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Isolation And Characterisation Of Beauveria And Metarhizium Spp. From Rice Fields Of Lower Brahmaputra Valley Zone, Assam

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Abstract:
Assam, the heartland of paddy cultivation in India, relies heavily on rice as the staple crop and primary source of livelihood. In order to protect rice production sustainable pest management is crucial to safeguard rice production, where entomopathogenic fungi (EPF) such as Beauveria bassiana and Metarhizium anisopliae play a promising role as biological control agents. The goal of the present study is to isolate and identify indigenous EPFs from Lower Brahmaputra Valley Zone (LBVZ) rice field of Assam. Soil samples were collected from three districts—Bongaigaon, Nalbari, and Barpeta—and processed through serial dilution and culture on selective media. Morphological characterization followed by molecular identification using ITS1 and ITS4 primers, PCR amplification, sequencing, and phylogenetic analysis confirmed the isolates as Beauveria bassiana and Metarhizium anisopliae. The findings demonstrate the diversity of LBVZ rice fields in native EPFs, underscoring their potential for use in eco-friendly pest management strategies. Harnessing locally adapted strains can provide effective, sustainable alternatives to toxic chemical pesticides, ensuring better crop protection while minimizing environmental risks.
Keywords: *Assam, Entomopathogenic Fungi, Lower Brahmaputra Valley Zone, molecular identification, Eco-friendly pest management.*

INTRODUCTION:

Assam known for its breath taking natural beauty is also the heartland of paddy cultivation in India. Paddy cultivation is the backbone of Assam economy, with agriculture being the prime source of income for a majority of population. More than half of the world's population depend on rice as the primary food source. Rice is the staple crop of Assam providing carbohydrates, proteins, and other vital nutrients. Rice contains 87% carbohydrates, 7-8% proteins, and very little fat (Selvakumar et al., 2014). Around 37.95% of Assam's total geographical area is under cultivation, according to data from the Agriculture Census 2015-16. The Lower Brahmaputra valley zone (LBVZ) of Assam has a rich agro climatic condition, it lies on the southernmost region of the river Brahmaputra and is characterized by fertile alluvial plains making this region richer for cultivation. Soil is the primary habitat for Entomopathogenic fungi (EPF), which provide a conducive environment for their growth and development. These fungi spend a significant portion of their lifecycle in soil, where they can persist and interact with insect hosts (Jackson et al., 2000).

EPF are very unique and earliest microbes to be employed for the treatment of biological management of insect pest. These groups of fungi are parasitic and can disable or kill insects. EPF species mostly belong to Ascomycota and Zygomycota division (Khan et al., 2012), there are more than 1000 entomopathogenic fungi species belonging to 100 genera in the world (Wang et al., 2017) of which 750 species of fungi from around 90 genera are known to be pathogenic to insects (St. Leger & Wang, 2010, Rajula et al., 2020).The Italian scientist Agostino Bassi was the first to propose that fungus could be used to infect silkworms and other species for the control of pest after identifying white muscardine disease in silkworms in 1835 (Rajitha & Savithri, 2015. Jiang & Wang, 2023). EPF can attack insects belonging to orders Coleoptera, Dermaptera, Homoptera, Lepidoptera and Orthoptera. A sequence of carefully coordinated actions initiates infection, which advances after spore attachment, penetration, proliferation inside the host's body for nutrition and leading to death within 3-7 days of infection (Lacey et al., 2015; Strasser et al., 2010). Metarhizium spp, Beauveria bassiana, Verticillium lecanii are some of the EPF species having most notable role in biocontrol programs, insect pest management as they are environmentally safe (Rachana

et al., 2016; Li & Sheng, 2007). Among various fungal species Beauveria bassiana and Metarhizium anisopliae are the most studied fungi because of their commercial production as biocontrol agents (Goettel et al., 2005; Meyling & Eilenberg, 2007).

EPFs have the ability to adapt to local environmental conditions, such as climate and habitat (Sevim et al., 2012). Therefore, it is essential to isolate and characterize indigenous fungal strains to develop effective biocontrol strategies against insect pests in specific areas. The present study aimed to isolate and characterize of Beauveria bassiana and Metarhizium anisopliae from soil samples collected from rice fields of Assam.

MATERIALS AND METHODS:

Sampling site:

Soil samples were collected from nine rice fields, three fields from each of districts of lower Brahmaputra valley zone of Assam were randomly selected, with geo coordinates of 26.470661° N, 90.776636° E; 26.475849° N, 90.712763° E; 26.474208° N, 90.539314° E for Bongaigaon District, 26.455374°N, 91.513754° E; 26.458311° N, 91.501456° E; 26.45307° N, 91.532694° E of Nalbari District and 26.286505° N, 91.070544° E; 26.314315° N, 91.047433° E; 26.407981° N and 90.976011° E of Barpeta District.

Sample collection:

A total of twenty-seven soil samples were collected, three from each site. All the soil samples were carefully mixed in the laboratory. Pebbles and other debris were removes (Sevim et al., 2010a)

Isolation of Fungi:

One g of collected soil sample was suspended in 10 ml of sterilized distilled water and vortexed for 10 minutes to achieve a uniform mixture. Serial dilutions ranging from 10^{-1} to 10^{-8} were prepared to facilitate the isolation of single fungal colonies (Fig1). Subsequently, 250-µL aliquots of the soil extracts were placed on Sabouraud Dextrose Agar (SDA) medium, containing iodine, chloramphenicol and streptomycin and kept in BOD incubator for fifteen days (Goettel & Inglis, 1997). At the end of incubation period whitish and greenish single colonies were again transferred to SDA to obtain pure colonies (Fig 2). Later the fungal isolates were again transferred to Potato Dextrose Agar media and incubated for another seven days and further investigation was performed (Songül et al., 2018).

Fig 1: (a) Serial dilution of collected soil sample and (b) Growth of soil microbes after two weeks of incubation.

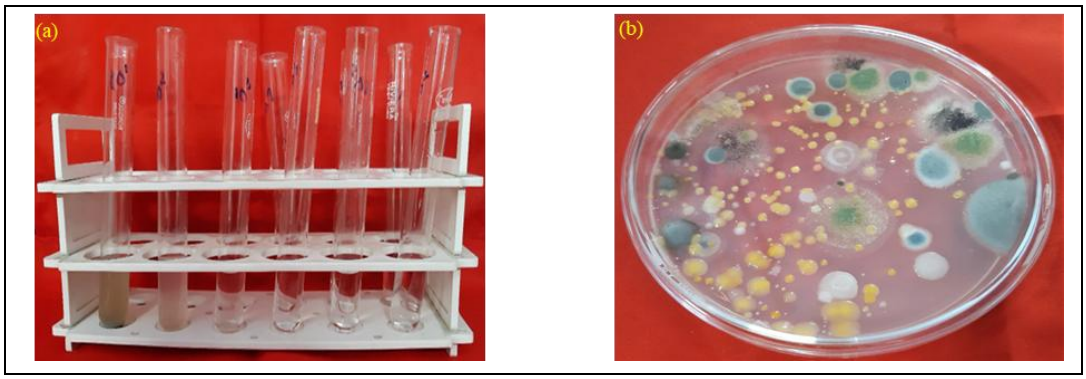
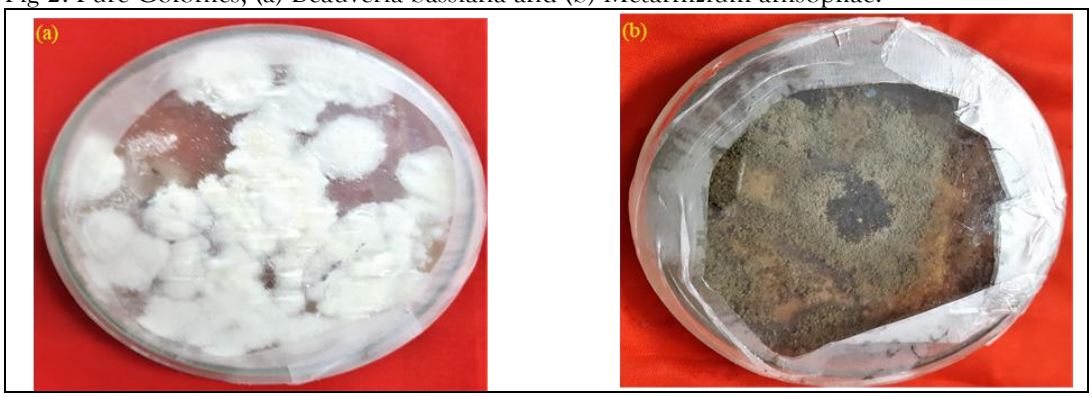


Fig 2: Pure Colonies; (a) Beauveria bassiana and (b) Metarhizium anisopliae.



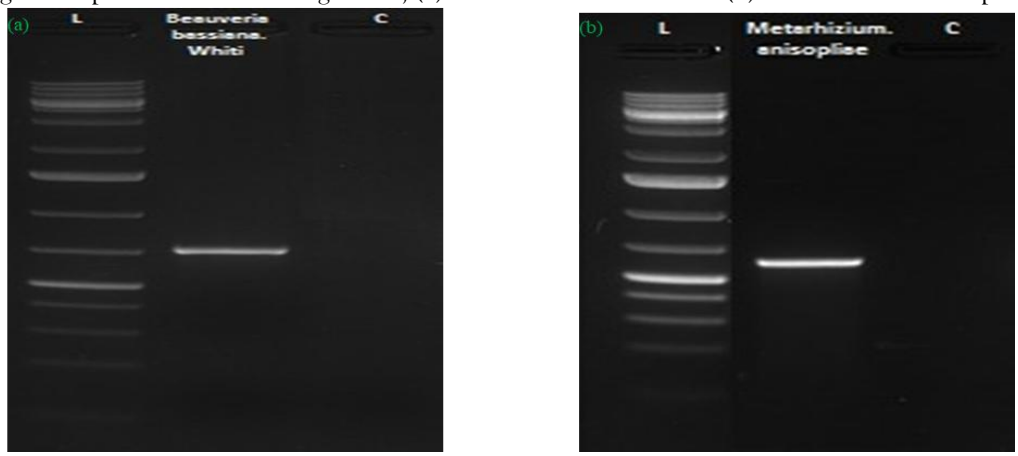
MICROBIAL IDENTIFICATION:

DNA was isolated and ITS region was amplified using ITS1 and ITS4 primers. Amplicon was electrophoresed in a 1% Agarose gel and visualized under UV. Concentration of the amplicon was checked in a Nano drop ND 2000. The amplicon was purified using Sure Extract PCR cleanup/Gel extraction kit (Genetix). Sequencing of amplicon with forward and reverse primers in ABI 3730xl cycle sequencer. Forward and reverse sequences were assembled and contig was generated after trimming the low-quality bases. The sequence analysis was carried out using Bioinformatic tool BLASTn of NCBI. Based on maximum identity score first few sequences were selected and aligned using multiple sequence alignment software ClustalO. Dendrogram was constructed.

RESULT AND DISCUSSION:

Isolated fungal strains were identified as Beauveria bassiana and Metarhizium anisopliae. The identification was confirmed by amplification of DNA fragments produced by polymerase chain reaction. (Fig 3) for both the fungal species.

Fig 3: Amplification DNA fragments, (a) Beauveria bassiana and (b) Metarhizium anisopliae



Identification for the species of the isolated fungus Beauveria bassiana and Metarhizium anisopliae was further confirmed by phylogenetic analysis. The samples isolated shows significant similarity with Beauveria bassiana and Metarhizium anisopliae based on homology and phylogenetic analysis. Thus, the EPF isolated from soil collected from the rice fields were confirmed as Beauveria bassiana and Metarhizium anisopliae (Fig 4 and Fig 5)

Fig 4: Phylogenetic tree of Beauveria bassiana. The scale on the top of the phylogram indicates the degree of dissimilarity.

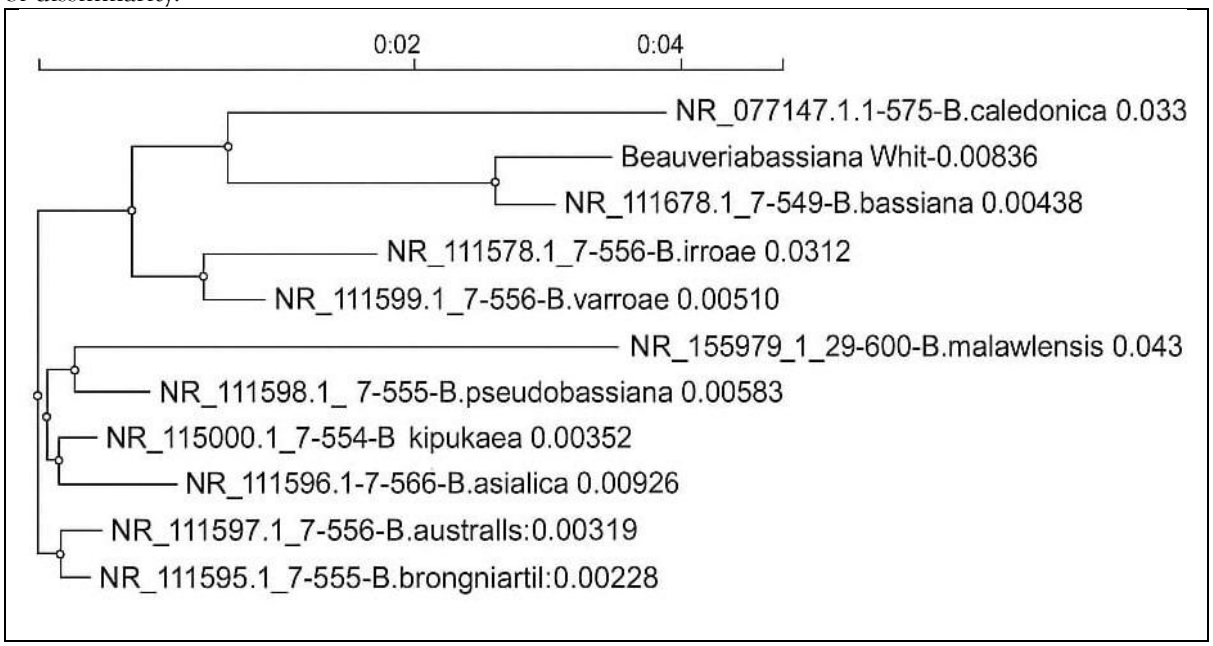
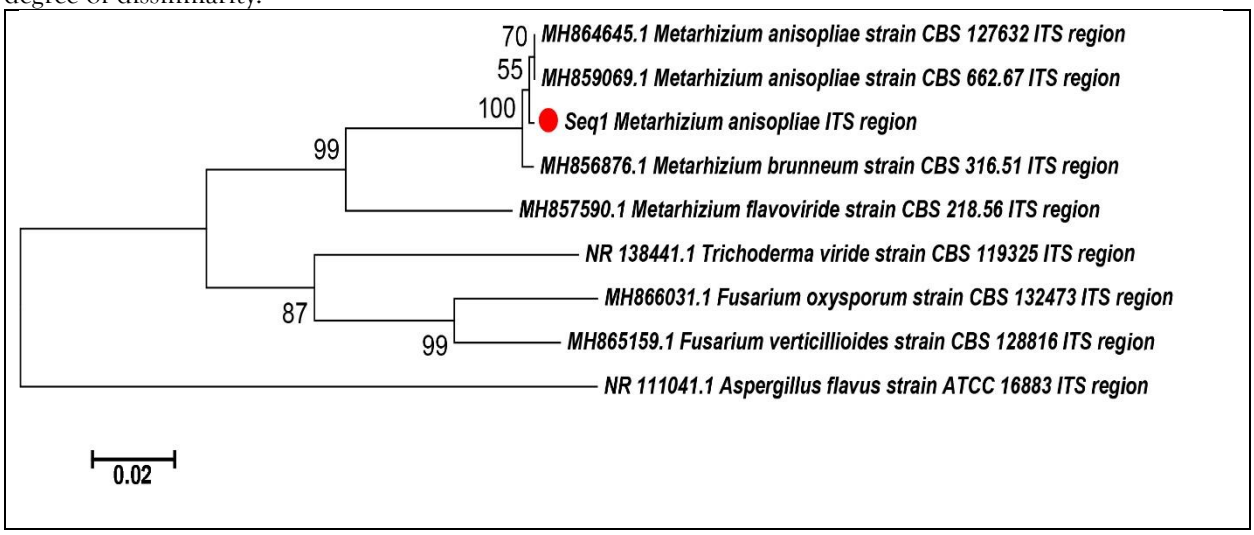


Fig 5: Phylogenetic tree of Metarhizium anisopliae. The scale on the top of the phylogram indicates the degree of dissimilarity.



EPFs play a crucial role in regulating insect populations in ecosystems, with numerous species being harnessed for biological control. Till date, over 170 commercial products have been developed from various EPF species, showcasing their potential as sustainable pest management tools (Lovett & St Leger, 2017). Among these, Beauveria bassiana and Metarhizium anisopliae are the most abundant species found in temperate region soils, highlighting their potential for controlling insect pests in agricultural and environmental setting (Perez-Gonzales et al., 2014). Studies have shown that Boverin, a commercially available biological insecticide based on Beauveria bassiana, effectively reduces populations of Cydia pomonella (Gardner & McCoy, 1992). Numerous studies have demonstrated the successful isolation and infectivity of entomopathogenic fungi (EPFs), particularly Beauveria bassiana and Metarhizium anisopliae, against larvae of various pest species (Lacey & Unruh, 2005; Zimmermann et al., 2013).

CONCLUSION:

As the isolated EPFs are confirmed as Beauveria bassiana and Metarhizium anisopliae, it can be concluded that the rice fields of Lower Brahmaputra districts are rich in EPF. These EPF are playing a significant role in pest control, but their value is often overlooked due to farmers' reliance on chemical pesticides. Furthermore, introduction of exotic EPF strains may struggle to adapt to the local environment, highlighting the importance of harnessing native EPFs for sustainable pest management as they are safer and more effective choice. They are naturally adapted to the local environment, soil conditions and less likely to harm non-target species, making them more potent against target pests.

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