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Isolation and characterization of spawn-contaminating microbes and their inhibitory impact on mycelial growth of *Pleurotus ostreatus*

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Mushrooms, celebrated for their culinary versatility and nutritional value, are gaining prominence as functional foods due to their medicinal properties. Among the 2,000 known mushroom species, only 25 are widely recognized as edible, with oyster mushrooms (*Pleurotus ostreatus*) ranking as the second most cultivated species globally. These mushrooms are rich sources of nutrients, antioxidants, and bioactive compounds, contributing to their therapeutic properties, including anti-inflammatory and antimicrobial effects. In India, mushroom cultivation presents a sustainable approach to converting agricultural waste into nutritious food, with West Bengal emerging as a significant hub for oyster mushroom farming. However, spoilage during spawn production and cultivation, caused by fungi such as *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus oryzae*, and bacteria like *Pseudomonas fluorescens*, severely impact their yield and profitability. This study focuses on the isolation and identification of pathogenic microorganisms responsible for oyster mushroom spawn spoilage in the Birbhum district. Pathogenicity tests revealed the most harmful pathogens affecting spawn and fruiting bodies. These findings identified key pathogens responsible for spawn spoilage in *Pleurotus ostreatus* (PO). *Rhizopus delemer* (RH1) emerged as the primary fungal pathogen, while four bacteria from the Enterobacteriaceae family exhibited the strongest inhibitory effects on spawn mycelia. These pathogens not only halted mycelial growth but also caused severe spawn deterioration, including texture damage. The FE-SEM study showed deterioration of PO spawns by selected spawn spoilage agents. Strategies to control fungal and bacterial contamination were explored, emphasizing biopreservation as a promising solution to enhance mushroom quality and marketability. By identifying the primary agents of spoilage and evaluating mitigation strategies, this research aims to support farmers in maintaining sustainability and profitability in mushroom cultivation.

Keywords: Distortion of fungal mycelia, Enterobacteriocea, FE-SEM, Mushroom spawn spoilage, *Pleurotus ostreatus*, *Rhizopus*

INTRODUCTION

Mushrooms have long been regarded as a culinary marvel and delicacy, celebrated for their unique flavor. However, only about 25 varieties are widely recognized as edible of the over 2,000 known mushroom species and few being commercially cultivated. The edible mushrooms are gaining attention for their nutritious and versatile qualities.

This naturopathic food contains multiple numbers of medicinal properties, economic value, and general acceptance. Globally, they are valued for their low fat and cholesterol content and rich supply of complex carbohydrates, proteins, vitamin D, and minerals (Jayachandran *et al*. 2017; Aida *et al*. 2009; Bell *et al*. 2022, Khatua et *al*. 2013; Jana and Acharya, 2022). Historically, mushrooms have been used for food and medicinal purposes across civilizations (Riaz *et al*. 2022). Their therapeutic properties-such as anti-inflammatory, anti-tumor, immune-boosting, antioxidant, and cholesterol-lowering effects—make them increasingly significant as medicine (Aida *et al*. 2009; Lyn *et al*. 2020). Like the other macrofungi, mushrooms also lack chlorophyll to harness solar energy and form distinct fruiting

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bodies both above and below the ground, relying on organic matter for nutrients. Cultivation of mushroom represents a sustainable method to convert agricultural and plant waste into high-quality food. Only in India its about 620 million tons of the organic waste (Thakur, 2020). Today, mushrooms are grown in nearly 100 countries, and 99% of global production is covered by varieties like oyster, button, shiitake, wood ear, and paddy straw mushrooms.

Oyster mushrooms are among the most widely cultivated mushroom species globally and ranked the second most farmed mushroom worldwide. China leads in oyster mushroom production, contributing 74% of the global output. Other significant producers include Italy, Poland, the Netherlands, Romania, the Republic of Korea, Spain, Lithuania, and India (Jarial *et al*. 2024). Oyster mushrooms are rich in nutrients with high levels of vitamin C, iron, potassium, zinc, calcium, phosphorus, folic acid, niacin, and vitamins B-1 and B-2. Their fruiting bodies are also abundant in phenols and flavonoids. These components help to reduce stress, possess strong antioxidant properties, and protect against various illnesses (Bhagarathi *et al*. 2023). Additionally, oyster mushrooms have antimicrobial and antioxidant effects, helping prevent damage to DNA and cell membranes (Bell *et al*. 2022).

In terms of cultivation and consumption, *Pleurotus ostreatus* (oyster mushroom) is the most popular in India, followed by *Agaricus bisporus* (button mushroom). In West Bengal, *Pleurotus* is the most cultivated mushroom due to the region's favorable environmental conditions (Raman *et al*. 2018; Bhargav *et al*. 2023). However, efforts must be made to raise awareness about the health benefits of consuming both farmed and wild mushrooms to boost local consumption.

During spawn production and mushroom cultivation, various spoilages have been observed and documented by several researchers (Bhargav *et al*. 2023; Agbagwa *et al*. 2020; Hassan and Ibrahim, 2022; Fadahunsi *et al*. 2013]. In India, spawn spoilage is reported to cause loss by 15–25% (Schill *et al.* 2021), primarily due to contamination by pathogenic fungi, like *Aspergillus niger*, *Fusarium chlamydosporum*, *Penicillium chrysogenum*, *Phoma exigua*, *Aspergillus fumigatus*, and *Fusarium pallidoroseum*. *Cladobotryum* species have also been reported to cause diseases in button mushrooms (Lee *et al*. 2011).

Pre- and post-distribution mushrooms are commonly affected by bacteria from the Pseudomonadaceae and Enterobacteriaceae families. *Pseudomonas fluorescens*, *Ewingella americana*, and *Pantoea beijingensis* have been identified as spoilage pathogens of oyster mushrooms by Schill *et al*. (2021). Other potential pathogens, such as *Clostridium*, *Enterobacter*, *Escherichia coli*, and *Bacillus*, can cause bacterial diseases in edible mushrooms, leading to financial losses and reduced marketability, ultimately impacting farmers' incomes (Sharma *et al*. 2007; Mwangi, 2016).

Oyster mushrooms (*Pleurotus* spp.) are highly susceptible to fungal pathogens that negatively impact their growth, yield, and quality. The growth of oyster mushroom mycelium is directly inhibited by several fungi, including *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Mucor* species, *Penicillium* species, *Rhizopus oryzae*, and other *Rhizopus* species. In a recent study, *Pleurotus sajor-caju* and other *Pleurotus* spp. have been found to be infected by *Aspergillus* sp., *Fusarium chlamydosporum, Phoma exigua, Aspergillus niger, Penicillium chrysogenum, Aspergillus fumigatus*, and *Fusarium pallidoroseum* (Bhargav *et al*. 2023). In another study, spoilage of *P. ostreatus* was observed during the cultivation by the *Fusarium oxysporum*, *Aspergillus flavus, Rhizopus oryzae*, and *Trichoderma harzianum* (Agbagwa *et al*. 2020). However, Suada *et al*. (2015) has reported the presence of *Fusarium* spp., *Mucor* spp., and *Trichoderma* spp. as the pathogenic fungi for *P. ostreatus*. Mwangi (2016) has reported the presence of the same pathogens. *Pleurotus pulmonarius* has been found to be susceptible to *Rhizopus arrhizus, Mucor hiemalis, Penicillium glabrum, Trichoderma longibrachiatum*, and *T. pleuroticola* (Hassan and Ibrahim, 2022). In addition, *P. sajor-caju* is highly infected by *Aspergillus fumigatus, A. niger, Botryodiplodia theobromae,* and *Rhizopus stolonifer* (Fadahunsi *et al*. 2013). Several reports highlight the infections in multiple

Pleurotus species, such as *P. ostreatus* and *P. pulmonarius* spoilage by *Aspergillus flavus* and *Trichoderma* (Sobowale *et al*. 2022), *Cladobotryum* (Jongman *et al*. 2018) and *Trichoderma* (Potoènik *et al*. 2015) infection on *Pleurotus* spp.. Additional studies have identified *Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus*, and *Trichoderma* as the dominant fungal pathogens of *P. ostreatus* (Omokaro and Ogechi, 2013). These findings also indicate the severity of contamination risks across different *Pleurotus* species that leads to significant yield lose and potential mycotoxin contamination affecting the mushroom cultivation in India.

Farmers are experiencing considerable losses due to microbial damage during mushroom cultivation and post-harvest period. Added to this, spoilage disrupts the profitability of farmers from cultivation. Therefore, preventing fungal and bacterial contamination is essential. To address this issue, commercial farms employ various methods, including drying, washing, UV treatment, irradiation, and use of chemical preservatives (Sharma *et al*. 2007; Hassan and Ibrahim, 2022; Agbagwa *et al*. 2020; Sobowale *et al*. 2022; Jongman *et al*. 2018; Mwangi, 2016). Effective disease management strategies are essential to mitigate these fungal infections and enhance the sustainability of oyster mushroom production.

However, controlling fungal pathogens poses a significant challenge, as both mushroom and pathogenic fungi belong to the same kingdom. Scientists have been attempting to develop many techniques to contemplate this problem. They have suggested the application of potential bio-preservatives as a better substitute for chemical agents. Bio-preservation with high selectivity only towards the fungal pathogen can present a promising solution, helping farmers to maintain mushroom quality while benefiting consumers by ensuring chemical-free safer food product.

In this study, spoiled oyster mushroom spawns were collected from local markets of the Birbhum district. The spoilage-causing microorganisms were isolated and identified from those samples. Furthermore, their pathogenicity level was observed to find out the most harmful pathogens affecting both the spawn and mushrooms. This can be helpful to decide the method of controlling the pathogens in this region, and ultimately support farmers in retaining their marketability and sustainability.

MATERIALS AND METHODS

Isolation and maintenance of fresh and contaminated Pleurotus spawn

Spawn of *Pleurotus* spp. were collected in both spoiled and fresh condition from the local spawn producers. Fresh samples were specifically collected from Huchuk Para, Adibashigram, situated under Ganpur Gram-Panchayet in Mohammad Bazar Block, Birbhum, West Bengal. The fresh spawn was immediately stored at 4°C to retain viability. For further study, the fresh samples were cultured on Potato Dextrose Agar (PDA) medium and incubated at 28°C for four days to get luxuriant fungal growth. After successful incubation, the cultures were preserved at 4°C for long-term use. This dual approach of collecting both fresh and contaminated spawn provides a robust foundation for analyzing factors affecting spawn quality and contamination.

Isolation of spoiled pathogenic fungi and bacteria

For isolating spoiled fungal pathogens, collected spawns with characteristic spoiled condition were placed aseptically on malt extract (ME) agar medium and incubated for 72 h at 28°C. The MEA medium was supplemented with 150 μg/mL streptomycin to avoid bacterial contamination. To isolate pure bacterial colonies from spoiled spawn, Nutrient Agar (NA) medium was added with 1 mg/mL fluconazole to inhibit the growth of any fungal contamination (Cao *et al*. 2024). NA plates were incubated for 48 h at 28 °C.

Screening of pathogenic fungi and bacteria based on inhibitory effect against Pleurotus spawn

The fungi and bacteria exhibiting the prominent inhibitory effect against the growth of *Pleurotus* sp. were assessed by culturing the organisms

on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) media. The growth inhibition by fungal pathogen against collected fresh *Pleurotus* was visualized using dual culture plate technique (Kim *et al.* 2023). The inhibition zones around the bacterial colonies were observed against the mushroom spawn. Only those demonstrating significant inhibitory effects were shortlisted for subsequent studies. Furthermore, preventive activities of CFS of spoiled pathogenic fungus and culture of bacteria were observed by collecting the fresh spawn mycelia and therefore treated with fixating agent [Glutaraldehyde-2% (v/v)] kept for prefixation for 30 minutes and dehydrated by alcohol gradation of 20% to 100% (v/v) (for 20 minutes for each gradation), followed by 100% (v/v) acetone for 20 minutes. The samples examined through FE-SEM (Carl Zeiss Gemini 450 (Gemini 2), UK/GB) after being gold coated (Quorum SC7620 Sputter Coater) for 105s (Maiti and Mandal, 2021).

DNA extraction from pure culture of potent Probiotic isolates and their molecular identification

Genomic DNA from the pathogenic bacteria, spoilage fungus, mushroom mycelia of isolated *Pleurotus* spp. were extracted following the manufacturer's protocol [bacterial genomic DNA isolation kit, Qiagen, Germany (Cat. No.-51304) and Fungal genomic DNA extraction kit-Himedia]. The presence of the DNA was visualized through 0.8 % (w/v) agarose gel electrophoresis. Spectrophotometric measurements were performed to determine the concentration and purity of DNA by measuring the A260 value and A260/A280 ratio using a UV spectrophotometer (Hitachi, Japan). DNA fragments were separated using electrophoresis. DNA samples were mixed with loading buffer (6x; 30% v/v glycerol, 0.25% w/v bromophenol blue), and electrophoresis was performed using 1xTAE buffer on 0.8 or 1% (w/v) agarose gels containing 0.5 µg/mL ethidium bromide (Duan *et al.* 2010). The gel was visualized in Chemi-Doc (BioRad, USA), and the molecular weight of the fragments was estimated using a DNA molecular marker (1 Kb ladder).

For the identification of bacterial isolates, PCR amplification of 16S rRNA gene was achieved using universal primers 27_F and 1492_R pair (Adie *et al*.2016). After purification of PCR product was then sequenced on Sanger sequencing platform (Guan *et al*. 2012). Pair-wise alignment of obtained sequence was executed by BLASTn tool of the nucleotide database (NCBI) to observe the sequence homology and sequences were then finally deposited therein.Extracted fungal DNA was PCR amplified by targeting the ITS region by using of universal primer pair ITS1/4 following lab standardized protocol. Purified PCR product was then sequenced on Sanger sequencing platform (Guan *et al.* 2012). Pair-wise alignment of obtained sequence was executed by BLASTn tool of the nucleotide database (NCBI) to observe the homology of sequence, and the sequence was deposited therein.

Sequence submission

Fastq format of the raw ITS 1/4 sequence of *Pleurotus* sp. and pathogenic fungi were deposited in NCBI database with the accession number POD81809.1 and OR271982.

RESULTS AND DISCUSSION

Isolation and characterization of fresh healthy Pleurotus spawn

Fresh mushrooms (*Pleurotus* sp.) with healthy spawn condition were collected from Huchuk Para, Adibashigram in Ganpur Gram-Panchayet, Mohammad Bazar Block, Birbhum, West Bengal. The identification of isolated mushroom was performed following the protocol recommended by Kotadiya *et al*. (2021).

Primarily, the collected spawn was mounted on PDA and after incubation of 4 days colony morphology was observed. Only the spawns that formed typical whitish mycelium with cottony texture of colony were screened further (Fig. 1A). The basidia was observed as slenderly clavate shaped containing 4 spores (Fig. 1B). Microscopic observation indicated that the cylindrical-oblong shaped spores were whitish-lilac grey in color (Fig. 1B). Finally, the spawn was inoculated in a cylindrical bed containing rice straw as substrate, and after 26 days of cultivation the structure of pileus, stipe and gills were recorded. Fruiting body having whitish grey colored

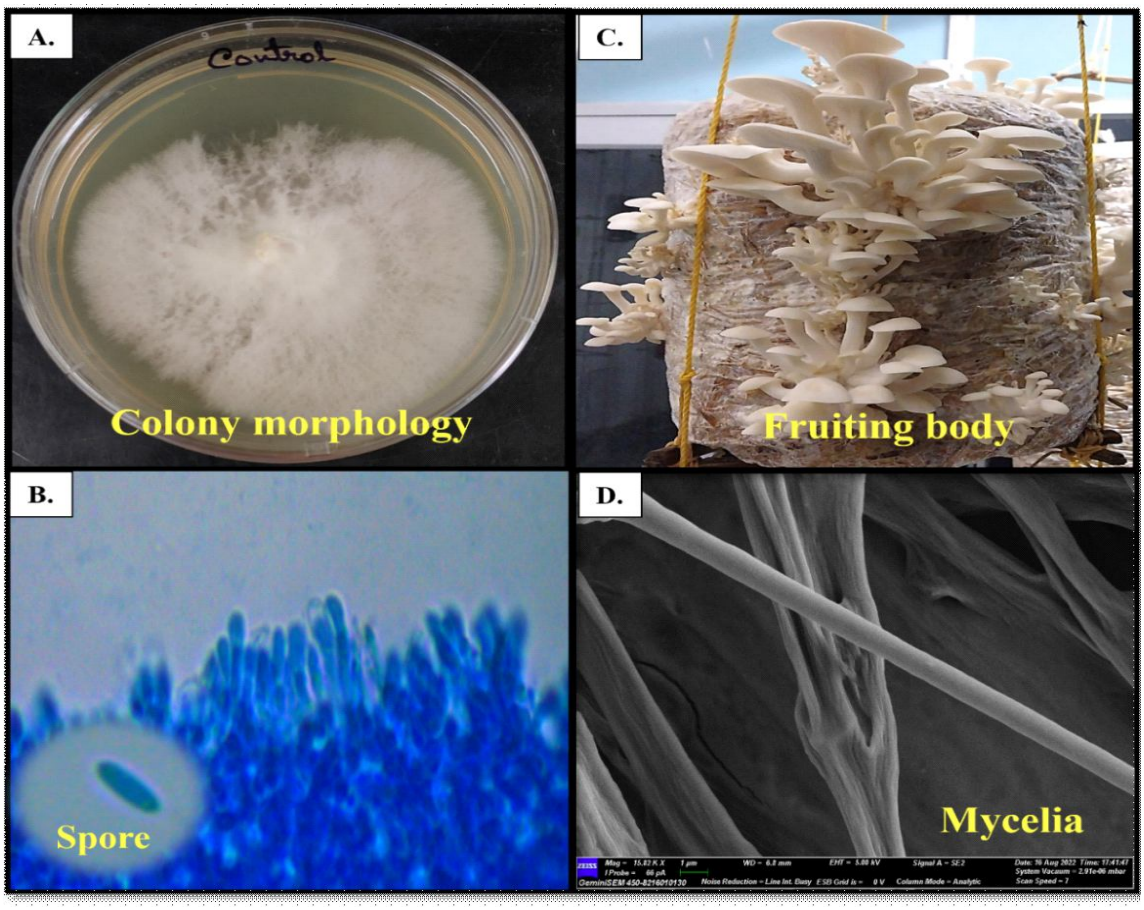


Fig. 1. Morphological characterization of isolated mushroom spawn. Fresh mushroom spawn marketed as *Pleurotus* sp. was collected from Huchuk Para, Adibashigram in Ganpur Gram-Panchayet, Mohammad Bazar Block, Birbhum, West Bengal. Morphological characterization and identification were performed following four techniques: **A.** Colony morphology on potato dextrose agar, **B.** Basidiospore character under compound microscope, **C.** Structure of fruiting body and its cultivation conditions **D.** Clear morphological study on FE-SEM. These characters of the isolated mushroom spawn were indicative of being *Pleurotus ostreatus*.

Table 1. Isolated mushroom spawn as identified by ITS gene sequence and their closest related species:

Isolate	Closest related species	Accession no of the isolate	Accession no of similar organism	Sequence Homology (%)
PO	*Pleurotus ostreatus*	POD81809.1	MK603976.1	100

pileus of diameter 5.6-11.2 cm with convex, smooth and soft edge that matured to a shell shape was demarked as the *Pleurotus* sp. (Fig. 1C). It was further confirmed by the white-cream-colored gills, and matured stipe of cream color with smooth surface texture measuring 4.7-7.2 cm (L) X 1.3-2.2 cm (d). For clear morphological analysis the mycelial structure was again visualized under FESEM (Fig. 1D). The structure supporting the microscopic view was finally selected and used for more downstream studies. These characters of the isolated and cultivated mushroom spawn PO were indicative of being *Pleurotus ostreatus* (Fig. 1).

Furthermore, molecular identification using the amplified ITS region sequence (using Sanger Technique) confirmed that the selected mushroom spawn (PO) was *Pleurotus ostreatus* (NCBI Accession no. POD81809.1) (Table 1).

The phylogenetic tree prepared based on the ITS sequence showed relatedness of the isolate with organism- *P. ostreatus* MK603976.1 (Fig. 2). The

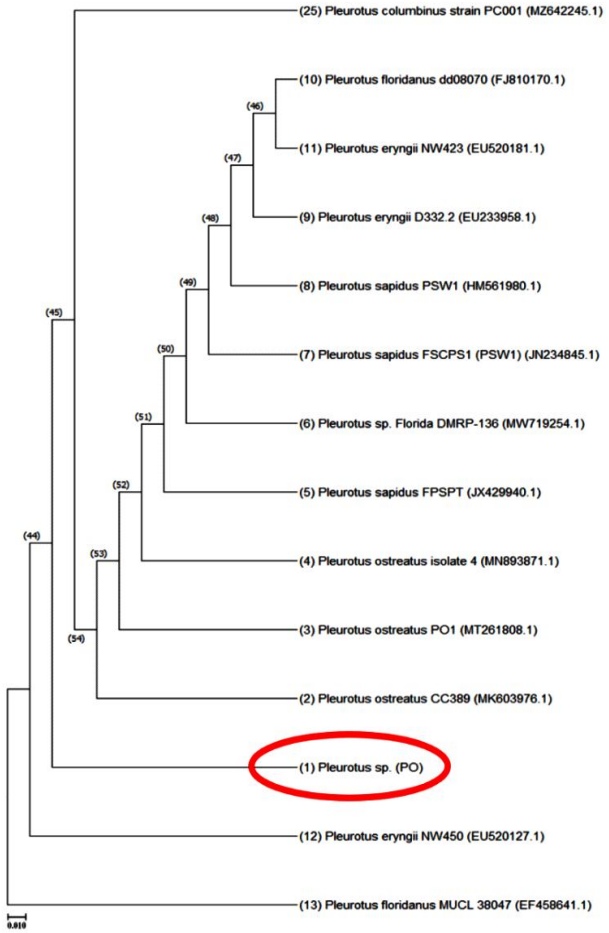


Fig. 2. Phylogenetic tree of isolated *Pleurotus*. Construction of Maximum likelihood phylogenetic tree elicited from the ITS1/4 region gene sequences of *Pleurotus ostreatus* PO. Bootstrap values (>50% are expressed as percentages of 1000 replications) are shown at branching points.

voucher specimens of the isolate are deposited in the Calcutta University Herbarium (CUH) with specimen no. of CUH AM 798.

Isolation of spoiled pathogenic fungi

Primarily, spoiled *Pleurotus* sp. spawn packets were collected from various spawn growers of Birbhum, West Bengal. Spoiled condition was recognized by the discoloration and bad odor in the spawn packets (Fig. 3). From ten such highly spoiled spawn packets fungal and bacterial pathogens were separated. Fungal (35) and bacterial (22) colonies were selected and characterized by their mycelial nature and colony morphology, respectively (Fig. 3).

Three types of different fungal colonies were detected from all collected samples, which were presumed to be *Penicillium* (5), *Aspergillus* (7) and *Rhizopus* (16) (Table 2, Fig. 4). This indicated that the main spoilage causing fungi in the collection area (Fig. 4) was *Rhizopus*, covering 57% of total fungal pathogens. Several studies on spoilage of *Pleurotus ostreatus* mushroom spawn also reported *Rhizopus* sp. as the most pathogenic fungus (Jarial *et al*. 2024; Bhagarathi *et al.* 2023).

On the other hand, *Penicillium* and *Aspergillus* were reported for causing spoilage of *Pleurotus sajor-caju* mushroom spawn (Thakur, 2020). In another study, *Botryodiplodia theobromae* proved to be an abundant pathogen of *P. sajor-caju* (Fadahunsi *et al*. 2013). Bhargav *et al*. (2023) found that the highest level of contamination for the genus *Pleurotus* occurred from June to September, and *Aspergillus* was the most detected pathogen. Their findings corroborated our findings with *Rhizopus* being the major player in causing spoilage of *Pleurotus ostreatus* spawn in the market of Birbhum during the same seasonal period.

Isolation of spoilage causing bacteria

In case of bacterial pathogens, 42 colonies were isolated from the 10 packets of spoilage spawn. The bacterial isolates were differentiated based on colony morphology, Gram character, motility, and cell shape. The characters indicated the existence of four groups of bacteria (Table 3). Previously, several reports also stated as contaminants or spoilage causing agent in mushroom spawn, although in lower number and with low pathogenicity (Bhagarathi *et al*. 2023; Raman *et al*. 2018).

Selection of pathogenic fungi and bacteria based on inhibition of PO spawn

The bacteria and fungi were chosen further because of their capacity to prevent the growth of *Pleurotus ostreatus* PO on PDA and NA medium, respectively. Isolate RH1 among the fungi showed the highest inhibition towards the mushroom spawn (Fig.5) [Kim et al., 2023].

Four bacterial pathogens (S2B2, S3B2, S3B3 and S3B6) showing prominent inhibition of PO

Fig. 3. Fresh and spoiled spawn packets: *Pleurotus* mushroom before (extreme left) and after (middle) bio-spoilage and number of bacteria (Right-Top) and fungi (Right-Below) isolated from 10 packets of spoilage mushroom.

Table 2. Identifying characters of the mushroom spoilage fungi

Genus*	Colony morphology	Microscopic study
Rhizopus	· N on - septate, fast growers · D ense cottony colonies · W hite, turns grey / golden brown	· Vegetative hyphae : stolon and rhizoids · Reproductive hyphae : sporangiophores - unbranched, elongated, columellate
Aspergillus	· Colonies are fast - growing · Texture velvety and cottony · B lack or green mould	· Vegetative hyphae and R eproductive hyphae · Unseparated conidiophore, L ar ge vesicle · Hyphae small, septate, hyaline · From m etullae or primary sterigmata, long conidiophores appeared · C onidiospores exist as round and unicell ular cells, in a chain form · S eparated, brush - like conidiophore
Penicillium	· Fast - growing and flat in appearance · Appears velvety or cottony · Mainly bluish - green colonies	

*The morphological characterization was performed as per the recommendation of Funder (Bhargav *et al*. 2023)

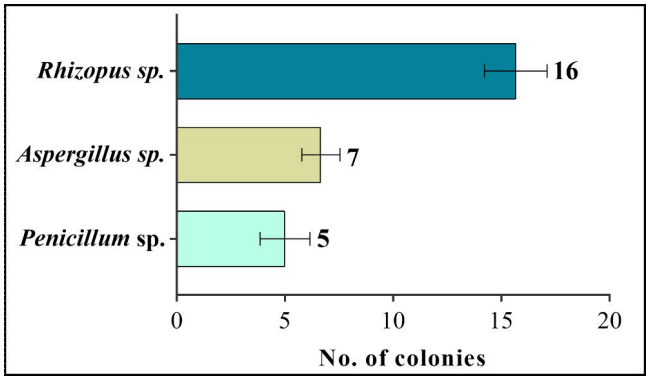
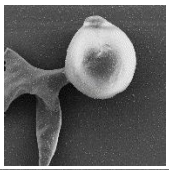
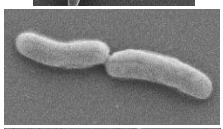
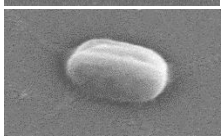
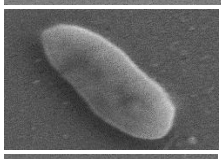
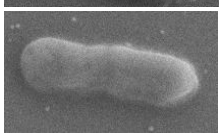


Fig. 4. Pathogenic organisms are isolated from spoilage mushroom spawns. The pathogenic fungi were isolated from spoiled mushroom spawn by mounted spoiled mushroom section on malt extract (ME) agar supplemented with 150 ìg/mL streptomycin and incubated for 72 hours at 28 °C. Primarily the pathogenic fungi were identified based on the colony morphology and mycelial structure under microscopic view. The study was performed from 10 spoiled mushroom spawn packets, and the mean value and standard deviation together of the data is presented in horizontal bars with error bar.

Table 4: Molecular identification of the isolated spawn spoilage microorganisms.

Pathogenic organism	Organism isolated	Closest related species	Accession no of similar organism	Homology (%)	FE- SEM Images
Fungus	RH1	*Rhizopus delemer*	PP756480.1	99.6	
Bacteria	S2B2	*Enterobacter hormaechei*	OM979076.1	100	
	S3B2	*Citrobacter freundii*	MN104593.1	100	
	S3B3	*Enterobacter hormaechei*	OM979076.1	99.8	
	S3B6	*Enterobacter cloacae*	MH266231.1	100	

growth were chosen for additional identification. The disintegration and distortion in mycelial structures of PO by the selected pathogens were examined further through FE-SEM study for better visualization (Fig. 6).

Molecular identification of selected spawn spoilage pathogens

Identification of fungal and bacterial pathogens were made based on the sequence similarity of ITS region 1 and 4, and 16S rRNA gene with the closest fungi and bacteria, respectively. The cellular morphology of the pathogenic fungi and bacteria was visualized under FE-SEM study. Amplified ITS1/4 region of RH1 genome showed maximum DNA sequence homology with *Rhizopus delemer* PP756480.1 (Table 4). Four bacteria showed sequence similarity with *Enterobacter hormaechei* OM979076.1, *Citrobacter freundii* MN104593.1, *Enterobacter hormaechei* OM979076.1 and *Enterobacter cloacae* MH266231.1 (Table 4). Interestingly all these bacteria were from Enterobacteriaceae group. This group is renowned for food spoilage and was isolated from several spoiled foods (Griffin *et al*. 2020; Dermesonluoglu *et al*. 2017]. Notably, Jiang *et al*. (2023) observed in the metagenomic study that Enterobacteriaceae group of bacteria was the most abundant population on deteriorated *Chanterelles* mushroom.

Spoiled *Pleurotus* sp. spawn packet was collected and used for the isolation of pathogenic fungi and bacteria on potato dextrose agar and nutrient agar, respectively. Extraction of the genomic DNA from the microorganism showing high level of repressive activity against the mushroom spawn. Genes for the ITS region (for fungi) and 16S rRNA (for bacteria) were amplified and sequenced on Sanger technique. DNA sequence homogeneity search using NCBI BLAST tool indicated the following closest related organism for each of the isolated pathogen. Characterization of morphology was carried out under FE-SEM study for better visualization regarding the cellular structure.

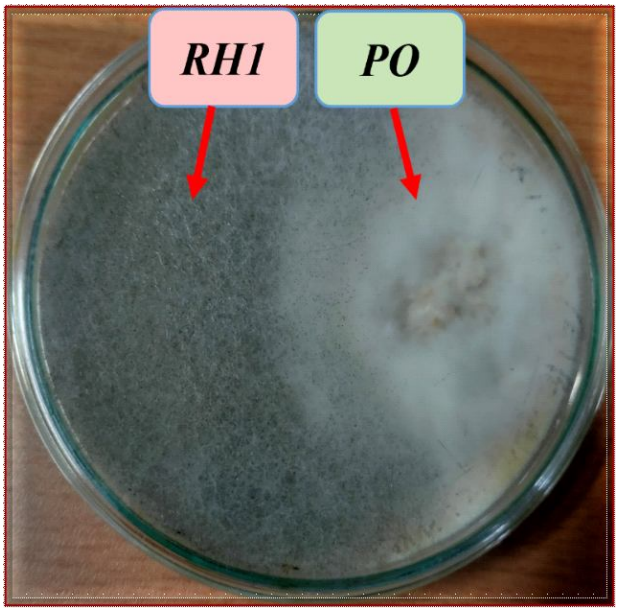


Fig. 5 : Selection of pathogenic fungus. Spoilage fungus *Rhizopus* RH1 (Left side) was selected based on inhibition of *Pleurotus ostreatus* PO (Right side) spawn as grown in co-culture on PDA at 28 C of incubation for 4 days.

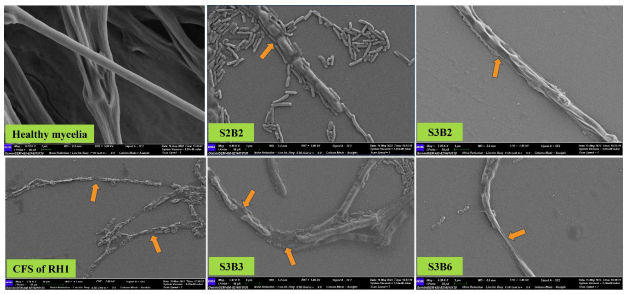


Fig. 6 : Spawn mycelia affected by pathogenic fungi and bacteria observed under FE- SEM. Intact mushroom spawn mycelia was observed under FE-SEM. The distortion in mycelial structure by pathogenic fungi (cell free supernatant or CFS of RH1) and four bacteria (S2B2, S3B2, S3B3 and S3B6) can be visible clearly as compared to the healthy one.

CONCLUSION

This study underscores the significant impact of both fungal and bacterial pathogens in causing spoilage of *Pleurotus ostreatus* spawn. Among the identified pathogens, *Rhizopus delemer* RH1 was recognized as the dominant fungal species responsible for spoilage, while bacteria from the Enterobacteriaceae family exhibited the strongest inhibitory effects on the mycelial growth of the mushroom. These pathogens not only inhibited the spawn growth but also caused severe deterioration in texture, and finally rendering the spawn unusable. Such spoilage events result in considerable economic losses for mushroom farmers, as they are often compelled to use chemical or physical preservatives to mitigate the problem. However, these methods can adversely affect the quality and yield of mushrooms, ultimately impacting consumer health and market acceptance.

In light of these challenges, researchers are exploring sustainable alternatives, with a focus on the development of bio-preservatives. In this way, additional efforts are being made to isolate *Lactobacillus* strains with probiotic properties, which could serve as natural inhibitors of spoilage pathogens (Research article under preparation). These bio-preservatives not only aim to prevent spoilage but also support healthier and more environmentally friendly mushroom production practices. This approach represents a significant step toward addressing the dual objectives of economic sustainability and high-quality mushroom cultivation.

DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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