EXPLORATION OF *SHIZIPHYLUM COMMUNE BASIDIOMYCETES (AGARICALS)* FROM DISTRICT MANSEHRA.

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Abstract

Research work was conducted on the examination and investigation of macro-fungi from the surrounding areas of Hazara University, Mansehra. Morphological and molecular characterization of the species was done. For molecular characterization, DNA was extracted from the isolated samples by kit method, and *ITS4* primer designed from conserved regions described in previous studies was used for PCR. The amplified PCR products were sequenced from Macrogen Korea via worldwide scientific Pakistan. Phylogenetic analysis of the obtained sequences was done based on the maximum likelihood method using Mega 6.0. Morphological examination showed that the species were observed on wood logs as well as living wild trees among forest trees. During our research work, our 2 collected specimen species from Hazara University and Village Kotkay Mansehra were identified as *Schizophylum commune*. This species was reported first time from the collection side of District Mansehra.

Keywords

Macro-fungi; Split gilled; Taxonomy; Basidiomycota; *Schizophylum*

1. INTRODUCTION

Described as a pathogenic fungus on plants as well as on humans causing apple rots and onychomycosis respectively. *Schizophyllum commune* is distributed worldwide and belongs to the phylum Basidiomycota, Schizophyllaceae family colonizes on trees and rotting wood [1,2]. The species was also studied for its anti-microbial activities against certain pathogens like *Armillaria tabescens* [3]root rot [3]. Deadwood is the common substrate for this rotting organism, as it is the decomposer of 150 genera of flowering plants [3]. Furthermore, the specie is described as a plant pathogen but is also concerned with human problems to some extent [4].

To distinguish. public strains and different enthralling traits require an estimation of hereditary and phenotypic multiplicity. Recent molecular phylogenetic examinations command displayed that the inner transcribed spacer (ITS) area of genomic DNA is authentically actionable to exact phylogenetic affiliations at lesser taxonomic degrees. The ITS of rDNA is accounted to exist as an adaptable zone among species and even among strains (5).

2. MATERIALS AND METHODS:

Periodical surveys were done to collect macro fungi and to record their prevalence from District Mansehra KPK Pakistan. Specimens of macro fungi were observed, photographed (Canon Power Shot A 460 5.0 MP Digital Camera), and collected in properly labeled paper bags from dead logs and living trees from forest areas and damp places. The specimens were preserved by sun and oven drying method and were then observed for morphological characteristics in the microbiology research laboratory, Hazara University Mansehra for proper identification and morphological studies. For Microscopy; Light Microscope was used for the differential observation of the spores (Compound Microscope with digital Camera CB, 10). Different characteristics were studied including sporocarp, shape, size, color, habitat, and GPS (Garmin e Trex 10 – GPS) value recorded. Micrometry was done with the help of a Light Microscope for the identification of specimens for their distinguishing characteristics, all the collected species were observed after slide preparation for drawing the mycelia structure.

2.1 RESEARCH METHODOLOGY:

During a survey of District Mansehra (KPK), the samples consisting of vegetative structures and sporocarp were collected. Morphological characteristics were observed, and data was taken on the following aspects like color, substrate, GPS location, date and time of collection, area, size of basidium, and spore size. The collected materials were initially placed in paper bags and were then shifted to glass jars in distilled water, then rinsed with tap water, air-dried, and sun-dried properly. After drying the specimens were labeled and packed for molecular identification. A small section from the sporocarp was taken from each collection for the spore size measurement and data was recorded (size, color, and shape of the spore).

The sporocarp, shape, size, color, and habitat observation were important in describing and identifying a specimen.

2.2 Molecular studies for identification:

For molecular identification of the collected species, DNA was extracted using EZ- 10Spin column Genomic DNA Minipreps kit, Fungi according to manufacturer's instructions. Confirmation of the integrity of the DNA was checked by subjecting the DNA to electrophoresis in 1% agarose gel.

For the amplification of the rDNA ITS region (*Ribosomal Internal Transcribed Spacer* region of the DNA), fungal-specific forward primer, ITS1F was applied in combination with the reverse primer, ITS4. PCR was performed in 20µl PCR tubes containing 10µl 2X Ready mix (Sigma-Aldrich), 8.3µl water, and 0.1µl of each primer with 1.5µl of DNA extract. PCR reactions were performed with 3.00 minutes of initial denaturation and 30s of final denaturation at 95ºC followed by 35 cycles at 53ºC for 35s, initial extension for 1.35+5s at 72º, and final extension for 2 minutes at 72º.

The products for which the PCR was done, were sequenced from Macrogen, Korea. The DNA sequences were edited. By using Sequence Scanner (v1.0) and Bioedit (v 7.0), the DNA sequences were edited. BLAST-searched for sequence comparison and identification by submitting the query in the GenBank database using default settings.

2.3 Phylogenetic Analysis

Sequences were aligned using Mega 6.0. The divergence in rDNA-ITS was measured by comparing sequence pairs reconstructed by using Meg Align (DNA STAR). For Phylogenetic

analysis, MEGA 6.0 was used. Employing the Tamura-Nai model, Maximum Likelihood (ML) trees were constructed and bootstrap consensus trees were generated. The bootstrap consensus trees were inferred from 1000 replicates and corresponding bootstrap values were cited in the tree. The samples were sequenced from Macrogen Korea via Worldwide Scientific Pakistan.

The sequences were submitted to NCBI and Gene Bank for accession numbers.

3. RESULTS

Schizophylum commune NJ40 and S. commune NJ41 were observed and identified from Hazara University and village Kotkay Mansehra.

3.1 Description of the Genera *Schizophylum:* Basidiomycota, Agaricomycetes, Shizophyllaceae

Schizophylum commune NJ40 *Schizophylum commune* var. *commune* Fr. 1815

NCBI Accession No. MZ044853

Thick in texture found on Sheesham tree trunk attach as creamy white growth in abundance layers.

The fungus was grown on tree stem in abundance, whitish in color, gilled, with small scales, and measured about 2.8cm broad towards margins and wide about 1.6cm on average. Basidiospores are hyaline, smooth-walled, and elongated, measuring 6.5 x 2.6 µm on average.

The specie was collected at noon at 29ºC with a relative humidity of 77%.

3.3 Specimen region: The specimen was examined from the densely populated area of different plants. The species *Schizophylum communeNJ*41 was observed on Kikar (*Acacia karoo)* tree branches in village Kotkay, 6-7km from Mansehra in July 2018.

Location Coordinates: N= 34º 28'0" E=73º 35'0" Alt= 1095m / 3593feet

Fungal Isolates: The same species *Schizophylum communeNJ*40*(*N= 34º 26'0" E=73º 15'0" Alt= 977m /3205feet) (NCBI Accession No. NJ0400852) was reported from the dead wood logs of trees grown in clusters as whitish growth with split gills near pheasant area of Hazara University, Mansehra during August 2018. The dead logs were observed as peach (*Prunus persica* L. Batsch.) trees on the campus.

3.3 Properties: Described as a pathogenic fungus on plants as well as on humans causing apple rots and onychomycosis respectively [6], A. Lahbib, 2016 and Kligman A.M., 1950). Characterization of the species revealed medicinal properties. The reports on S. commune could be analyzed for its medicinal characteristics that included anticancer, antitumor, and immunomodulating properties [7].

Remarks: No Published reports from Hazara Region but the species is given the accession number (GenBank (MN178555); in "A compendium of macro-fungi of Pakistan by ecoregions" [8].

Photographs of the Mushrooms on the substrate

Fig.1.Schizophylum commune NJ41 grown on living trees

Fig.2.Schizophylum commune NJ40 grown on dead wood logs

Fig.3.Schizophylum commune NJ40 preserved by sun drying.

Fig.4. Gills under the surface of the species *S. commune*

3.4 Evolutionary relationships of taxa

The Maximum Likelihood method and the Kimura 2-parameter model were used to infer the evolutionary history [9]. The bootstrap consensus tree was established to infer from 1000 replicates [10]. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branch replicates. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and Bio NJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2344)). This analysis involved 28 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 624 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [11].

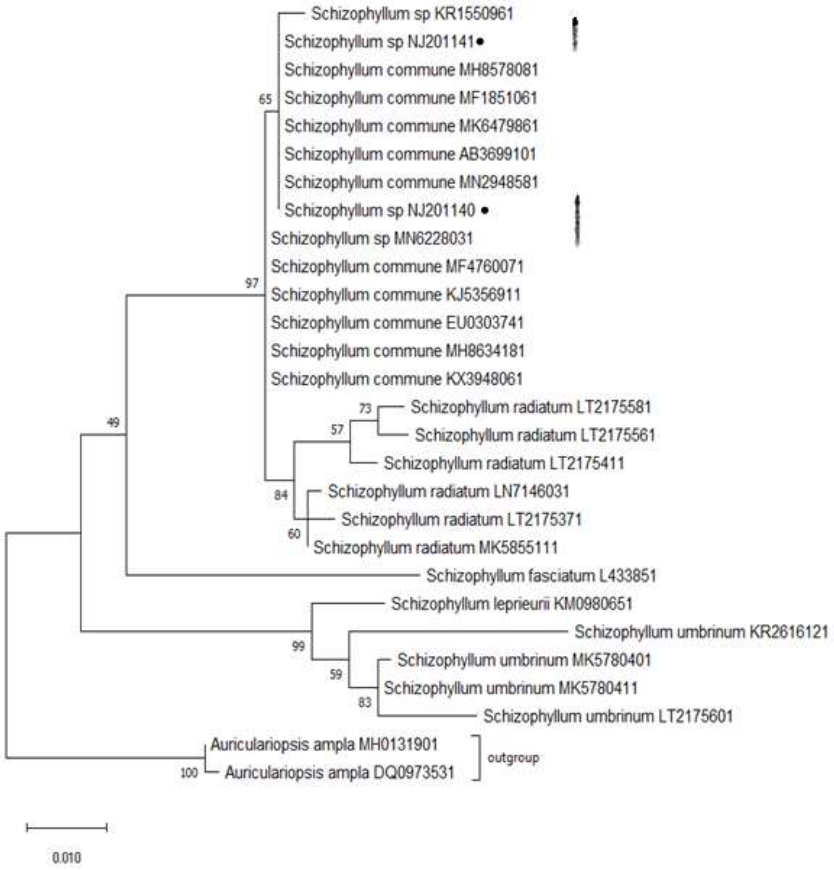

Figure .4. Phylogenetic tree of *Schizophyllum commune*. Generated with 1000 bootstrap values based on sequences of different species and *Auriculariopsis ample* an outgroup. The collection is represented by • in the Phylogenetic tree.

Conclusion: The species *Schizophylum communeNJ*40 and *Schizophylum communeNJ*41 make the clade among the same species so it is confirmed that the species sequenced are the same. The species was widely distributed on dead logs as well as a living tree so further this can be studied for characterization for active ingredients for the treatment of different diseased as shown antioxidant activities.

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