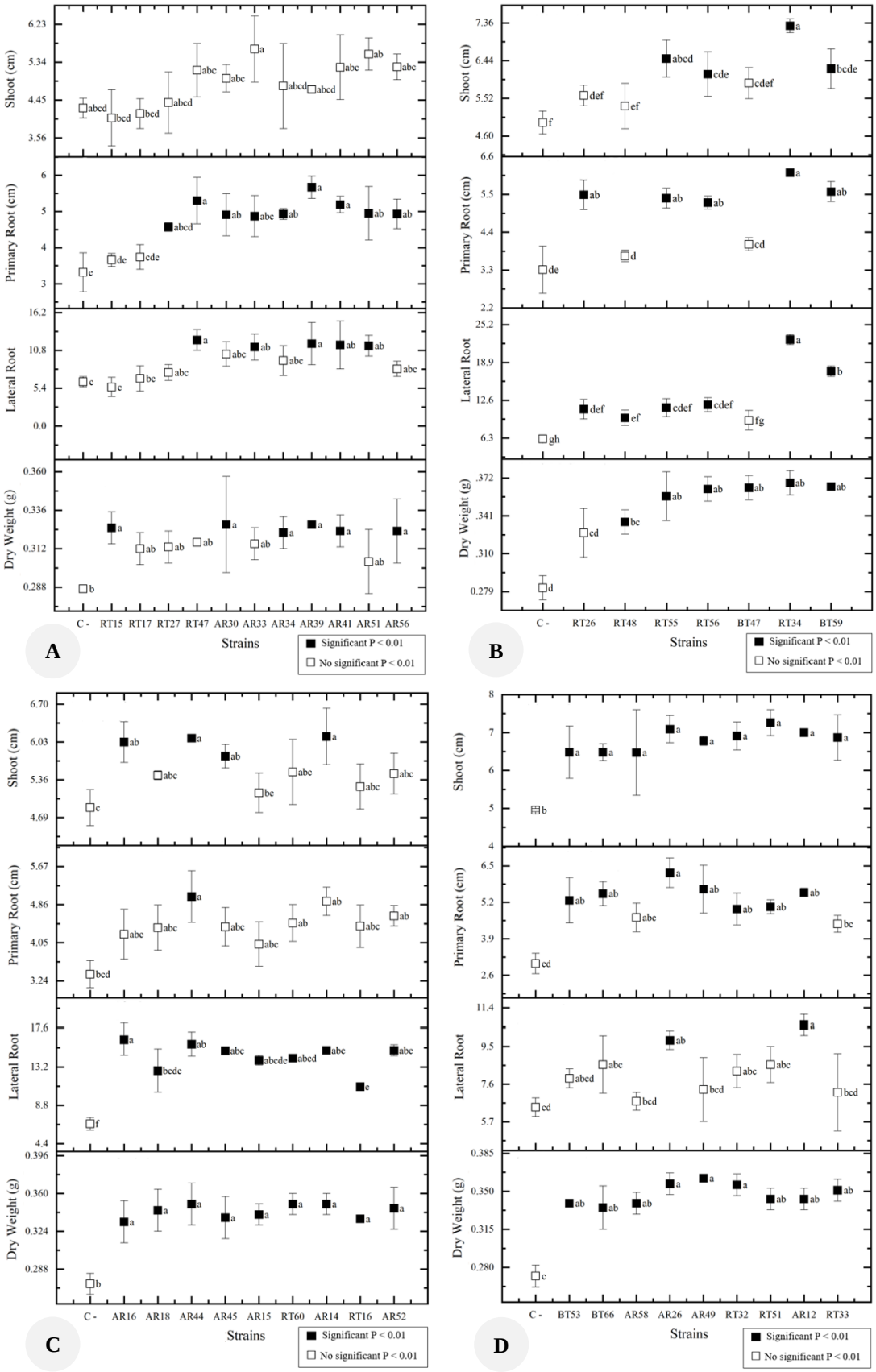

Figure 3. Plant growth-promoting activity of actinomycetes isolates in promoting shoot length, primary root length, number of lateral roots, and dry weight of edamame sprouts. A. Group 1: RT15, RT17, RT27, RT47, AR30, AR33, AR34, AR39, AR41, AR51, AR56. B. Group 2: RT26, RT48, RT55, RT56, BT47, RT34, BT59. C. Group 3: strain AR16, AR18, AR44, AR45, AR15, RT60, AR14, RT16, AR52. D. Group 4: strain BT53, BT66, AR58, AR26, AR49, RT32, RT51, AR12, RT33

Table 1. Screening for the best plant growth-promoting strains based on the value growth promoters

Strains	IAA (µg/mL)	Value of growth promoters (%)			
		Δ Primary root (%)	Δ Lateral roots (%)	Δ Shoot (%)	Δ Dry weight (%)
RT34	3.78 ± 2.49 lmno	85.22 a	269.28 a	47.97 a	30.56 abcd
AR26	9.47 ± 1.64 fghijklm	84.88 a	52.3 hijklmnopqr	43.08 abcd	31.16 abc
AR39	8.27 ± 1.26 ghijklmn	70.68 abc	86.47 cdefghijkl	10.35 hijkl	13.86 fghijklm
BT59	7.55 ± 2.18 ghijklmn	68.65 abcd	183.74 b	26.69 abcdefghi	29.44 abcd
AR49	46.84 ± 9.07 a	67.58 abcd	13.79 opqrs	36.95 abcdefg	32.79 ab
RT26	4.62 ± 1.68 klmno	65.96 abcd	80.72 defghijklm	13.38 fghijkl	12.67 hijklm
AR12	8.53 ± 0.89 ghijklmn	64.07 abcde	63.79 fghijklmnop	41.43 abcde	26.00 abcdef
RT55	3.56 ± 0.65 lmno	63.16 abcdef	84.94 defghijkl	30.90 abcdefgh	26.78 abcd
BT66	9.71 ± 2.27 efghijkl	62.87 abcdef	33.33 klmnopqrs	30.81 abcdefgh	23.14 abcdefghi
RT47	8.78 ± 0.50 fghijklmn	59.53 abcdefg	94.71 cdefghij	20.87 bcdefghijk	10.24 jklmn
RT56	6.47 ± 2.68 ijklmno	59.13 abcdefg	92.77 cdefghijz	24.14 abcdefghij	28.89 abcd
AR41	4.24 ± 0.16 klmno	56.41 bcdefgh	83.53 defghijkl	22.61 abcdefghijk	12.61 hijklm
BT53	11.86 ± 1.42 defghij	55.97 bcdefgh	22.41 mnopqrs	30.81 abcdefgh	24.33 abcdefghi
RT51	14.17 ± 4.29 cdefg	49.18 cdefghi	33.33 klmnopqrs	46.52 ab	26.11 abcdef
AR51	2.84 ± 0.19 mno	49.05 cdefghi	81.76 defghijkl	29.82 abcdefgh	6.14 mn
AR34	5.35 ± 1.00 jklmno	48.50 cdefghi	48.24 jklmnopqr	12.26 ghijkl	12.21 ijklmn
AR56	4.35 ± 0.61 klmno	48.50 cdefghi	29.41 lmnopqrs	22.78 abcdefghij	12.53 hijklm
AR30	10.73 ± 1.71 defghijk	47.83 cdefghi	62.94 fghijklmnopq	16.35 efghijkl	13.98 efghijklm
RT32	2.95 ± 0.05 lmno	46.77 cdefghij	28.16 lmnopqrs	39.64 abcde	30.86 abc
AR33	15.55 ± 0.98 cdef	46.60 cdefghij	78.82 defghijklmn	32.61 abcdefgh	9.85 klmn
AR58	3.44 ± 0.19 lmno	37.68 efghijkl	4.60 qrs	30.74 abcdefgh	24.70 abcdefgh
RT27	2.61 ± 0.43 no	37.68 efghijkl	21.18 nopqrs	3.13 ijkl	9.20 klmn
AR44	16.63 ± 1.21 cd	36.03 fghijkl	137.22 bcd	27.74 abcdefghi	27.74 abcd
AR14	19.86 ± 4.08 c	32.85 ghijklm	126.67 bcde	27.47 abcdefghi	27.47 abcd
RT33	2.42 ± 0.43 no	30.89 hijklm	11.50 opqrs	38.82 abcdef	29.02 abcd
AR52	50.07 ± 0.48 a	23.87 ijklmn	126.67 bcde	26.31 abcdefghi	26.31 abcde
BT47	4.99 ± 0.26 klmno	22.40 ijklmn	50.60 ijklmnopqr	19.55 cdefghijkl	28.99 abcd
RT60	3.44 ± 0.09 lmno	19.49 jklmn	113.33 cdefg	27.77 abcdefghi	27.77 abcd
RT16	8.78 ± 2.50 fghijklmn	17.30 klmn	63.89 fghijklmnop	22.48 abcdefghijk	22.48 abcdefghij
AR45	9.69 ± 1.73 efghijkl	16.97 klmn	126.11 bcde	22.96 abcdefghij	22.96 abcdefghi
AR18	7.04 ± 0.62 hijklmn	16.31 klmn	91.67 cdefghijk	25.61 bcdefghijk	25.61 abcdefg
RT17	16.43 ± 3.97 cde	12.60 lmn	7.65 pqrs	-2.96 kl	8.64 lmn
AR16	3.48 ± 0.28 lmno	12.26 lmn	145.00 bc	21.43 abcdefghijk	21.43 bcdefghijk
RT48	12.27 ± 2.76 defghi	12.21 lmn	57.23 ghijklmnopqr	8.27 hijkl	19.35 cdefghijkl
RT15	3.22 ± 0.33 lmno	10.03 lmn	-11.76 s	-5.48 l	13.36 ghijklm
AR15	13.35 ± 1.34 cdefgh	6.02 mn	109.44 cdefghi	24.17 abcdefghij	24.17 abcdefghi

Figure 4. Growth response of edamame sprouts on A. Uninoculated ISP4 medium; B. RT34; C. AR26; D. AR39; and E. BT59 culture

Strain RT34 not only produced the highest primary root length but also performed the best in enhancing the number of lateral roots and shoot length compared to all other strains. This strain significantly promoted root growth in terms of both primary root elongation and lateral root number. The enhancement is expected to increase the root surface area, aiding plants in the early growth stages to absorb more water (Bhat et al. 2020). The increase in root surface area also leads to higher secretion of root exudates, which facilitates greater colonization of the rhizosphere by microorganisms (Upadhyay et al. 2022). Consequently, elevated microbial populations in the rhizosphere can improve plant health, with the RT34 strain showing the most potential in this regard (Dlamini et al. 2022).

Molecular identification based on 16S rRNA gene

Molecular identification using 16S rRNA gene sequencing revealed that four selected isolates belong to the genus *Streptomyces* (Table 2). Based on BLAST partial sequence 16S rRNA gene alignment, strain RT34 had 99.93% similarity with *Streptomyces griseoruber* strain NBRC 12873.

The closest taxonomy was confirmed based on the phylogenetic tree (Figure 5). Alignment of the 16S rRNA gene sequence with BLAST program from the NCBI gene-bank database revealed that AR26 had 99.18% similarity with *Streptomyces glaucescens* strain NRRL B-2706 (Table 2). Strain AR26 also had a close relationship with *Streptomyces glaucescens* based on the phylogenetic tree (Figure 5). Based on BLAST partial sequence 16S rRNA gene alignment, strain AR39 had 99.26% similarity with *Streptomyces lusitanus* NBRC 13464 as the closest relative species (Table 3). In the same case, the BT59 strain, which had the closest partial sequence 16S rRNA gene to *Streptomyces nigra* 452 of 99.78% based on BLAST results. Species of the genus *Streptomyces*, which comprise the vast majority of taxa within the family Streptomycetaceae, are a predominant component of the microbial population in soils worldwide (Labeda et al. 2012).

Phosphate solubilizing assay

The selected strains achieved phosphate solubilization indices ranging from 1.16 to 1.95 and quantitative soluble phosphate concentrations of 527.76 µg/mL, 292.02 µg/mL, 512.94 µg/mL, and 258.31 µg/mL, respectively (Figure 6). The observed solubilization capacities align with previous findings, where *Streptomyces* species have been reported to solubilize phosphate within a range of 70.36 to 1916.12 µg/mL (Chouyia et al. 2022). Interestingly, *Streptomyces* sp. RT34 had achieved the highest phosphate solubilization (Figure 6). Actinomycetes from edamame-cultivated peatlands possess an important trait for plant growth promotion, namely phosphate solubilization (Bargaz et al. 2021). These findings indicate their capacity to promote plant growth by improving the availability of phosphate, an essential nutrient.

Nitrogen fixation assay

Acetylene reduction assay (ARA) results revealed that the four selected strains exhibited nitrogenase activity ranging from 19.92 to 28.1 nmol h^{-1} $tube^{-1}$ (Figure 7). Interestingly, the highest nitrogenase activity was observed in RT34 and AR39 strains that had nitrogenase activity as high as that of *Azotobacter* sp. as the positive control. *Streptomyces* has been distributed in nature as a free-living nitrogen fixer (Dahal et al. 2017). However, the nitrogenase activity of *Streptomyces* for validation as a significant free-living nitrogen fixation capacity is still limited. *Azotobacter* sp. is widely recognized for its robust nitrogen-fixing ability and plant hormone production, which are beneficial in plant health improvement (Sumbul et al. 2020).

Table 2. The homology analysis of four strains based on 16S rRNA sequences

Strain codes	Closest relative species	Query cover (%)	E-value	Similarity (%)	Accession no.
RT 34	*Streptomyces griseoruber* NBRC 12873	100	0.0	99.93	NR_041086.1
AR 26	*Streptomyces glaucescens* NRRL B-2706	100	0.0	99.18	NR_115773.1
AR 39	*Streptomyces lusitanus* NBRC 13464	100	0.0	99.26	NR_041143.1
BT 59	*Streptomyces nigra* 452	100	0.0	99.78	NR_179868.1

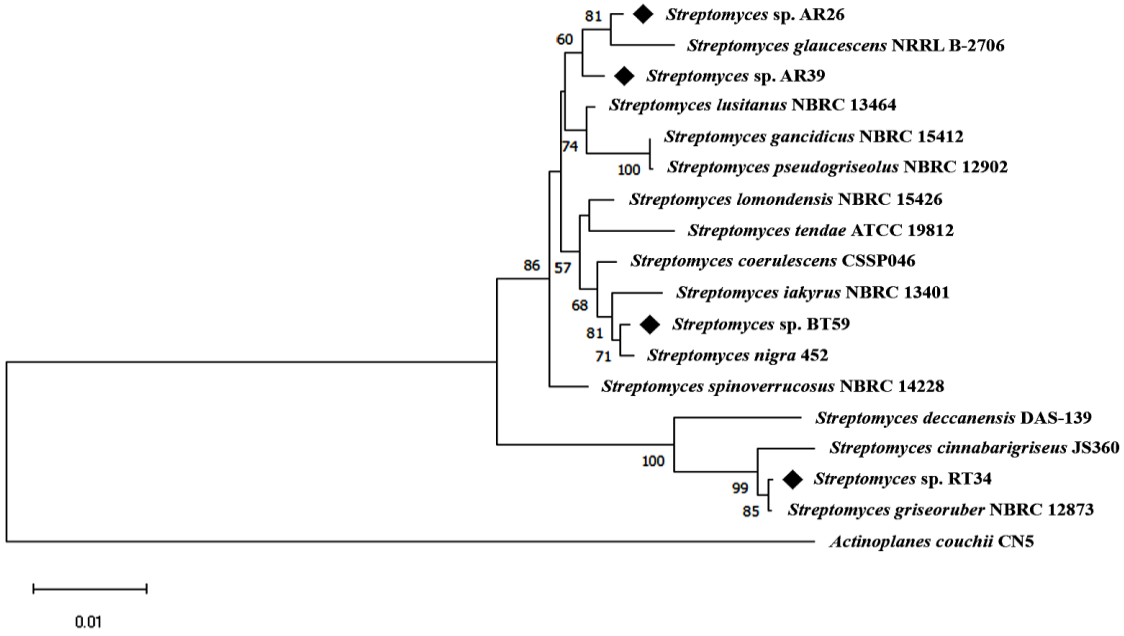

Figure 5. Phylogenetic tree of *Streptomyces* isolated from edamame-cultivated peatland that was constructed using the neighbor-joining method with 1000x boostrap value

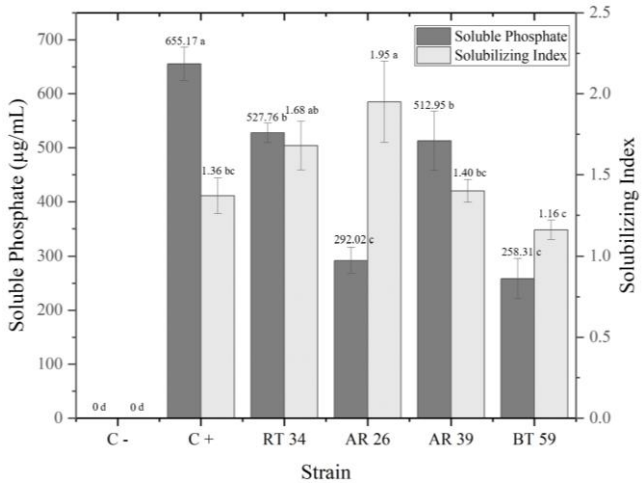

Figure 6. Quantitative phosphate solubilization ability of four selected *Streptomyces* isolates based on solubilizing phosphate in Pikovskaya medium and solubilizing index (P-value 5.62 x 10^{13}). C -: uninoculated ISP4, C +: *Streptomyces collinus* ARJ 38

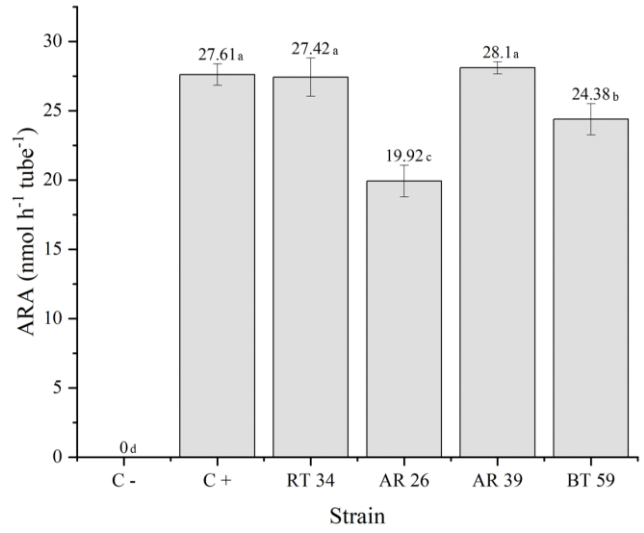

Figure 7. Nitrogenase activity based on the acetylene reduction assay (ARA) of four selected *Streptomyces* isolates (P-value 5.48 x 10^{11}). C -: Uninoculated NFB; C +: *Azotobacter* sp.

Typically, *Streptomyces* sp. is used in co-inoculants with *Azotobacter* sp. to leverage their combined nitrogen-fixing strength (Kaur et al. 2024). This discovery marks a breakthrough in recognizing *Streptomyces* as a viable single inoculant with nitrogen-fixing capabilities comparable to those of *Azotobacter* sp. Specifically in peatlands, formation of nodules by symbiont nitrogen fixers can be inhibited by the phenolic acid's high toxicity (Batish et al. 2007). Therefore, free-living nitrogen fixers are the optimal choice for plant growth promoter inoculants in cultivated peatlands. However, plant growth-promoting inoculants commonly used as free-living nitrogen fixers are commonly recognized diazotroph genera, a group of bacteria capable of fixing atmospheric nitrogen, including *Azotobacter* sp. and *Azospirillum* sp. (Khan et al. 2021). These findings highlight the insight of these free-living nitrogen fixers derived from *Streptomyces* as a single-inoculant nitrogenous fertilizer with robust nitrogen fixation capabilities, supporting the growth of edamame, particularly under peatland conditions.

In conclusion, the results of present findings provide compelling evidence that the four selected *Streptomyces* strains possess significant plant growth-promoting properties. These strains were highly effective in promoting primary root elongation and were validated for indole-3-acetic acid production. Additionally, RT34, AR26, AR39 and BT59 can solubilize phosphate in the range of 258.31 to 527.76 µg/mL. As essential characteristics of leguminous inoculants, these *Streptomyces* strains also exhibit free-living nitrogen fixation, with two of them demonstrating nitrogenase activity as high as that of *Azotobacter* sp.. These findings pave the way for the development of edamame biostimulants specifically designed for peatland agriculture. Additionally, they provide valuable insights into establishing sustainable agricultural practices in peatland ecosystems. Future investigation into *in planta* application should be required for a comprehensive study. To our knowledge, this is the first report on *Streptomyces* isolated from edamame-cultivated peatlands that have plant growth-promoting properties.

ACKNOWLEDGEMENTS

The authors thank the Center for Higher Education Fund from the Indonesian Ministry of Education, Culture, Research and Technology and the Indonesia Endowment Fund for Education for supporting funding through the Indonesian Education Scholarship (ID Number 202101121289). The authors would like to thank the Soil Biotechnology Laboratory of IPB University for providing the *Azotobacter* sp. isolate used in this study.

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