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Research Article

Edamame (*Glycine max* (L.) Merr) Seedling Growth Promotion by *Streptomyces* sp. RT52 and Analysis of Its *pho*D Gene

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ABSTRACT

The excessive use of synthetic fertilisers in edamame (*Glycine max* (L.) Merr) cultivation has raised environmental and health concerns, necessitating sustainable alternatives. This study aimed to characterise the plant growth-promoting properties of RT52 strain, an isolate from acidic peat soil, by assessing its indole acetic acid production, phosphate solubilisation activity, nitrogenase activity, *in vivo* seedling growth-promotion and analysis of *pho*D partial sequences. Based on the results of molecular identification of the 16S rRNA gene, strain RT52 was closely related to the genus *Streptomyces*. Interestingly, seedling growth-promotion revealed that *Streptomyces* sp. RT52 could significantly increase primary root length 104.64 %, shoot length 29.69 %, number of lateral roots 63.35 % and dry weight 18.75 % of edamame sprouts. Colorimetric assays confirmed that *Streptomyces* sp. RT52 produced 30.73 µg mL^{-1} IAA. Its phosphate solubilisation reached 153.50 ± 23.57 µg mL^{-1}, while nitrogenase activity, determined via acetylene reduction assay, was 21.29 ± 1.04 nmol C_2H_4 h^{-1} $tube^{-1}$. Nonetheless, partial sequence of PhoD *Streptomyces* sp. RT52 reached 99.19 % similarity with alkaline phosphatase from *Streptomyces*. Superposition analysis of this sequence confirmed its structural similarity to alkaline phosphatase D from *Bacillus subtilis* (2YEQ), supporting its role in phosphate solubilisation under acidic conditions. These findings demonstrate the potential of *Streptomyces* sp. RT52 as a biofertiliser candidate for promoting edamame growth.

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INTRODUCTION

Edamame (*Glycine max* (L.) Merr) is a high-protein legume widely consumed as a nutritious food source. However, its cultivation relies heavily on synthetic fertilisers (Sari et al. 2021), which pose environmental and health concerns. The excessive use of nitrogen-based fertilisers, such as urea, contributes to soil acidification and nitrate leaching, leading to groundwater contamination and greenhouse gas emissions (Adalibieke et al. 2023). In China, long-term use of phosphate fertilisers has led to an estimated total Cd accumulation of approximately 10.52 tons over a decade (Li et al. 2020). Soluble agrochemical residues were detected, including 95 % Cd, 95 % Pb, and 35 % Ni, in water resources (Neh et al. 2023). These agrochemical residues pose significant health risks, including neurological disorders, cancer, and reproductive issues (Anjaria & Vaghela 2024). Thus, growing consumer awareness of the health benefits of organic edamame has driven research into alternative strategies to reduce chemical fertiliser dependency, highlighting the potential of Plant Growth-Promoting Rhizobacteria (PGPR) as a sustainable solution.

The ideal PGPR for edamame cultivation should enhance nitrogen fixation and phosphate solubilisation while promoting overall plant growth. *Streptomyces* spp. are promising biofertiliser candidates due to their ability to capture atmospheric nitrogen, solubilise phosphate, and produce plant growth hormones (Nazari et al. 2023). They belong to the *Bacteria* domain, *Actinobacteria* phylum, *Actinobacteria* class, *Streptomycetales* order, and *Streptomytaceae* family (Nazari et al. 2023). *Streptomyces corchorusii* CASL5 increased root and shoot growth by 41.73 % and 14.49 %, respectively (Silambarasan et al. 2022), while *Streptomyces hydrogenans* DH16 enhanced pea root and shoot growth by 45.93 % and 38.28 %, respectively (Kaur & Manhas 2022), highlighting their biofertiliser potential.

One key genetic marker associated with phosphate solubilisation is the *pho*D gene, which encodes alkaline phosphatase responsible for hydrolising organic phosphorus into bioavailable forms (Amri et al. 2022). While *pho*D-mediated phosphate solubilisation is well-documented in neutral to alkaline soils, its role in acidic environments remains underexplored. The discovery of a *pho*D-harboring *Streptomyces* strain from acidic peatland presents a novel opportunity to understand phosphate mineralization under low pH conditions. This is particularly relevant for edamame cultivation in acidic soils where phosphate availability is restricted.

In this study, we focused on characterising the plant growth-promoting traits of *Streptomyces* sp. RT52, an isolate obtained from peatland. The strain was previously isolated from the rhizosphere of edamame-cultivated peatland from the Microbiology Laboratory, IPB University collection. We aim to evaluate its ability to produce IAA, nitrogenase activity and solubilised phosphate via *pho*D. To validate its PGPR potential, we assessed its effects on edamame seedling growth, including shoot length, primary root elongation, lateral root formation, and dry biomass accumulation. By elucidating the mechanisms underlying its plant growth-promoting properties, this study provides insights into the suitability of *Streptomyces* sp. RT52 as a biofertiliser for sustainable edamame cultivation.

MATERIALS AND METHODS

Materials

The strain RT52 that used in this study was isolated from edamame rhizosphere. This strain and other *Streptomyces* isolates used in this study include *Streptomyces* sp. ARJ 11 and *Streptomyces collinus* ARJ 38 were maintained on International Streptomyces Project 4 medium (7 days, 29 °C) (10 g Soluble Starch, 1 g $MgSO_4$ x $7H_2O$, 1 g NaCl, 2 g $(NH_4)_2SO_4$, 2 g $CaCO_3$, 1 L aquadest, 1 mL Trace Salts solution (0.1 g $FeSO_4$ x $7H_2O$, 0.1 g $MnCl_2$ x

$4H_2O$, 0.1 g $ZnSO_4$ x $7H_2O$, 100 mL aquadest) and 20 g agar). Control isolates, including *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* DH5α ATCC 8739, were maintained on a nutrient agar medium (24 hour, 29 °C). In addition, *Xanthomonas oryzae* was maintained in Wakimoto agar medium (24 hour, 29 °C) (g L^{-1} composition: $Ca(NO_3)_2$ 0.5 g, $Na_2HPO_4.12H_2O$ 2 g, peptone 5 g, Sucrose 15 g, $FeSO_4.7H_2O$ 0.5 g, Agar 15 g, distilled water 1 L) (Rahma et al. 2023), while *Azotobacter* sp., was maintained on Nitrogen-Free Bromothymol Blue medium (NFB) (5 days, 29 °C) (g L^{-1} composition: malic acid (5 g, K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1 g), $CaCl_2.2H_2O$ (0.02 g), micronutrient solution ($CuSO_4.5H_2O$ 0.04 g; $ZnSO_4.7H_2O$ 0.12 g; H_3BO_3 1.4 g; $Na_2MoO_4.2H_2O$ 1 g; $MnSO_4.H_2O$ 1.175 g in 1000 mL of distilled water) 2 mL; 2 mL bromothymol blue; 4 mL FeEDTA solution; 1 mL vitamin solution KOH 4.5 g; agar 15 g; aquadest 1000 mL with pH 6.5) (Sondo et al. 2023). All isolates were sourced from Prof. Aris Tri Wahyudi's collection at the Microbiology Laboratory, IPB University, except for *Azotobacter* sp., obtained from the Soil Biotechnology Laboratory, IPB University.

Methods

Molecular Identification Based on 16S rRNA Gene

The RT52 strain was identified by molecular sequencing using 16S rRNA gene. The Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA) was used for genome extraction following the manufacturer's instructions. Cells and spores of the *Streptomyces* sp. RT52 (100 mg), cultivated on ISP4 agar medium for 7 days, were resuspended in 200 µL of sterile water or Phosphate-Buffered Saline (PBS) and transferred into a ZR BashingBead™ Lysis Tube containing 750 µL BashingBead™ Buffer. Mechanical lysis was performed using a bead beater at maximum speed for 5 min, followed by centrifugation at 10,000 ×g for 1 min. The supernatant (400 µL) was purified using a Zymo-Spin™ III-F Filter and Zymo-Spin™ IICR Column, with subsequent DNA elution in 100 µL of DNA Elution Buffer and storage at −20°C for 16S rRNA and *pho*D genes analysis. The PCR protocol included predenaturation (95°C, 5 min), denaturation (95°C, 30 s), annealing (55°C, 30 s), elongation (72°C, 1 min 30 s), and post-elongation (72°C, 10 min) for 35 cycles, with specific actinomycete primers, 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-TAC GGT TAC CTT GTT ACG ACT T-3') (Aqlinia et al. 2025). PCR products were electrophoresed on a 1 % (w v^{-1}) agarose gel at 70 V for 30 minutes and stained with Fluorosave. Sequencing was performed by First Base Sequencing Services (Malaysia). Sequences were aligned using the Basic Local Alignment Search Tool-program Nucleotide (BlastN) from the National Centre for Biotechnology Information (NCBI) website. The phylogenetic tree was constructed using the Neighbour-Joining (NJ) method with 1000X bootstrap in MEGA 11.0 software.

Hypersensitivity and Hemolytic Assay

Hypersensitivity assays were conducted on 2-month-old tobacco plants. A 7-days-old actinomycete culture on agitated (150 rpm) ISP4 broth was injected into tobacco leaves and observed for 3 to 5 days post-inoculation (Fatmawati et al. 2019). Positive results were indicated by the occurrence of necrosis on tobacco leaves, which classified the isolate as a plant pathogen. A 24-hour-old culture of *Xanthomonas oryzae* was used as a positive control. Meanwhile, cultures of *Escherichia coli* DH5α and uninoculated ISP4 medium served as negative controls. A hemolytic assay was done by inoculating 7-days-old actinomycete into blood agar (supplemented with 5 % sheep blood) and incubating for 3-5 days at room temperature (Fatmawati et al. 2019). *Staphylococcus aureus* was used as a positive control, and *Escherichia coli* DH5α was used as a negative control. A clear zone around the colony indicated hemolytic activity,

suggesting the isolate's potential pathogenicity.

Indole Acetic Acid Production

Indole acetic acid production of *Streptomyces* sp. RT52 was determined based on a colorimetric assay in ISP4 supplemented with 0.2 % L-Tryptophan (L-Trp), according to Wahyudi et al. (2019). A ten-day-old culture was incubated at 150 rpm in a rotary shaker (Fatmawati et al. 2019), and centrifuged at 11,000 rpm for 15 min. A 0.5 mL supernatant aliquot was mixed with 2 mL of Salkowski reagent (composition: 150 mL H_2SO_4, 7.5 mL 0.5 M $FeCl_3 \cdot 6H_2O$, and 250 mL distilled water), and pink colour development indicated a positive reaction. IAA concentration was measured spectrophotometrically at 535 nm 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.

Phosphate Solubilisation Activity

Phosphate solubilisation activity was conducted quantitatively. The quantitative assay was performed according to Amri et al. (2022). *Streptomyces* sp. ARJ 38 isolate was used as a positive control and uninoculated Pikovskaya supplemented with $Ca_3(PO_4)_2$ was used as a negative control (Amri et al. 2022). Soluble phosphate concentration was determined using a standard curve of K_2HPO_4 with the range of concentrations 0, 20, 40, 60 and 100 µg mL^{-1}.

Nitrogen Fixation

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). Seven-days-old isolate was used as a sample on Nitrogen-Free Bromothymol Blue (NFB) semi-solid 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 determined using gas chromatography (GC) (Hitachi 450, Hayasep Porapak N 2 m column). Chromatograms were analysed, and an ethylene standard curve was generated to quantify ethylene concentrations in the tested samples based on peak area measurements. Positive control of nitrogenase activity was conducted using *Azotobacter* sp.

Plant Growth Promoting Activity

International Rules for Seed Testing was used for the seedling's growth promotion assay (ISTA 2018). *Streptomyces* sp. RT52 was cultured on ISP4 medium supplemented with 0.02 % L-trp for ten days and agitated at 150 rpm. Surface sterilisation of Edamame seeds (cv. Biomax 1) was conducted by 5 min immersion in 96 % alcohol, 30 sec in 2.5 % sodium hypochlorite, and rinsed with sterile distilled water (Fatmawati et al. 2019). The sterilised seeds were then immersed in the actinomycete culture (10^7 CFU mL^{-1}) for 30 min. The ISP4 medium without inoculation was used as a negative control. Shoot length, primary root length, lateral root length, and dry weight were observed as growth parameters. Shoot length was measured from the stem base to the seedling tip, while primary root length was recorded from the stem base to the root tip. The number of secondary roots per seedling was counted. Dry weight was measured after drying at 60°C until constant weight. Germination tests were conducted in triplicate, each replicating nine seeds.

Detection of *pho*D genes

Amplification of the *pho*D gene was done by PCR using *pho*D primers (ALPSF730: 5′-CAGTGGGACGACCACGAGGT-3′ and ALPSR110:5′-GAGGCCGATCGGCATGTCG-3′) and the method described by Amri et al.

(2022). The amplicons were visualised using 1 % (b v^{-1}) gel agarose electrophoresis and sequenced by First Base Sequencing Services (Malaysia). The Blast X program on NCBI aligned the sequence of nucleotides. Construction of the phylogenetic tree was done by MEGA.11 program using the Neighbour-joining method (1000x bootstrap).

Partial amino acid sequence alignment was conducted using Clustal Omega (Walker 2020). Domain prediction and a 3D protein structure model construction were carried out using Swissmodel (Waterhouse et al. 2024). Visualisation of the 3D protein model was conducted using Swissmodel and superposition analysis was performed using PyMol (Schrödinger & Delano 2020).

Statistical Analysis

Data were statistically analysed using One-Way Analysis of Variance (ANOVA) followed by Tukey's test using R software (Nanda et al. 2021). Normality was assessed using Jarque-Bera test, while homogeneity of variances was evaluated using Levene's test. All the experiments were conducted in triplicate. Significant differences were determined by 99.00 level confidence, with a p value less than 0.01 described as significant treatment.

RESULTS AND DISCUSSION

Isolate identification

The edamame rhizospheric strain RT52 was identified based on a partial 16S rRNA gene and spore chain type. Homology analysis of the 16S rRNA partial gene (1381 bp) revealed that strain RT52 was closely related to the genus *Streptomyces* (Table 1 and Figure 1). This result was confirmed using a phylogenetic tree (Figure 2). The 16S rRNA gene sequences information of RT52 isolate were deposited in GenBank under the accession number PQ793833. Isolate RT52 has a white-grey spore in ISP4 and an *open-loop* chain-spore type that confirmed the genus *Streptomyces* (Figure 1). *Streptomyces* sp. is known to have complex spores that can be classified as straight to flexuous (*rectus-flexibilles*), open loops (*retinaculam-apertum*), open or closed spirals (*spira*), or *verticillate* (Barka et al. 2016).

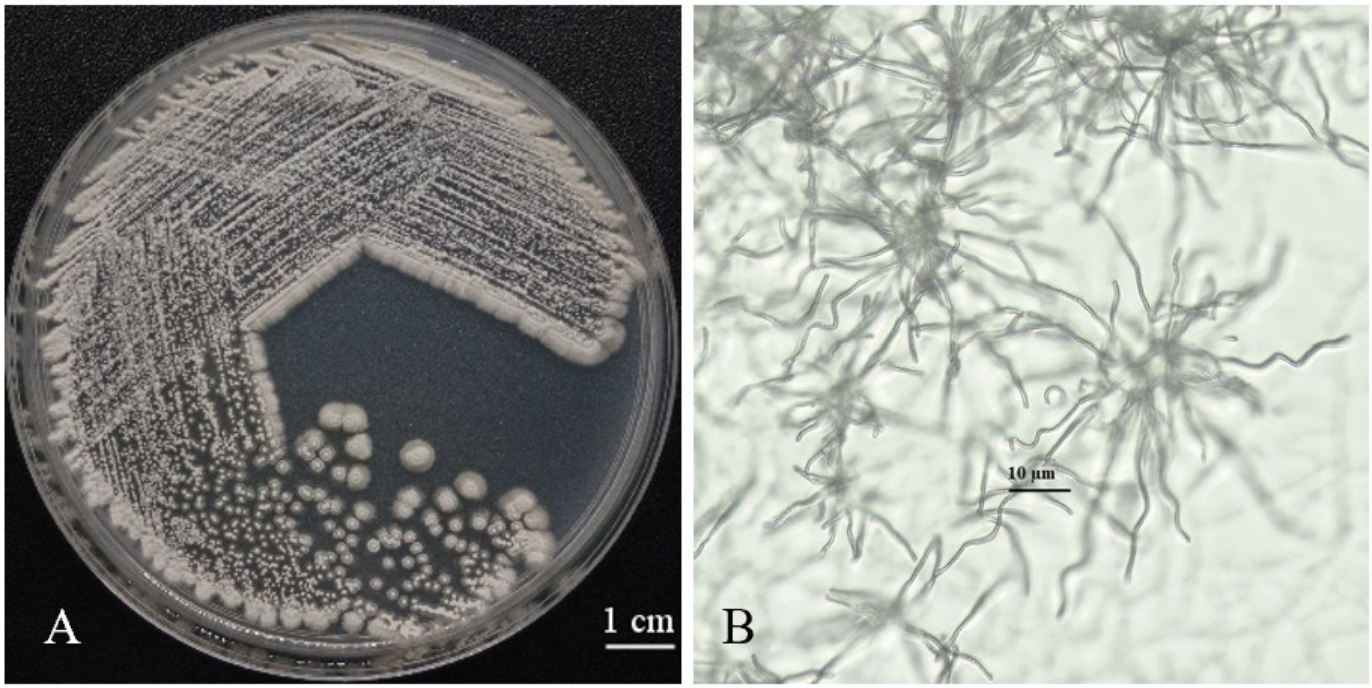

Figure 1. *Streptomyces* sp. RT 52 colony morphology (A) and *open-loop* chain-spore type (B).

Table 1. The homology analysis of RT52 isolate based on 16S rRNA gene sequence.

No	Closest relative species	Length	Query cover (%)	E-value	Similarity (%)	Accession no.
1	*Streptomyces europaeiscabiei* CFBP 4497	1441	100	0.0	99.93	NR_116533.1
2	*Streptomyces scabiei* ATCC 49173	1441	100	0.0	99.85	NR_116531.1
3	*Streptomyces europaeiscabiei* KACC 20186	1521	100	0.0	99.85	NR_042790.1

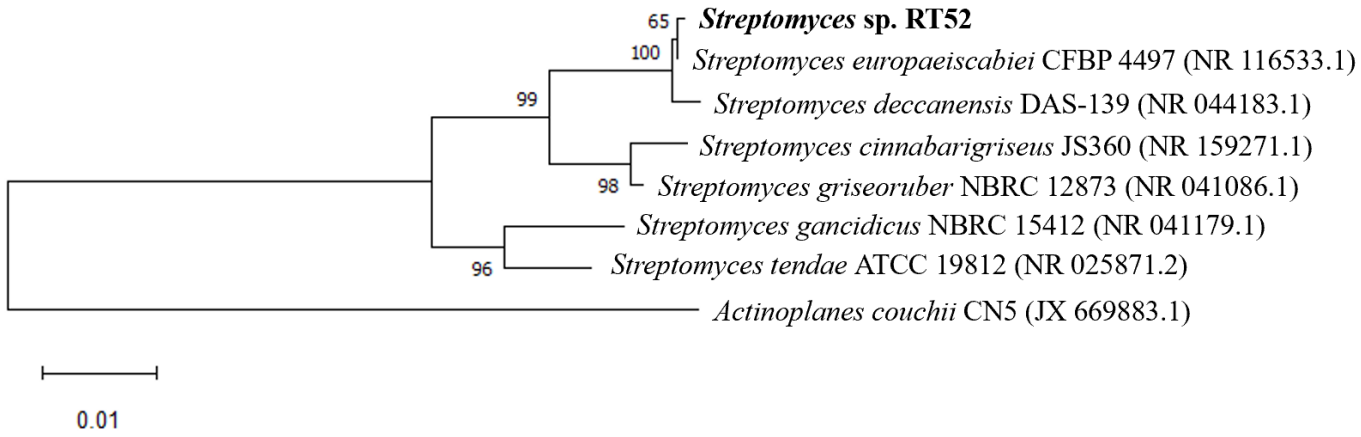

Figure 2. Phylogenetic tree of *Streptomyces* sp. RT52 strain compared to their closest relative species constructed with the neighbour-joining method with 1000x bootstrap value.

Plant Growth-Promoting Activity of *Streptomyces* sp. RT52

Hypersensitivity and hemolytic assays were performed to assess the pathogenicity of the isolates in plants and mammals. *Streptomyces* sp. RT52 tested negative for both assays, indicating the absence of pathogenic traits. These results confirmed that *Streptomyces* sp. RT52 is a non-pathogenic strain suitable for further research.

Streptomyces is a potential plant-growth promoter. Evaluation of the plant growth promoter traits of *Streptomyces* sp. RT52 was confirmed in this study by IAA production, phosphate solubilisation, and nitrogen fixation (Table 2). Therefore, the *Streptomyces* sp. RT52 produced IAA at a concentration of 30.73 µg mL^{-1}, which was higher than the of the positive control (Table 2). Compared to other PGPR, such as *Pseudomonas* and *Bacillus*, which produce IAA in the range of 2.15–26.47 µg mL^{-1} (Lata et al. 2024), *Streptomyces* sp. RT52 exhibited higher IAA production at 30.73 µg mL^{-1}. IAA is a fundamental phytohormone categorised as an auxin that influences plant growth and can stimulate plant growth (Etesami & Glick 2024).

In this study, *Streptomyces* sp. RT52 exhibited nitrogenase activity of 21.29 ± 1.04 nmol C_2H_4 h^{-1} $tube^{-1}$, which was higher than that of *Bacillus*, reported to range from 0.45 to 2.89 nmol C_2H_4 h^{-1} (Henagamage 2022). Nitrogenase activity was determined by calculating the concentration of ethylene produced using the ARA method. This nitrogenase activity confirms that *Streptomyces* sp. RT52 is a free-living nitrogen fixer that can convert atmospheric nitrogen to ammonia and nitrite, which is taken up and utilised by plants. However, as long as the high use of urea fertiliser in agriculture can have a negative impact on the environment, nitrogen fixation is important trait for plant growth-promoting rhizobacteria.

Additionally, the phosphate solubilisation capacity of *Streptomyces* sp. RT52 (153.50 ± 23.57 µg mL^{-1}) exceeded that of plant growth-promoting bacteria such as *Pseudomonas* and *Serratia*, which solubilised between 66.2 and 89.5 µg mL^{-1} (Blanco-Vargas et al. 2020). Specific criteria for determining a potent PGPR include its ability to produce phytohormones at sufficient lev-

Table 2. Plant Growth Promoting Traits of *Streptomyces* sp. RT52.

Isolate	Hypersensitivity Assay	Hemolytic Assay	IAA Production ($\mu g\ mL^{-1}$)	Phosphat Solubilisation ($\mu g\ mL^{-1}$)	Nitrogenase Activity (nmol $C_2H_4h^{-1}tube^{-1}$)
C -	-	-	0.00 ± 0.00 [b]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]
C +	+	+	12.68 ± 1.38 [ab]	12.68 ± 1.38 [ab]	655.17 ± 30.98 [a]
RT 52	-	-	30.73 ± 9.83 [a]	30.73 ± 9.83 [a]	153.50 ± 23.57 [b]

*Notes: C+ hypersensitivity assay: *Xanthomonas oryzae*; C+ hemolytic assay: *Staphylococcus aureus*, C+ IAA production: *Streptomyces* sp. ARJ11, C+ phosphate solubilisation: *Streptomyces* sp. ARJ38; C+ nitrogenase activity: *Azotobacter* sp.; C- hypersensitivity assay, IAA production, phosphate solubilisation and nitrogenase activity: uninoculated medium; C- hemolytic assay: *E. coli* DH5α. Significant 0.01; P value (IAA) 0.00501; P value (phosphate solubilisation) 2.64×10^{-7}: P value (nitrogenase activity) 6.24×10^{-8}.

Table 3. Activity *Streptomyces* sp. RT52 in promoting edamame sprouts growth include shoot length, primary root length, number of lateral roots and dry weight.

Isolate	Shoot Length (cm)	Primary Root Length (cm)	Number of Lateral Root	Dry Weight (g)
C -	4.95 ± 0.04 [b]	3.02 ± 0.36 [b]	6.44 ± 0.47 [b]	0.272 ± 0.01 [b]
RT 52	6.42 ± 0.47 [a]	6.18 ± 0.80 [a]	10.52 ± 1.72 [a]	0.323 ± 0.01 [a]

*Notes: C-: uninoculated medium. Significant 0.01; P value (shoot length) 1.37×10^{-6}; P value (primary root length) 4.72×10^{5}; P value (number of lateral root) 0.00168; P value (dry weight) 5.56×10^{-8}.

els, fix atmospheric nitrogen, and solubilise essential nutrients while demonstrating efficacy under diverse soil conditions. However, the optimal threshold levels for enhancing edamame growth vary depending on soil physicochemical properties and environmental factors.

Figure 3. Edamame seedling promoted by culture of *Streptomyces* sp. RT52 after 5 days. A. Uninoculated ISP 4 treatment. B. Culture of *Streptomyces* sp. RT52 treatment.

The plant growth-promoting activity was validated using germination assay of edamame seedlings (Figure 3). Interestingly, *Streptomyces* sp. RT52 significantly promoted all growth parameters tested in this study, including shoot length, primary root length, number of lateral roots and dry weight, at a confidence level of 99.00 (Table 3 and Figure 3). The increase in primary root length reached 104.64 % (Table 1), exceeding the growth promoted by *Streptomyces alfalfae* XN-04 (81.56 %) (Chen et al. 2021) and *Streptomyces hydrogenans* DH16 (45.93 %) (Kaur & Manhas 2022). Similarly, lateral roots formation increased by 63.35 % (Table 1), surpassing the 62.52 % increase induced by *S. alfalfae* XN-04 (Chen et al. 2021). A larger root surface area enhances water absorption during early plant growth (Bhat et al. 2020) and promotes higher root exudate secretion, which facilitates microbial colonisation in the rhizosphere (Upadhyay et al. 2022). Additionally, shoot length increased by 29.69 % (Table 1), exceeding the 15.49 % increase reported in *Streptomyces corchorusii* CASL5 (Silambarasan et al. 2022).

Phosphate solubilisation is a key trait of plant growth-promoting rhizobacteria (PGPR), particularly in soils where phosphorus availability is a major limiting factor for plant growth. Phosphate-Solubilising Bacteria (PSB) convert bound soil phosphate into bioavailable forms, enhancing plant nutrient uptake efficiency (Asril et al. 2023). PSB exhibit varying phosphate solubilisation efficiency among strains due to differences in gene regulation, enzyme activity, and environmental adaptability. In this study, *Streptomyces* sp. RT52, isolated from acidic peatland, demonstrated a significant phosphate solubilisation capacity of 153.50 ± 23.57 µg mL^{-1} (Table 2), suggesting its potential as biofertiliser in phosphorus-deficient conditions.

Microbial phosphate solubilisation improves phosphorus bioavailability through organic and inorganic acid production, enzymatic hydrolysis, pH reduction, siderophore secretion, and H_2S production (Chouyia et al. 2022). Organic acids, such as gluconic, citric, and oxalic acids, chelate metal ions (Asril et al. 2021), while inorganic acids (HCl, H_2SO_4, HNO_3) also solubilise phosphate (Chouyia et al. 2022). Alkaline phosphatases hydrolise organic phosphorus into inorganic phosphate (Amri et al. 2022). Some *Streptomyces* solubilises phosphate by reducing pH, secreting siderophores, and producing H_2S, which dissolves ferric phosphate, forming ferrous sulphate and releasing phosphorus for plant uptake (Chouyia et al. 2022).

Detection of *pho*D gene of *Streptomyces* sp. RT52

The *pho*D gene analysis showed that the partial PhoD sequence of *Streptomyces* sp. RT52 exhibited 99.19 % similarity to alkaline phosphatase from *Streptomyces* (Table 4). Phylogenetic analysis further confirmed that *Streptomyces* sp. RT52 is closely related to alkaline phosphatase D and belongs to the PhoD family of phosphatases (Figure 4). Alkaline phosphatase synthesised in bacteria is commonly encoded by three homologous genes, *pho*A, *pho*X and *pho*D (Rodriguez et al. 2014). Alkaline phosphatase D abundance is higher in soil bacteria, including Actinomycetes, whereas alkaline phosphatases A and X are commonly found in marine bacteria. Alkaline phosphatase D is a member of the metallophosphatase superfamily, which is characterised by metal ions bound to the active site (Rodriguez et al. 2014). The cofactors in alkaline phosphatase D were Fe^{3+} and Ca^{2+}. This enzyme is synthesised during phosphate deficiency in bacteria. The presence of *pho*D gene by molecular identification confirmed that *Streptomyces* sp. RT52 can synthesise alkaline phosphatase D that is involved in phosphate solubilisation. Alkaline phosphatase can catalise the hydrolysis of esters and phosphoric acid anhydrides and increase the availability of phosphate in soil (Amri et al. 2022).

In this study, structural modelling confirmed homology with *Bacillus subtilis* PhoD (2YEQ) (Figure 5), a well-characterised enzyme in phosphate

Table 4. Homology analysis of partial alkaline phospatase (*pho*D) encoding genes from *Streptomyces* sp. RT52.

No	Homologous sequence	Query Cover (%)	E-value	Similarity (%)	Accession number
1	Alkaline phosphatase (*Streptomyces caniscabiei*)	99	8e-70	99.19	WP_192362777.1
2	Alkaline phosphatase (*Streptomyces caniscabiei*)	99	8e-70	99.19 %	WP_086804156.1
3	Alkaline phosphatase (*Streptomyces deccanensis*)	99	2e-69	98.37 %	WP_239752249.1

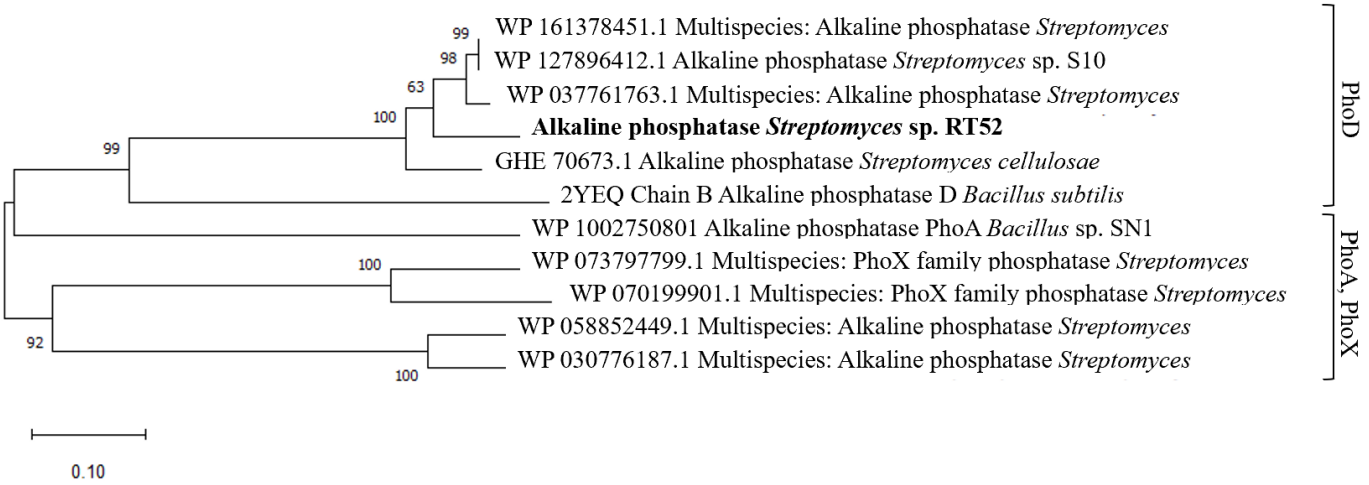

Figure 4. Phylogenetic tree based on partial PhoD amino acid sequences of *Streptomyces* sp. RT52.

metabolism. Prediction of the partial amino acid sequence of PhoD *Streptomyces* sp. RT52 was visualised using the Swissmodel software (Figure 5). The Ramachandran plot showed that the most favoured region was reach 87.7 % (Supplementary). This value indicates that the structure is sufficiently consistent. The G-factor value reach -0,10, G factor value higher than -0.5 showed the usual conformation. Superposition analysis predicted protein with the original protein from *Bacillus subtilis* (2YEQ) showed high similarity in topological structure with an RMSD value below 2 Å, namely 0.84 Å. Notably, overlapping structures were observed, particularly in the partial residue region (206–336) (Figure 5). Therefore, the partial amino acid sequence of alkaline phosphatase D of *Streptomyces* sp. RT52 is predicted to have similar

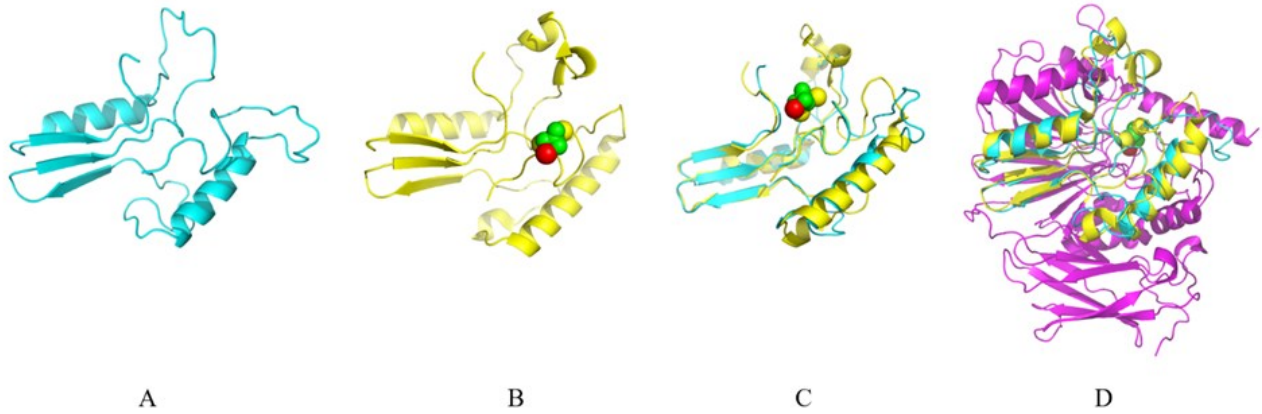

Figure 5. Prediction of the 3D structure of partial phoD *Streptomyces* sp. RT52 and superposition of PhoD *B. subtilis* (2YEQ). A. Partial PhoD of *Streptomyces* sp. RT52 (cyan) with an RMSD 0.84 Å. B. Specific residue (206-336) of PhoD of *B. subtilis* (2YEQ) (yellow), including Fe^{3+}(green), Ca^{2+}(yellow) and phosphate (red) molecules. C. Superposition partial PhoD of *Streptomyces* sp. RT52 (cyan) and specific residue (206-336) of PhoD of *B. subtilis* (2YEQ) (yellow). D. Superposition partial PhoD of *Streptomyces* sp. RT52 (cyan) and PhoD of *B. subtilis* (2YEQ), yellow: specific PhoD residue of *B. subtilis* (2YEQ) (206-336) and magenta: all residues of PhoD of *B. subtilis* (2YEQ).

properties and functions as the experimental protein alkaline phosphatase D from *Bacillus subtilis* (2YEQ).

Investigating the structure of the *pho*D gene in *Streptomyces* sp. RT52 is essential for elucidating its functional role in phosphate solubilisation, particularly in acidic peatland environments. The *pho*D gene encodes alkaline phosphatase, an enzyme that hydrolises organic phosphate esters into bioavailable inorganic phosphate, thereby enhancing phosphorus availability for plant uptake (Amri et al. 2022). The findings suggest that *Streptomyces* sp. RT52 employs enzymatic hydrolysis as a phosphate solubilisation mechanism, with alkaline phosphatase playing a crucial role in catalysing the breakdown of organic phosphate compounds, thereby facilitating phosphorus acquisition by plants.

CONCLUSIONS

In conclusion, this study demonstrated that *Streptomyces* sp. RT52 plant growth promoter traits, included IAA hormone production, phosphate solubilisation activity and nitrogenase activity. The plant growth-promoting activities of *Streptomyces* sp. RT52 confirmed by significantly promoted edamame sprout included shoot length, primary root length, number of lateral roots and dry weight parameter. IAA production was validated at a concentration of 30.73 µg mL^{-1}. *Streptomyces* sp. RT52 has nitrogenase activity that revealed by ARA assay, of 21.29 nmol $C_2H_4h^{-1}tube^{-1}$. A phosphate-solubilising assay confirmed a soluble phosphate capacity of 153.50 µg mL^{-1}. The presence of the *pho*D gene confirmed the ability to produce alkaline phospatase, which plays an important role in solubilising phosphates. Further investigation is necessary for the comprehensive plant growth promoter activity of *Streptomyces* sp. RT 52 by *in planta* application.

AUTHOR CONTRIBUTION

IE: Helped in planning the research subject, conceptualisation, methodology, data curation, examination, revising the data, interpretation of the results, data analysis, reviewing the work and writing the original manuscript. AT: Conceptualisation, methodology, data curation, supervision, writing-review. RIA: Conceptualisation, methodology, data curation, supervision, writing-review. EH: Conceptualisation and methodology. ATW: planning for the research subject, conceptualisation, methodology, data curation, supervision, investigation and writing-review.

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CONFLICT OF INTEREST

Authors declares there is no conflict of interest.

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