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First Report of Entomopathogen *Beauveria felina* as a Fungal Endophyte in Banana Plants

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ABSTRACT

This short article reports the isolation and identification of *Beauveria felina*, an entomopathogen as a fungal endophyte of banana, isolated from the healthy roots of locally cultivated banana from Nagaland, a northeastern state of India. Identification was done on the basis of morphological characteristics and molecular identification using ITS1 (forward) and ITS4 (reverse) primers. In Petri plates, the colony was observed as white creamish, the aerial hyphae from the colonies produced erect synnematous structures which were hairlike, long and abundant. Microscopically, the conidia were single celled, oval to ellipsoidal, hyaline, appearing at the tip of conidiogenous cell, 10×14 µm in size under 40× objective lens. Molecular identification confirmed that the closest identity of the isolate was *Beauveria felina*. To our best knowledge, this is the first report of *B. felina* as a fungal endophyte associated with banana or any other plant in the world.

Key words: Banana, *Beauveria felina*, Entomopathogen, First report, Fungal endophyte.

Banana is considered to be among the highly important fruit around the world having its origin in the South East Asia (Hazarika *et al*., 2021). With an average production of 33 million metric tonnes per year, India is the largest producer of banana in the world (FAO, 2024). Despite being the largest producer of banana, there are several diseases that hampers the growth, production and yield of banana. Out of all the diseases, utmost attention received from plant pathologists around the globe in the last 2 decades is due to Panama wilt, also known as Fusarium wilt, which is a major destructive disease of banana (Ordonez *et al*., 2015; Westerhoven *et al*., 2022). In India, the disease is widespread throughout the growing regions with race 1 being the most common (Prasadji and Smith, 2007). Presently, there are no successful approach to combat the disease other than using tissue culture plants that are pathogen free and quarantine schemes (Kema *et al*., 2021) which are laborious. Use of chemical pesticides are known to have disastrous effects on the quality of soil and also causes degradation of the environment (Roopashree *et al*., 2024). In recent years, there is increased interest in novel approaches based on microbial inoculation as a way to lessen the adverse effects of standard farming procedures (Roopashree *et al*., 2024). Both microorganisms and plants benefit from their symbiotic interaction with one another. More importantly, plant microbe symbiosis has an impact on plant growth and health, which successfully improves agricultural qualities and increases soil quality and nutrient cycling (Sahu *et al*., 2017). To preserve and increase their yield, safe and environmentally friendly substitute techniques must be created (Roy *et al*., 2021). With this in mind, collection, isolation and identification of fungal endophytes from healthy leaves and roots of wild and cultivated banana was carried out in four districts (Chumoukedima, Kohima, Peren and Mokokchung districts) of Nagaland, India.

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The sample collection/survey and experiment were conducted during the year 2021-2024. All the laboratory works were conducted in the Department of Plant Pathology, School of Agricultural Sciences, Medziphema Campus, Nagaland University, Nagaland, India.

Collection and isolation of the fungal endophytes

The healthy leaves and roots samples were collected from four districts of Nagaland (Chumuokedima, Kohima, Peren and Mokokchung districts) and isolation of the fungal endophytes was carried out under *in vitro* conditions as per the method given by (Zakaria and Aziz, 2018; Zakaria *et al*., 2016), respectively.

Morphological and molecular identification of the fungal isolate

The isolated fungal endophyte was identified based on their cultural, morphological and molecular characteristics. For molecular identification, the fungal endophytes were grown in sterilized Potato dextrose broth of 100 ml containing 10 µl of Tween 80 and incubated at shaker incubator for 7 days at 28°C. The mycelium was sieved through a sterile Whatman filter paper No. 1 and the excess broth was drained out. Mycelium weighing about 5-10 g was taken and grounded with the help of a mortar and pestle in liquid nitrogen. DNA extraction was carried out using HiMedia DNA extraction kit. After DNA extraction, PCR amplification was also carried out using HiMedia PCR amplification kit and PCR was performed in Thermal Cycler (Bio-Era, Model ADEPT, India). Sequencing of ITS region was done using ITS1 (Forward) (5′- TCCGTAGGTGA ACCTG CGG-3′) and ITS4 (Reverse) (5′-TCCTCCGCTTAT TGATATGC- 3′) primers (White *et al*., 1990). The PCR products were purified and sequenced from BioKart, Bangalore, India. The sequences were lined up with the help of BioEdit Sequence Alignment Editor Version 7.0.5 software by (Hall, 1999) to achieve consensus sequences. Comparison of the consensus sequences with other DNA sequences was done in GenBank using BLAST (Basic Local Alignment Search Tool) in NCBI (National Centre for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/). The nucleotide sequences of ITS 18S rRNA gene were deposited in NCBI GenBank for acquiring the accession number.

Based on the cultural, morphological and molecular characterization, the fungal endophyte was identified as *Beauveria felina*. In Petri plates, the colony were observed as white creamish, later the aerial hyphae from the colonies produced erect synnematous structures which were hairlike, long and abundant (Fig 1). Microscopically, the conidia were single celled, oval to ellipsoidal, hyaline, appearing at the tip of conidiogenous cell, 10×14 µm in



Fig 1: *B. felina* as observed in culture plate.

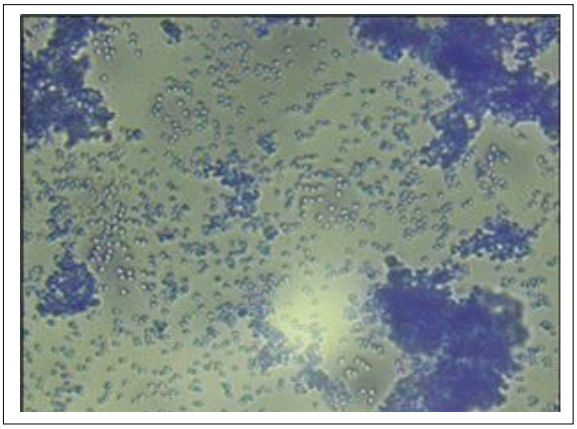
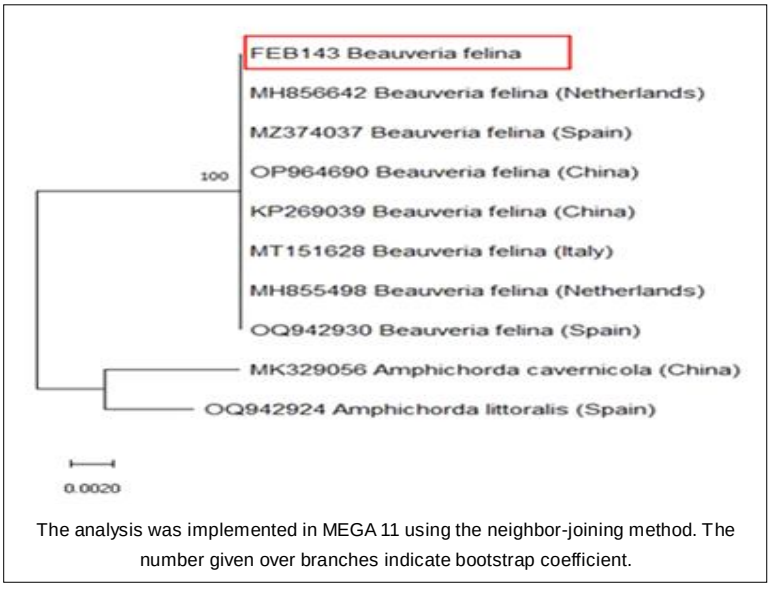


Fig 2: The conidia of *B. felina* as observed in microscope under 40×.



The analysis was implemented in MEGA 11 using the neighbor-joining method. The number given over branches indicate bootstrap coefficient.

Fig 3: Phylogenetic analysis of ITS sequences of *B. felina* with reference sequences retrieved from NCBI (National Center for Biotechnology Information).

size under 40× objective lens (Fig 2). Molecular identification revealed that the sequence was 100% similar to *Beauveria felina* (Fig 3). The sequence was submitted to GenBank and the accession no. acquired is PP715981. This particular isolate was collected from the healthy roots of locally cultivated banana called Tepfesi from farmers' field in Kohima district, of state Nagaland, India. The collection site is located at 94.1086°E longitude, 25.6751°N latitude and 1489 msl altitude. *B. felina* was found to show positive reaction for amylase and siderophore production when tested *in vitro* and also found to produce IAA in considerable amount. However, a thorough investigation is required to check its efficacy against various plant pathogens and insect pest. To our best knowledge, this particular entomopathogen has not been reported from any other plant as a fungal endophyte and is a first report as a fungal endophyte from banana or any other plant in the world.

Ramanujam *et al*. (2021) isolated entomopathogenic *B. felina* from naturally infested fall army worm (FAW), *Spodoptera frugiperda* and stated that in SDY extract agar medium, the colony was creamish white, flat initially and later produced erect synnematous structures and using ITS primers, it was identified as *B. felina*, which confirms the present findings. Several researchers have reported *Beauveria bassiana* as fungal endophyte having antifungal properties. Barra-Bucarei *et al*. (2020) stated that *B. bassiana* could colonize tomato and chili pepper tissues and provide antagonistic effect against *Botrytis cinerea* when tested *in vitro*. It has also been reported to demonstrate antifungal activity against pathogens like *Fusarium* spp. and *Cladosporium herbarum* (Orole and Adejumo, 2009) and *Rhizoctonia solani* (Ownley *et al*., 2009). Du *et al*. (2014) also reported on the isolation of *Beauveria felina* from marine bryozoan.

CONCLUSION

Beauveria felina has been reported as an entomopathogen against fall army worm that affects the maize crop and have also been isolated from marine bryozoan. However, this present study shows evidence that *Beauveria felina* can also exist as a fungal endophyte. The endophyte *Beauveria felina* was isolated from the healthy roots of locally cultivated banana plants of Nagaland, India. Currently, there are very less work done on this particular entomopathogen and there is no work done to check its effectiveness as a biocontrol agent. Therefore, future research should focus on checking its effectiveness and its benefits as a biocontrol agent against important plant diseases.

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Informed consent

No animals or animal procedures were used in this research.

Conflict of interest

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