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Article

Isolation and characterization of entomopathogenic fungus *Beauveria tenella* from the mushroom of larch forest ecosystem

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Abstract

A pure culture of the entomopathogenic fungus Beauveria tenella was isolated from mushroom tissues and evaluated for its plant growth-promoting properties. The fungal isolate was identified by sequencing of the internal transcribed spacer (ITS) region, which showed 99% sequence similarity to reference sequences deposited in GenBank. The ability of the isolate to solubilize inorganic phosphate was assessed on Pikovskaya's agar medium, where clear halo zones were observed around fungal colonies.

The phosphate solubilization index was 2.19 ± 0.05, indicating effective phosphate-solubilizing activity. In addition, the isolate produced indole-3-acetic acid (IAA), a phytohormone associated with plant growth and root development, at a concentration of 8.12 μg mL^{-1} as determined by the Salkowski colorimetric method. These results demonstrate that B. tenella possesses plant growth-promoting traits through phosphate solubilization and IAA production and may have potential applications as a biofertilizer or multifunctional microbial inoculant in sustainable agriculture.

Keywords: Pure culture, entomopathogenic, phosphate solubilization, IAA production

Introduction

Entomopathogenic fungi are an important group of microorganisms that naturally regulate insect populations and have been widely investigated as biological control agents in sustainable agriculture. Among them, species of the genus Beauveria are recognized for their pathogenicity against a broad range of insect pests and their potential use in biopesticide development [1]. Several species, including Beauveria bassiana, B. brongniartii, B. amorpha, and B. caledonica, have been studied extensively for their insecticidal activity and ecological importance [1].

In addition to their role in biological control, some Beauveria species can establish endophytic associations with plants and contribute to plant growth and health. These fungi have been reported to promote plant development through the production of phytohormones, such as indole-3-acetic acid (IAA), and by enhancing nutrient availability through phosphate solubilization [2].

IAA is one of the most important auxins involved in cell division, cell elongation, root development, and other physiological processes in plants [3,4]. Similarly, phosphate-solubilizing microorganisms can improve phosphorus availability in soil, thereby enhancing plant growth and productivity [5–7]. Among the species within this genus, Beauveria tenella is known as an entomopathogenic fungus capable of producing cuticle-degrading enzymes and bioactive secondary metabolites that contribute

to insect pathogenicity [8]. Previous studies have demonstrated the effectiveness of B. tenella as a biological control agent against several insect pests, including Anoplophora malasiaca in citrus orchards [9]. Despite its importance as an entomopathogenic fungus, information regarding its occurrence in natural forest ecosystems and its potential plant growth-promoting properties remains limited.

Mongolia possesses extensive forest ecosystems dominated by larch (Larix sibirica), which harbor diverse fungal communities. However, studies on the isolation, molecular identification, and functional characterization of entomopathogenic fungi from forest mushrooms are scarce. Understanding the diversity and ecological functions of these fungi may provide valuable information for the development of biological control agents and microbial inoculants for sustainable agriculture.

Therefore, the objective of this study was to isolate and molecularly identify an entomopathogenic fungal strain obtained from mushroom tissues collected in a larch forest ecosystem and to evaluate its plant growth-promoting traits through phosphate solubilization and indole-3-acetic acid (IAA) production.

Materials and methods

Sample collection

Mushroom samples of Suillus luteus were collected in 2024 from a larch (Larix sibirica) forest located in Erdene soum, Tuv Province, Mongolia. Each sample was placed in a sterile plastic bag, labeled with collection information, transported to the laboratory, and stored at 4 °C until processing. Fungal isolation was initiated within 48 h after collection [14,15].

Fungal isolation

Mushroom samples were washed thoroughly under running tap water to remove surface debris. Tissue samples were surface-sterilized by immersion in 70% ethanol for 30 s, followed by 1% sodium hypochlorite solution for 2 min, and then rinsed three times with sterile distilled water. Sterilized tissues were cut into approximately 0.5 × 0.5 cm segments using sterile scalpels and forceps and placed on potato dextrose agar (PDA) supplemented with ampicillin (50 μg mL^{-1}). Plates were incubated at 26 ± 2 °C for 14 days. Emerging fungal colonies were sub-cultured repeatedly on fresh PDA until pure cultures were obtained.

Plant growth-promoting phosphate solubilization

The phosphate-solubilizing ability of the fungal isolate was evaluated using Pikovskaya's (PVK) agar medium [16]. The medium was sterilized at 121 °C for 15 min and dispensed into 60 × 15 mm Petri dishes. A 5-mm mycelial plug from an actively growing PDA culture was inoculated onto the center of each plate and incubated at 25 °C for 10 days.

Phosphate solubilization was assessed by measuring the diameter of the clear halo zone surrounding the fungal colony. Colony diameter and halo diameter were recorded on days 1, 3, 5, and 7 after inoculation. Each assay was conducted in triplicate. The phosphate solubilization index (SI) was calculated according to the following equation [17]:

$$\text{Solubilization index(SI)} = \frac{\text{colony diameter} + \text{clear zone diameter}}{\text{colony diameter}}$$

Indole-3-acetic acid (IAA) production

IAA production was determined using the colorimetric method with Salkowski reagent [18]. Fungal isolates were cultured in potato dextrose broth (PDB) supplemented with 1% L-tryptophan and incubated at 25 °C with shaking at 120 rpm for 14 days.

Following incubation, 1.5 mL of culture broth was centrifuged at 10,000 rpm for 15 min. One milliliter of the supernatant was mixed with 2 mL of Salkowski reagent consisting of 50 mL of 35% $HClO_4$ and 1 mL of 0.5 M $FeCl_3$. The reaction mixture was incubated in darkness at room temperature for 30 min. Development of a pink coloration indicated IAA production. Absorbance was measured at 535 nm using a spectrophotometer, and IAA concentration was quantified using a standard calibration curve.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from pure fungal cultures using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.PCR amplification was performed in a 25 µL reaction volume containing 12 µL of 2× Power Taq PCR Master Mix, 1 µL each of forward and reverse primers, 1 µL of genomic DNA template, and 9.5 µL of nuclease-free water. The fungal internal transcribed spacer (ITS) region was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [19]. PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 2 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min.

Amplified products were separated on 1% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide. PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Germany) and sequenced using the Sanger sequencing method.

Sequence chromatograms were edited using BioEdit software and aligned in MEGA 11. Species identification was performed by comparing ITS sequences with reference sequences deposited in the GenBank database using BLAST.

Phylogenetic analysis

The ITS sequence obtained in this study and related reference sequences retrieved from GenBank were aligned using MEGA 11 [20]. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method based on the Kimura two-parameter model with 1,000 bootstrap replications.

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean ± standard deviation (SD). Descriptive statistical analyses were conducted using Microsoft Excel 2010.

Results

Isolation and Identification of Beauveria tenella

A fungal isolate was recovered from the internal tissues of *Suillus luteus* collected from a larch (*Larix sibirica*) forest in Erdene soum, Tuv Province, Mongolia (47°44′50.0″ N, 107°38′27.0″ E). Repeated subculturing on potato dextrose agar (PDA) resulted in a pure fungal culture, which was subsequently identified as *Beauveria tenella* based on molecular characterization.

The isolate produced white, cottony colonies with abundant aerial mycelium on PDA (Figure 1). Colony morphology was characterized by dense white mycelial growth on the upper surface and pale yellow to cream pigmentation on the reverse side of the culture plate, which is consistent with morphological characteristics reported for species of the genus *Beauveria*.

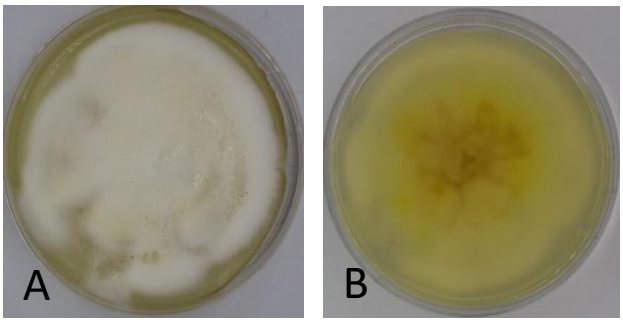

Figure 1. Colony morphology of Beauveria tenella cultured on potato dextrose agar (PDA). (A) Front view showing dense white aerial mycelium; (B) Reverse view showing pale yellow pigmentation.

Indole-3-acetic acid (IAA) production by *Beauveria tenella*

The ability of *B. tenella* to produce indole-3-acetic acid (IAA) was evaluated using the Salkowski colorimetric assay. Following incubation with Salkowski reagent, the culture supernatant developed a pink coloration, indicating the presence of IAA (Figure 2). IAA concentration was quantified spectrophotometrically at 535 nm using a standard calibration curve ($y = 0.0161x + 0.0289$). The isolate produced 8.12 ± 0.42 μg mL^{-1} of IAA. The development of a pink color in the reaction mixture confirmed the production of IAA by *B. tenella*, whereas no color change was observed in the uninoculated control (Figure 2).

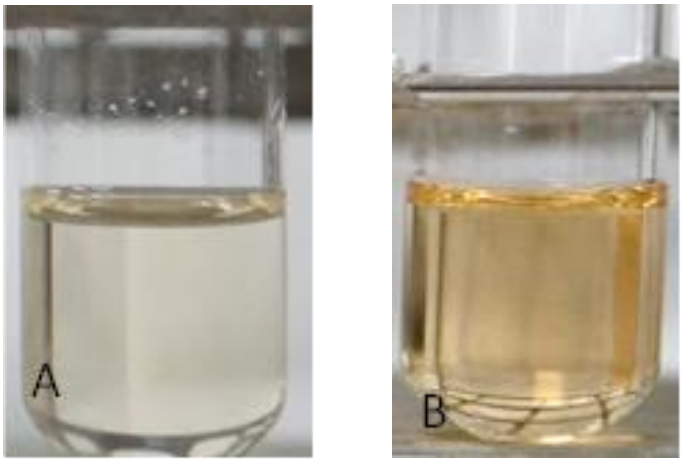

Figure 2. Detection of indole-3-acetic acid (IAA) production by *Beauveria tenella* using Salkowski reagent. The pink coloration indicates positive IAA production, whereas the control remained unchanged.

Formaton of clear zones and phospate solubilization index on PVK agar

The clear zone became visible in the isolates of *Beauveria tenella* on the first day of incubation. Phosphate solubilization reached its peak on the third day, after which the phosphate solubilization index declined beginning on the fifth day, while the fungal biomass continued to increase (Figure 3,4). In addition to parasitizing insects, some strains of *Beauveria tenella* have the ability to solubilize both organic and inorganic phosphate compounds. Phosphate solubilization increased during the middle of the incubation period, which is in agreement with the results reported by [21], who also demonstrated a gradual increase in mobilized phosphorus by fungal isolate F1 in liquid cultures. However, toward the later stages of incubation, a decline in phosphate solubilization was observed. This decrease is consistent with the findings of [22]. According to [23], the reduction could be attributed either to the accumulation of soluble phosphate, which exerts an inhibitory effect on further solubilization of TCP or RP, or to the depletion of the carbon source, which limits both the production of organic acids and microbial activity.

Phosphate Solubilization Activity of Beauveria tenella

The phosphate-solubilizing ability of *Beauveria tenella* was evaluated on Pikovskaya's (PVK) agar medium. Clear halo zones surrounding the fungal colony were observed on the first day of incubation, indicating the solubilization of insoluble phosphate compounds (Figure 3). The phosphate solubilization index (SI) increased during the early stages of incubation and reached its maximum value on the third day. Thereafter, the SI gradually declined from the fifth day onward, despite continued expansion of fungal biomass (Figure 4). The mean phosphate solubilization index of *B. tenella* was **2.19 ± 0.05**, confirming its ability to solubilize insoluble phosphate under *in vitro* conditions.

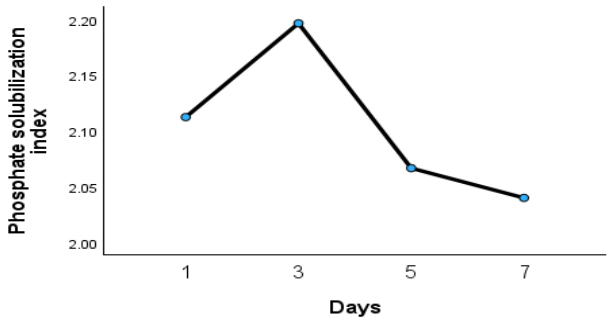

Figure 3. Phosphate solubilization index (SI) of Beauveria tenella grown on Pikovskaya's (PVK) agar containing tricalcium phosphate (TCP) during 7 days of incubation. Values represent the mean of three replicates.

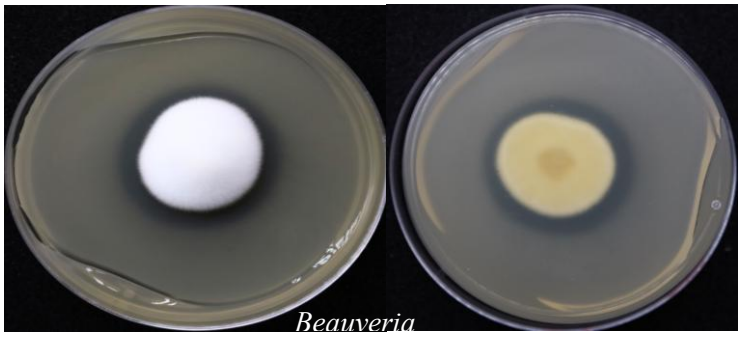

Figure 4. Colony morphology and halo-zone formation of *Beauveria tenella* on PVK agar containing tricalcium phosphate (TCP) after 7 days of incubation. Left: front view; right: reverse view.

Molecular identification of fungal isolates using molecular markers

The ITS region of the fungal isolate was successfully amplified and sequenced. BLAST analysis of the obtained ITS sequence revealed 99% sequence similarity with reference sequences of *Beauveria tenella* deposited in the GenBank database, confirming the taxonomic identity of the isolate.

Phylogenetic analysis based on ITS sequences further supported the molecular identification of the isolate (Figure 5). The isolate clustered within the *Beauveria tenella* clade together with closely related reference strains obtained from GenBank. High bootstrap support values (>70%) confirmed the stability of the major branches. In contrast, *Penicillium polonicum*, which was used as an outgroup, formed a distinct lineage separate from the *Beauveria* isolates.

The combined BLAST and phylogenetic analyses confirmed that the fungal isolate recovered from *Suillus luteus* belonged to the species *Beauveria tenella*.

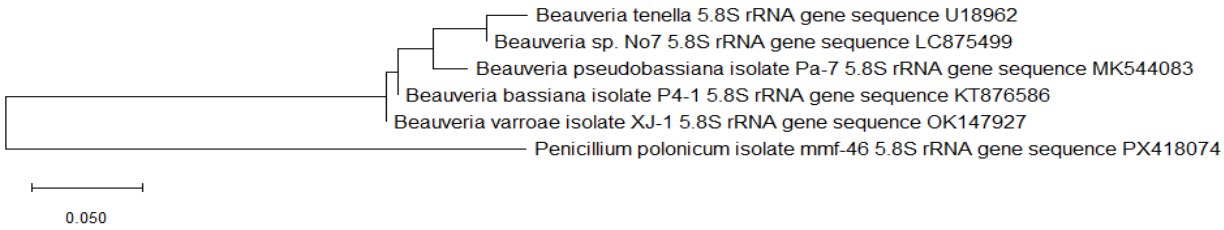

Figure 5. Maximum Likelihood phylogenetic tree based on ITS sequences showing the relationship between the fungal isolate obtained in this study and related Beauveria reference sequences retrieved from GenBank. Bootstrap values based on 1,000 replications are indicated at branch nodes. Penicillium polonicum was used as the outgroup.

Discussion

In this study, an entomopathogenic fungus was successfully isolated from the fruiting body of *Suillus luteus* collected from a larch (*Larix sibirica*) forest ecosystem in Mongolia. Molecular identification based on ITS sequence analysis confirmed the isolate as *Beauveria tenella*. The isolate exhibited morphological characteristics typical of the genus *Beauveria*, including dense white aerial mycelium and cream-yellow pigmentation on the reverse side of colonies. Similar morphological characteristics have been reported for *B. tenella* isolated from soil and insect hosts [24].

Besides their role as biological control agents, entomopathogenic fungi have increasingly been recognized for their plant growth-promoting properties. One of the most important mechanisms is phosphate solubilization, which increases the availability of phosphorus for plant uptake. In the present study, *B. tenella* formed clear halo zones on Pikovskaya's agar and exhibited a phosphate solubilization index of 2.19 ± 0.05, indicating its ability to mobilize insoluble phosphate compounds. Similar phosphate-solubilizing activity has been reported in other *Beauveria* species, including *B. bassiana* and *B. caledonica* [25–27]. The ability of these fungi to release phosphorus has been attributed to the production of organic acids such as citric, oxalic, lactic, formic, and orotic acids, which reduce the pH of the surrounding medium and facilitate phosphate dissolution [28]. Therefore, the phosphate-solubilizing activity observed in *B. tenella* may contribute to improved nutrient availability in the rhizosphere.

The fungal isolate also demonstrated the ability to produce indole-3-acetic acid (IAA), with a concentration of 8.12 ± 0.42 μg mL^{-1}. IAA is one of the most important auxin phytohormones involved in root development, cell elongation, and overall plant growth [29]. The production of IAA by *B. tenella* suggests that this species may possess plant growth-promoting potential in addition to its entomopathogenic activity. Similar findings have been reported for *B. bassiana*, which has been shown to produce IAA and enhance plant growth parameters such as plant height, root development, biomass accumulation, and stress tolerance in several crop species [30–33]. Although the effects of *B. tenella* on plant growth have been less extensively studied, the present results indicate that this species may play a comparable ecological role.

The combination of phosphate solubilization and IAA production observed in *B. tenella* suggests that this fungus may function as a multifunctional microorganism capable of contributing to both pest management and plant growth promotion. However, the present study was conducted under laboratory conditions, and further investigations are required to evaluate its effectiveness under greenhouse and field conditions and to determine its potential application as a biofertilizer or microbial inoculant.

Conclusion

An entomopathogenic fungal isolate obtained from a mushroom collected in a larch forest ecosystem in Mongolia was identified as *Beauveria tenella* based on ITS sequence analysis. The isolate demonstrated plant growth-promoting traits through phosphate solubilization (SI = 2.19 ± 0.05) and production of indole-3-acetic acid (8.12 ± 0.42 μg mL^{-1}). These findings suggest that *B. tenella* has potential as a multifunctional microorganism with applications in sustainable agriculture. Further studies are needed to evaluate its effects on plant growth and stress tolerance under greenhouse and field conditions.

Author's contribution

Ts.Lkh. conducted the fungal isolation experiments, analyzed the data, and interpreted the results. E.V. and G.A. supervised the study, contributed to the experimental design, literature review, and manuscript preparation. E.Z. and A.T. carried out field surveys and sample collection. O.O. contributed to the literature review. U.B. performed the molecular identification and phylogenetic analysis of the fungal isolate. U.J. assisted with polymerase chain reaction (PCR) analysis. All authors reviewed and approved the final manuscript.

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