



Wood-decay fungi associated with decaying of coarse woody debris in Hail region, North of Saudi Arabia

Nawaf Alshammari^{1*}, Alshammari Bader Fraih Z¹, Mona Saleh Al Tami², Tarun Kumar Upadhyay³

¹Department of Biology, University of Hail, Hail, Saudi Arabia

²Department of Biology, College of Science, Qassim University, Qassim, Saudi Arabia

³Department of Biotechnology, Parul Institute of Applied Sciences and Centre of Research for Development, Parul University, Vadodara 391760, Gujarat, India

ARTICLE INFO

Original paper

Article history:

Received: December 10, 2022

Accepted: April 07, 2023

Published: May 31, 2023

Keywords:

DNA Sequencing, Hail region, incubation chambers, ITS, wood-decay fungi

ABSTRACT

The present study was conducted in order to describe the assemblage of wood decay fungi and mycomycetes associated with the Hail region in the North of Saudi Arabia by using incubation chambers. There was a collection of coarse woody debris from the study area, AL baithe, which was brought to the laboratory and placed inside a series of incubation chambers in which water was added. Over a period of several weeks, fruiting bodies appeared in these chambers which were morphologically analysed via AmScope stereomicroscope and molecular identification was carried out via ITS-1F and ITS-4R DNA sequencing methods. The obtained sequences after successful identification via BLAST analysis were submitted to the Genebank NCBI database. During the course of the study, a total of 20 species of different 14 families of wood-decay fungi were identified of which mycelium has been generated in incubation chambers. Among the identified wood-decay fungi, Phanerochaetaceae (03 species), Bionectriaceae (02 species), Hypocreaceae (02 species) and Chaetomiaceae (02 species) being the most frequent. Hence, the present study was the first one to report on the diversity of wood-decay fungi of Saudi Arabia and was successful in using incubation chambers to describe the specimens of wood-decay fungi associated with coarse wood debris.

Doi: <http://dx.doi.org/10.14715/cmb/2023.69.5.7>

Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

In the world, fungi are one of the most significant organisms due to their vital role in ecosystem functions, their potential impact on the environment and their impact on humans (1). There is no doubt that fungi play an indispensable part in the daily lives of humans, not only because they are beautiful, but also because they play a significant role in industry, agriculture, and medicine (2; 3). In addition, fungi contribute to the bio-remediation of soil, the recycling of nutrients and the decay of dead organic matter in soil and litter, as well as to the production of biofertilizers (4, 5). Although many wild mushrooms are edible and can be used in cooking, identifying them correctly in the field can be a challenge. Aside from that, there are poisonous and even deadly when eaten (6). People from rural areas are often threatened with death if they consume wild mushrooms unwisely, which is a common occurrence.

Fungi that cause wood decay are usually associated with woody hosts or soils rich in humus and organic matter. In forests, such fungi are responsible for the decay and recycling of carbon and nitrogen as well as the conversion of plants and animals into humus (7). Wood decay fungi have the capability to digest wood composition like cellulose and lignin. The wood-decay fungi play an essential role in many aspects, since they are important in soil development, nutrient recycling, and the carbon budget of these ecosystems and serve as food sources (8). Wood-decay fungi are rare in the Hail region due to harsh weather

and therefore the diversity assessment of fungi associated with the deserts of Saudi Arabia has never been conducted in intensive study. In this context, the present study was carried out with objectives including a brief summary of the fungal species that can be found in the desert habitats of the North region of Hail, Kingdom of Saudi Arabia and the utilization of an incubation chamber to investigate the growth characteristics of wood-decay fungi.

Materials and Methods

Study area

The purpose of the present study was to describe wood-decaying fungi occurring in the North area of the Hail region (26°06'48.1" N 41°54'24.9" E), Saudi Arabia. In this area of the world, the climate is hot during the summer months and cold during the winter months. In the winter, the daily temperatures can vary between 0°C to 16°C, while in the summer; the daily temperatures can range from 34 °C to 44 °C. The average amount of precipitation in the region varies from 10 - 20 cm per year. There is a high chance of extreme weather conditions such as storms and droughts in this area. The vegetation in this area is dominated by a variety of plants including *Acacia*, *Amarix articulata*, *Culotropis procera* etc. (Fig 1, A).

Sample collection

As part of this study, coarse woody debris was collected from the period of February 2021 to March 2021 by

* Corresponding author. Email: naib.alshammari@uoh.edu.sa

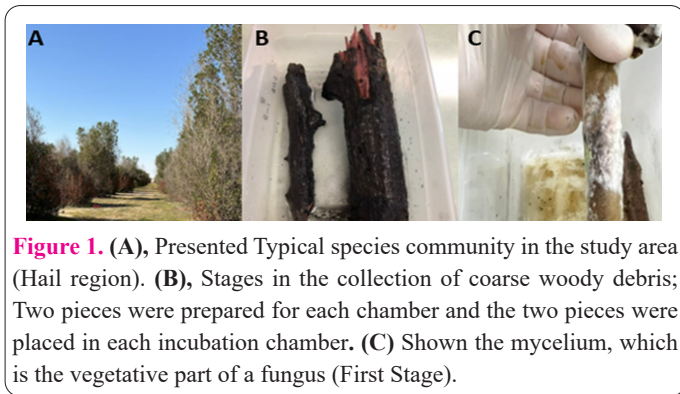


Figure 1. (A), Presented Typical species community in the study area (Hail region). (B), Stages in the collection of coarse woody debris; Two pieces were prepared for each chamber and the two pieces were placed in each incubation chamber. (C) Shown the mycelium, which is the vegetative part of a fungus (First Stage).

visiting different regions of Hail, thereafter returning to the laboratory and then incubating them in plastic incubation chambers of $30 \times 12 \times 6$ cm in which extra water was added during incubation (Fig. 1, B). For two months, the samples were observed, and if necessary, extra water was added to ensure that the samples retained moisture throughout the observation period. Whenever fruiting bodies were found in the coarse woody debris (CWD), they were photographed and collected for further study.

Morphological description

The description of morphology refers to the study of many characteristics such as shape, size and structure that are characteristic of fungi. Fungi are characterized by a specific design in which the growing process starts from hyphae and then proceeds to mycelium. Mycelium develops into fruiting bodies whenever it is provided with a favorable environment such as adequate moisture and sufficient water. In the present study, the morphological description of different wood-decay fungi obtained from incubation chambers was determined by using an AmScope stereomicroscope (9,10). A greater emphasis was placed on the external features of the fruiting body, such as its cap, stalk, and color, as well as its internal features. As soon as the morphology of the fruiting body had been described, small tissues were collected from the fruiting body and placed in Eppendorf tubes and stored at 4°C for their molecular identification.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from the sterilized collected fungal materials using a Wizard® genomic purification kit (Promega Corporation, Madison, WI). The optical density of the isolated genomic DNA was measured at 260 and 280 nm in order to determine its quantity (UV-1800, Shimadzu Spectrophotometer, Japan). As a further confirmation of the quality of the extracted genomic DNA, it was electrophoresed on agarose gel (0.8%) in order to determine its purity. The amplification of ITS DNA sequences ITS-1F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4R (5'-TCCTCCGCTTATTGATATGC-3') was carried out using a protocol with published primers (11). In order to perform the PCR amplification, a total volume of 20 mL was used, containing 1x ReadyMix™ Taq PCR reaction mix (Sigma), 10 pmol of each primer, 10 ng of the genomic DNA template and nuclease-free water in order to make up the volume. The PCR cycling conditions were: 95°C for 5 min, 35 cycles of 95°C for 45s, 50°C for 45s, and 72°C for 1 min 50s, and a final extension step at 72°C for 10 min. In order to detect amplified PCR products, we have performed agarose gels (1%) were electrophoretically

stained with ethidium bromide and visualized under ultraviolet light. Successful PCR amplification was obtained using a GenElute™ PCR clean-up kit, and the purified PCR products were sequenced by GeneWiz (South Plainfield, NJ), a commercial PCR sequencing company. By performing nucleotide blast searches against the NCBI database (www.ncbi.nlm.nih.gov), the sequences obtained from the latter company were cleaned up and then identified by performing nucleotide blast searches against the NCBI database.

Results

In total, 20 fungal fruiting bodies were collected from the study area, each of which was grown on different small coarse woody debris and was given a unique reference number. Mycelium was generated on all coarse wood debris (Fig. 1, C). A number of factors play a significant role in the growth of fungi including environmental conditions. In order to encourage the growth of wood-decaying fungi, it is important to maintain a suitable temperature, moisture, and water supply.

In order to identify the collected fungi, amplification and sequencing of ITS-1F and ITS-4R were carried and obtained sequences were blasted against the NCBI database with the BLAST option. For the proper identification, sequences with a 97% sequence similarity with an existing sequence in NCBI were considered as species-identified; at a lower sequence similarity, however, sequences would be considered genus-identified only. Many studies in the kingdom of Fungi have not been able to establish a universal cut-off value for identifying species; however 97% sequence similarity has been used in a number of other publications (12). The identified species are listed in (Table-1).

In total, there are at least 14 families represented by the taxa identified, with the Phanerochaetaceae (03 species), Bionectriaceae (02 species), Hypocreaceae (02 species) and Chaetomiaceae (02 species) being the most frequent. Other identified fungi were belongs to the Meripilaceae, Psathyrellaceae, Strophariaceae, Catathelasmataceae, Meruliaceae, Pluteaceae, Inocybaceae, Auriculariaceae, Agaricaceae and Trichocomaceae families with 01 species each. Despite the fact that this study focused on wood-decay fungi, some of the taxa identified in this study have a different ecological role and are only associated with the decomposition of wood.

Discussion

In forest ecosystems, the decomposition of wood is one of the most influential processes for nutrient recycling, soil formation, and carbon budgets (13). There are two major significant aspects of this concept that make it an essential functional component of all ecosystems. It is, first and foremost, a process that ensures that primary producers get access to the essential nutrient elements in a timely manner while, secondly, it plays a major role in the formation of humus molecules due to the process of decomposition. There are a wide range of forest wastes and agricultural materials in nature that contain high levels of lignocelluloses and that could be converted into useful products by bioconversion. As a renewable resource, they are a source of many useful biological and chemical products that can

Table 1. List of wood-decay fungi recorded from north of Hail region, Saudi Arabia.

No	Taxon	SGB	Family
1.	<i>Rigidoporus vinctus</i>	AY336752	Meripilaceae
2.	<i>Nectriopsis violacea</i>	AY489687	Bionectriaceae
3.	<i>Coprinopsis atramentaria</i>	EU888583	Psathyrellaceae
4.	<i>Agrocybe smithii</i>	DQ115779	Strophariaceae
5.	<i>Bionectria pityrodes</i>	AY249898	Bionectriaceae
6.	<i>Calcarisporium arbuscula</i>	AY271796	Catathelasmataceae (Tricholomataceae)
7.	<i>Phlebia subochracea</i>	AB084604	Meruliaceae
8.	<i>Trichoderma reesei</i>	CP021300	Hypocreaceae
9.	<i>Climacodon septentrionalis</i>	AY705964	Phanerochaetaceae
10.	<i>Ceriporiopsis gilvescens</i>	AY219403	Phanerochaetaceae
11.	Uncultured fungus	AB534507	-
12.	<i>Trichocladium asperum</i>	AM292054	Chaetomiaceae
13.	<i>Chaetomium globosum</i>	ON689376	Chaetomiaceae
14.	<i>Pluteus romellii</i>	KM983699	Pluteaceae
15.	<i>Hyphodermella rosae</i>	MN749631	Phanerochaetaceae
16.	<i>Inocybe sp.</i>	MN964335	Inocybaceae
17.	<i>Trichoderma viride</i>	KM458804	Hypocreaceae
18.	<i>Exidia recisa</i>	LC098751	Auriculariaceae
19.	<i>Lycoperdon pyriforme</i>	MF161171	Agaricaceae
20.	<i>Aspergillus ruber</i>	MN749935	Trichocomaceae

(Note: SGB = Sequence in GenBank).

be derived from them (14).

Increasing amounts of biomass are being accumulated in the environment every year, resulting not only in the degradation of the environment, but also in the loss of potentially valuable materials that can be used to produce energy, food, and chemicals in the future. The role of myxomycetous fungi, which have the ability to decompose lignocelluloses in wood decay, plays a very significant role in the decomposition process of wood. There has been a great deal of interest among scientists in a variety of disciplines regarding the biology of wood-decaying myxomycetous, for example in forest ecology (13), forest pathology (14, 15), biotechnology (16) and bioremediation (17).

In spite of the fact that numerous studies have been carried out on wood-decaying fungi throughout the world (18, 19, 20, 21) the current study is the first of its kind undertaken in the north region of Hail, Saudi Arabia. According to the total number of fruiting bodies reported in the study area (22) which clearly indicates that different wood-decaying fungi in the study area will grow whenever they have given a suitable environment (5) (Fig. 2). In addition, there are no published studies that have been conducted which have used incubation chambers as a method to report the species that are present in Saudi Arabia. A final point that should be mentioned is that incubation chambers have been found to be an effective method in the laboratory for growing fungal growth.

Acknowledgements

The authors are thankful to Department of Biology, University of Hail, Hail, Saudi Arabia for providing research facility to conduct this research. This research has been funded by Scientific Research Deanship at University of Ha'il – Saudi Arabia through project number GR-22 088.

Interest conflict

The authors declare no conflict of interest. Consent for publications The author read and approved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article Authors' Contribution All authors had significantly contributed to the manuscript. Nawaf Alshammari, Alshammari Bader Fraih Z, Tarun Kumar Upadhyay designed the concept, Nawaf Alshammari wrote the initial draft, and Alshammari Bader Fraih Z performed the experiment. Data analysis, reviewing, and editing all authors contributed in this manuscript.

Ethics approval and consent to participate

Ethical review and approval were waived for this study due to the severity level of the applied procedure, which is defined as "Not an experiment" according to the ethical statement.

References

- Bhunjun CS., Niskanen T, Suwannarach N, Wannathes N, Chen

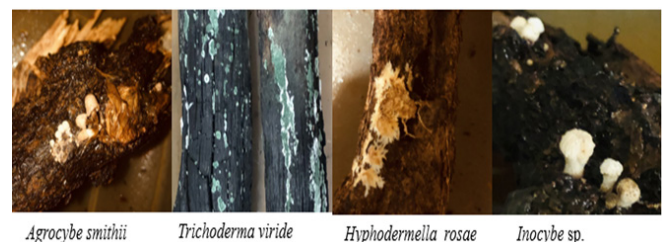


Figure 2. Different species of wood-decay fungi were documented in the incubation chambers.

1. YJ, McKenzie EH, Lumyong S. The numbers of fungi: are the most speciose genera truly diverse?. *Fungal Divers* 2022; 1-76.
2. Waktola G and Temesgen T. Application of mushroom as food and medicine. *Adv Biotechnol Microbiol*. 2018;113, 1-4.
3. Panahi HKS, Dehhaghi M, Guillemain GJ, Gupta VK, Lam SS, Aghbashlo M, Tabatabaei M. A comprehensive review on anaerobic fungi applications in biofuels production. *Sci Total Environ* 2022; 154521.
4. Yadav AN, Kour D, Kaur T, Devi R, Yadav N. Agriculturally important fungi for crop productivity: current research and future challenges. *Agriculturally Important Fungi for Sustainable Agriculture*. 2020; 275-286.
5. Alshammari N, Ameen F, AlKahtani MD, Stephenson S. Characterizing the assemblage of wood-decay fungi in the forests of northwest Arkansas. *J Fungi* 2021; 7(4), 309.
6. Das K. Diversity and conservation of wild mushrooms in Sikkim with special reference to Barsey Rhododendron Sanctuary. *NEBIO* 2010; 1(2), 1–13.
7. Rocha KS, Queiroz MS, Gomes BS, Dallago R, de Souza RO, Guimarães DO, Leal IC. Lipases of Endophytic Fungi *Stemphylium lycopersici* and *Sordaria* sp.: Application in the synthesis of solketal derived Monoacylglycerols. *Enzyme Microb Technol* 2020; 142, 109664.
8. Eriksson KEL, Blanchette RA, Ander P. In: *Microbial and enzymatic Degradation of wood and wood components*, Springer-Verlag, Berlin Heidelberg 1990; 407pp.
9. Gilbertson RL, Ryvarden L. *North American Polypores*, Volume I. Fungi flora, Oslo, Norway, 1986.
10. Sotome K, Akagi Y, Lee SS, Ishikawa NK, Hattori T. Taxonomic study of *Favolus* and *Neofavolus* gen. nov. segregated from *Polyporus* (Basidiomycota, Polyporales). *Fungal Divers* 2013; 58, 245–266.
11. Toju H, Tanabe AS, Yamamoto S, Sato H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PloS One* 2012; 7(7), e40863.
12. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural products research community. *J Nat Prod* 2017; 80: 756–770.
13. Tiwari CK, Parihar J, Verma RK. Additions to wood decaying fungi of India. *J Threat Taxa* 2010; 970-973.
14. .Asiegbu FO, Nahalkova J, Li G. Pathogen-inducible cDNAs from the interaction of the root rot fungus *Heterobasidion annosum* with Scots pine (*Pinus sylvestris* L.). *Plant Sci* 2005; 268: 365-372.
15. Ofodile LN, Uma NU, Attah LE, Simmonds MSJ, Popoola OE. Cnemomorphological study and antimicrobial activity of *Daedalea Quercina*. *Acta SATECH* 2010; 3(2): 102-107.
16. Cohen R, Persky L, Hadar Y. Biotechnological applications and potential of wood conservation needs and management options. *Eur. J. For. Res.* 2002;127: 1-22.
17. 17.Kaushik P. and Malik A. Fungal dye decolourization: Recent advances and future Potential. *Environ Int* 2009; 35, 127-141.
18. Leiniger TD, Wilson AD, Lester DG. Hurricane Andrew damage in relation to wood decay fungi and insects in bottomland hardwoods of the Atchafalaya Basin, Louisiana. *J Coast Res* 1997; 13, 1290–1293.
19. Alemu F. Assessment of wild mushrooms and wood-decay fungi in Dilla University, Main Campus, Ethiopia. *Int J. Adv Res* 2013; 1, 458–467.
20. 20. Lyngdoh A, Dkhar MS. Wood-rotting fungi in East Khasi Hills of Meghalaya, Northeast India, with special reference to *Heterobasidion perplexa* (a rare species–new to India). *Curr Res Environ Appl* 2014; 4, 117–124.
21. Bhutia LP. A preliminary survey on diversity of wood decay fungi from East Sillim. *J Mycopathol Res* 2016; 54, 255–261.
22. Alshammari NI., Veettil VN., Sulieman AME., & Stephenson SL. Impact of Field and Laboratory Environmental Conditions on the Diversity of Wood-Decay Fungi in the Forests of Northwest Arkansas. *J Pure Appl Microbiol* 2020; 14, 1801-1808.