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RESEARCH ARTICLE

MOLECULAR BASED IDENTIFICATION OF BOLETUS EDULAIS FOUND IN JHARKHAND

Kiroom Rashmi Topno[1] and Dr. Anjani Kumar Srivastava[2]

1. Research Scholar, PG Department of Botany, Ranchi University, Ranchi, Jharkhand.
2. University Professor,PG Department of Botany, Ranchi, Jharkhand.

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Abstract

Food industry is dynamic and in search of innovation and nutrition content. Nutrition and food supplements demand can be fulfilled by mushroom.*Agaricus*(Button mushroom), *Pleurotus* (Oyster mushroom)and *Volvariella*(Paddy straw mushroom)are only popularly species cultivated and commercialized. There are many other wild mushrooms which can be commercialized with ethnomedicinal and rich nutrition content. In Jharkhand many wild mushroom species are found but they identified with many synonyms.(Srivastava and Soreng 2014) Identification is done on the basis of morphological structures and microscopic study of spores.(Pegler 1981, Wu2014) This study is to identify the wild mushroom locally called as "Jamun khukhari" through molecular characterization and report molecular data to NCBI GeneBank.

Introduction:-

Jharkhand state is rich inbiodiversity and its physical feature constitutes the Plateau. Tree *Shorearobusta*(Sal) covers more percentage of the Jharkhand's Forest. The other percentage of forest contains trees like*Madhuca indica* (Mahua),*Terminalia tomentosa*(Asan), *Millettia pinnata* (Karanz).(Kumar and Saikia 2020) Forest products are usedby tribal communities for food, medicine and shelter.Monsoon bring lots floral treasure of land for upliftment of local and tribal community. Mushroom is popularly consumed non timber product of forest. There are rare mushroom and puffball species like*Macrolepiota*, *Termitomyces*, *Lentinula*, *Volvariella*, *Boletus*, *Calocybe*, *Astraeus*,*etc.* aresold in high price in village weekly market during monsoon. (Panda and Tayung 2015) The reason for high price is manual harvesting and non-culturable in synthetic media.

Boletus edulais is known asJamun khukhari by local and tribal people in Jharkhand. This name was given because it is belief that it is ectomycorrhizal associated with roots of *Syzygiumcumini*(black plum) Jamun in Hindi.Thisspecies of mushroom is entitledas king mushroom. This was placed in family Boletaceae. This is easily noticed and distinguished by the absence of gills, colourful caps, pores hard thick stipe. (Verma and Pandro, 2018) Wounded parts of some species of Boletaceae turns blue due oxidation of pulvinic acid derivatives (Nelson, 2010). Few species of this groups are found to be poisonous but many are safe for consumption. (Singer 1986)

Wild mushroom can be commercialised after proper identification and phytochemical analysis. Morphological features are misleading the identification of closely related mushrooms reported from different region of India. In present era molecular characterization for fungal identification is made easy and accurate with ITS (Internal transcribed spacer) regions by finding similarities in Genetic database BLAST (Basic Local for Alignment Search Tool). (Rajaratnam, *et. al*., 2012)The present study describes the DNA sequencing for attempting to identify the wild

Corresponding Author:- Mrs. Kiroom Rashmi Topno
Address:- Research Scholar, PG Department of Botany, Ranchi University, Ranchi, Jharkhand.

edible mushrooms collected from Jharkhand, using genomic DNA from fruiting bodies and also looking for a match of sequences for molecular based *Boletus edulais* on NCBI database.

Materials And Methods:-

Collection and survey

Jamun khukhari was collected form weekly village market of Khunti district, Jharkhand. The Sample was photographed and survey was conducted with the tribal people belonging to around villages during collection.

DNA isolation and Purification

Sample were brought to lab cleansed with tap water distilled for removing muddy content in outer surface. Then it was cleansed with 70 % of alcohol. Sample were treated with Liquid nitrogen and crushed in motor pestle making fine powder. Powdered sample were mixed with CTAB and Centrifuged as per Doyle and Doyle protocol. (Doyle and Doyle 1987) The genomic DNA was checked by agarose gel electrophoresis in 1.5 % agarose gel. The obtained DNA content was purified by RNase treatment at 37℃ for 60 minutes followed by Phenol- Chloroform-isomylalcohol (25:24:1 ratio). Centrifugation at 12,000 rpm for 10 min of purified supernatant with equal volume of 70% ethanol. The resultant DNA pellet were air dried and suspended to ice box after dissolving in 1X TE buffer. (Aamir, *et. al.*, 2015)

Amplification

PCR reactions was performed for amplification with Forward Primer 5'-ACT GAA CCT TAT CAT TTA GAG- 3' and Reverse Primer 5'- AAG TCC ACT GAA CCT TAT CAT- 3' in a 50 µl reaction volume with 30 ng template DNA and ran on programme with initial denaturation at 94℃ for 5 minutes, denaturation at 94℃ for 30 seconds, annealing at 60℃ for 40 seconds, extension at 72℃ for 1 minute and final extension at 72℃ for 2 minutes. The amplicons were eluted and Agarose gel run with Ethidium Bromide stain for 90 minutes 50V and photographed.

Sequencing and Blast analysis

ITS (Internal transcribed spacer) sequence of conserve region amplicons containing were processed to Sequencing by Sanger Sequencing method with ABI 3730 genetic analyzer in Bunshi Bioscience Pvt Ltd. The resultant ITS Sequence was obtained and analysed by finding the similarities in BLAST. **(**Altschul*et al*., 1997) The resultant ITS sequence was deposited in Database of NCBI GENBANK.

Results:-

The present study survey reviled different report from earlier research documentation. Jamun khukhari found in Jharkhand are having dark black colour cap with large hard stipe in scales. The size of cap is found to in maximum size than reported in this study. The local villagers denied the fact about the association with the roots of *Syzygiumcumini* they notified the location of this species near the edges ofSal (*Shorearobusta)* forest.Local name Jamun Khukhari is given due to its black colour of pileus. It is been reported and bitter tasting mushroom often cooked with tamarind to enhance the bitter taste. It having high ethnomedicinal importance as remedy for stomach disorders.The photograph of market- place of collection and morphological structure were recorded (Fig 1).

The sample collected was processed for DNA isolated and ITS was amplified by PCR for sequencing (Fig 2). The resultant ITS sequence was matched with BLAST Database and found to be 99.71% similarities and 0 E value (Fig 3). (Table 1) and identified as *Phlebopusportentosus*. ITS sequence of length 905 bp was deposited in NCBI GENBANK and obtained accession number MT611108.

Figure 1a:- Place of Collection- market b. morphological structure.

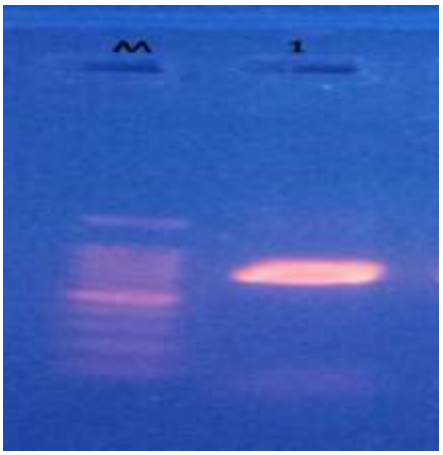

Fig2:- M -500 bp DNA ladder 1 - amplified DNA of ~ 500 bp.

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BLAST® » **blastn suite** » results for RID-7VHH90R401R

Your search is limited to records that exclude: uncultured/environmental sample sequences
Your results are filtered to match records with percent identity between 99.71 and 100.

Job Title	Nucleotide Sequence ...
RID	7VHH90R401R Search expires on 03-28 17:23 pm
Program	BLASTN
Database	nt
Query ID	lcl|Query_3543
Description	None ...
Molecule type	dna
Query Length	1096

Descriptions

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Phlebopus portentosus isolate FG-DF2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1249	1249	62%	0.0	99.71%	MN962526.1
Phlebopus portentosus strain CMU51-210-1 18S ribosomal RNA, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA, partial sequence	1247	1247	62%	0.0	99.71%	JQ695906.1
Phlebopus portentosus isolate FG-LC1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1242	1242	61%	0.0	99.71%	MN962556.1
Phlebopus portentosus isolate FG-MH1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1242	1242	61%	0.0	99.71%	MN962548.1
Phlebopus portentosus isolate FG-YX1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1242	1242	61%	0.0	99.71%	MN962545.1
Phlebopus portentosus strain CMU51-271-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1242	1242	61%	0.0	99.71%	JQ623568.1

Graphic Summary

Distribution of the top 6 Blast Hits on 6 subject sequences

Query

1 200 400 600 800 1000

Fig 3:- BLAST analysis of mushroom sample (*Phlebopusportentosus*).

Table 1:- Resultant ITS sequence of Jamun Khukhari.

Sample	Resulted ITS sequence
Jamun khukhari	GGAAACACCCTCCAACGGTTCACGGGTTTTAACCATACGCCAAAGGAGAGGGCGGGCCAG GTTTTGACGGCACACGCTTACATATATGCCAGCAACGTTTTCCCACCCAGCATCACCCTCTT TTTTTTTCCCCCCTTCGGACTTTGTGGCTTTCATAATGATCCTCCTTCCCCCAGGGGAACCTG CGGAAGGATCATTATTGAAAGCAACAAAGTCCGAAGGGGGGAAAAAAAAAAAGAGGGTGAT GCTAGGTGGGACGACTGTCGCTGGCATATATCGTATGCATGTGCACGTCGAAACCTTGGCT CGCCCCTCTTCCTTTCGGCGTAATGCTTAATACACCTGTGAACCTGTTGTAGGTTGTTCCCCT ACGAGCAGTAGGAGGACGATCATGTCTTTCCATCACACTACATGTATGTCTACAGAACGTT GAAAGTCGTCTCGACCCTTGACGGGGTTGGACGCAAAACCATAGTACAACTTTCAGCAACG GATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGC AGATTTTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGC ATGCCTGTTTGAGTGTCATCGAATTCTCAACCATGTCTTGATGTTAACTTTCGAGGCATGGC TTGGACTTGGGAGCTTTGCTGGTTGGACCCCCCTCTCTTCGAGGGGGGAATGTCAGCTCTCC TCAAAAGCATTAGCAAAAGGGACGTGTTCGTCGCATGAACTGACGGCCTTCGACGTGATAA TGATCGTCGTGGCTGGAGGGAAAAGTGTGATGGCGAAGGTCTGTCCTAGCTTCTAATCAAA GGCGAGGGGCTTGTGTCACGCATGCCCTCTGTCTTATCGAACCC

Conculsion:-

This study went through morphological features to previous research document of *boletus edulis*, different reports were found. The accurate identitywas obtained by ITS for conserved region through sequencing. The mushroom species known to be *boletus edulis* was concluded as *Phlebopusportentosus.Phlebopus*earlier was placed under the family Boletinellaceae (suborderSclerodermatineae of the Boletales) (Binder and Hibbett2006) as a Boletus by Heim (1936), and gave the status of genus by Singer (1936)This mushroom has recorded associated with Salforest of Central India (Verma and Pandro 2018). In southern China, it is commonly known as "Black Boletus". It is found under shade of *Ficus Sp*. and *Eriobotrya japonica*people of China has successful in cultivating and established profitable market.(Zhang et al., 2017)Now this mushroom can be further processed for culture and commercialization as identification by molecular characterization has concluded this mushroom species as *Phlebopusportentosus*.

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