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Isolation, Characterization of Bacterial Diseases of Paddy Straw Mushroom and its Prevention by Antibiotics in Comparison with Plant Extracts

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ABSTRACT

Paddy straw mushroom is widely cultivated in Odisha for their unique aroma, texture, and growth habit. Bacterial diseases contributed to the low production and storability of *Volvariella* spp. The present study was conducted on bacterial diseases of *Volvarirella* spp. Different types of symptoms were recorded to be associated with marketable mushroom buds of *Volvarirella* spp. leading to rotting of the buds within 24-48 hours of harvest. The bacterial species *Klebsiella pneumonia* was found to be associated with the symptoms. From all the plant extracts *Laurus nobilis* and *Amomum sabulatum* were shown to be most efficient at preventing the development of bacterial species. It was followed by *Zingiber officinale*, which inhibits the growth of the bacterial species. Among12 antibiotics Cefoperazone, Cefadroxil, Amoxyclav, Erythromycin, Clarithromycin, and Ampicillin could not inhibit the growth of any bacterial species. The rest of the antibiotics differ in their action against each of the bacterial pathogens. Cefaclor and Cefuroxime inhibited the maximum growth of the bacterial species.

Key words: *Paddy Straw Mushroom Diseases, Yellowish browning rot, Klebsiella pneumonia, Plant Extract, Antibiotics*

Introduction

The primary types of the worldwide mushroom industry are edible and therapeutic mushrooms. 2,000 species are found in nature, yet just 25 are commonly consumed as food (Chatterjee and Patel, 2016). Mushroom production worldwide surged to 10.4 million metric tons in 2016, led by China and the USA. Mushrooms are valued for their health benefits, with production increasing 27-fold since 1978, outpacing global population growth (Royse, 2014). On average, consumers now enjoy about 5 kg of mushrooms per person per year (Royse *et al.*, 2014). Mushrooms are a rich source of nutrients, particularly proteins, minerals vitamins as well as bioactive constituents, such as phenolic compounds, terpenes, steroids, and polysaccharides (Okhuoya *et al.*, 2010). Due to their elevated potassium-to-sodium ratio, mushrooms are beneficial for those with heart disease and hypertension. Various mushroom species contain metabolites with diverse health-promoting properties, including anti-tumor, antioxidant, anti-toxic, antiplatelet aggregation, antihyperglycemic, antimicrobial, and antiviral effects (Nongthombam *et al*., 2021). In India, Punjab, Haryana, UP, Rajasthan, J&K, and Odisha are the top mushroom-producing states. The industry grew 4.3% annually from 2010-17. White button mushrooms (73%) lead in production, followed by oysters (60%), paddy straw (7%), and milky mushrooms

(3%). Per capita mushroom consumption was less than 100 g/year in 2016-17. India exported 1054 quintals of white button mushrooms, boosting the industry (Sharma *et al.,* 2017). In today's health-conscious society, mushrooms are emerging as the future of vegetables due to their medicinal, nutritional benefits, and delicious taste. Their high digestibility makes mushrooms a potential protein alternative (Pavel, 2009) in addition to protein mushrooms have an excellent sense of Vitamin D which is not available in other food supplements (Pehrsson *et al*., 2003). In India, there are five mushroom species cultivated commercially: White button mushroom (*Agaricus bisporus*), Oyster mushroom (*Pleurotus* spp.), Paddy straw mushroom (*Volvariella* spp.), Milky mushroom (*Calocybe*), and Shiitake (*Lentinula edodes*). However, *A. bisporus*, *Pleurotus* spp., and *Volvariella* spp. dominate the commercial market, accounting for a combined 96% of mushroom production in India (Sharma *et al.,* 2017). Similar to other crops, mushrooms face numerous biotic and abiotic challenges. Mushroom cultivation is vulnerable to various competing organisms and parasitic fungi (Brown, 1937; Davis, 1938). Bacteria and nematodes can harm mushrooms both directly and indirectly, leading to various prevalent contaminants in mushroom cultivation. *Trichoderma harzianum*, *Coprinus* spp., *Aspergillus niger*, and *Penicillium sp*. have been identified as contaminants during the incubation and cultivation stages of oyster mushroom (Pleurotus ostreatus) spawn (Akhter *et al.,* 2017). Apart from causing specific diseases in mushroom sporophores, numerous other fungi thrive within the mushroom bed as competitors, substantially impacting mushroom yield. Commercial mushroom crops are susceptible to various fungal diseases (Fletcher and Gaze, 2008). Similarly, a few viral diseases have been reported to be associated with edible mushrooms (Sharma, 2017) (Sharma Kumar, 2007). However, bacteria are considered a major threat to mushroom production. Numerous bacterial diseases have been documented worldwide in the fruit bodies of *A. bisporus, A. bitorquis, Pleurotus* spp., *Volvariella* spp., *Lentiluna edodes, Flamulina velutipes*, and *Auricularia* spp. Bacterial blotch (*Pseudomonas tolaasii*) leads to estimated losses of 5-10% in mushroom production, with an additional 10% reduction in market value due to spoilage (Fermor, 1987). On the other hand, the traditional cultivation methodology of *Volvariella* spp. provides enough scope for the development and colonization of mushroom mycelium and fruiting bodies by a wide range of bacterial pathogens. The prevalence of high moisture and warm temperatures provides the ideal ecological condition for the colonization of bacterial pathogens within the mushroom substrate. The research on bacterial pathogens is limited to bacterial rot only of *Volvariella* spp. caused by *Pseudomonas* spp. (Kannaiyan, 1978). Insufficient research on bacterial diseases in *Volvariella* spp. results in decreased quality and shelf life. This study identifies responsible pathogens

Materials and Methods

Isolation and purification of bacteria associated with different symptom categories

Disease samples from various sources displayed distinctive symptoms in affected mushrooms, including stipe deformities, yellowing, soaked yellowish-brown streaks in the inner tissue, cavity formation, vulva sliminess, foul odor, and cap rot. Bacteria linked to these symptoms were isolated using surface disinfection and tissue sampling techniques (Fig. 1). These isolates were subsequently purified and assigned unique identification numbers (Fig. 2).

Fig. 1. Symptoms showing yellowish browning of the bulbus base

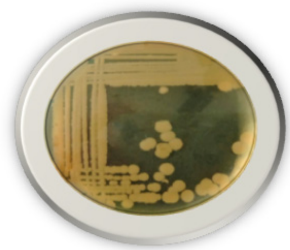

Fig. 2. Isolates of *Klebsiella pneumonia)*

Pathogenicity test

To assess the pathogenicity of bacterial isolate, we obtained fresh, apparently healthy mushroom buds at the egg stage from mushroom growers and transported them in new polythene bags to our laboratory. The pathogenicity test was conducted as described by Kumar *et al.,* (2018).

Characterization of isolated bacterial pathogens for their identification

The pure bacteria colonies obtained from the colonies growing on the nutrient agar were later identified based on morphological and biochemical tests, as reported by CMI (2010).

Molecular characterization of strains by the presence of 16S rRNA

BLAST analysis of 16S rDNA sequence homology was conducted as described by (Joko *et al*., 2014). This sequence was submitted to the GenBank database under accession number OQ415529.

Study of plant extracts on growth of the test bacteria

Water-based crude extracts were collected from 14 plant species chosen for their common use in Ayurvedic medicine. Plant extracts on the growth of test bacteria were conducted according to (Gonelimali *et al.*, 2018).

Study of effects of antibiotics on the growth of test bacteria

Twelve commercially available antibiotic discs (Himedia) were tested against the bacteria. Bacterial suspension was spread on NA plates, and three antibiotic discs were placed in the center. Inhibition zones were measured in millimeters after 24-48 hours.

Results and Discussion

Bacterial isolates associated with symptoms

Bacterial isolate such as, IAS1002 wasfound to be associated with symptoms in which yellowish browning of the bulbus base of the *Volvariella* spp was the major diagnostic symptom. In contrast to the diseased fruiting body of the mushroom, none of the pathogenic bacteria could be found to be associated with the healthy fruiting bodies whose internal tissue from the base to the stipe looked white. Yellowish browning of the bulbus base was found to be the most important symptom of bacterial disease as it is connected to the vulva and fungal mycelium. Vulvas being the outermost covering are more likely to be exposed to infection by several pathogens. Also, the mycelium growing within the substrate of the mushroom bed might be the source of primary infection. As mushroom cap grows out of the vulva by rupturing the vulval wall, possibly transferring the pathogen onto the cap and/or stipe resulting in the development of symptoms on such parts. Therefore, the result indicated an association of more than one pathogen with each symptom type. However, the ultimate symptom, yellowish browning of the bulbus base showed to be associated with one isolate. More systematic studies are required in this aspect are required for better conclusive results.

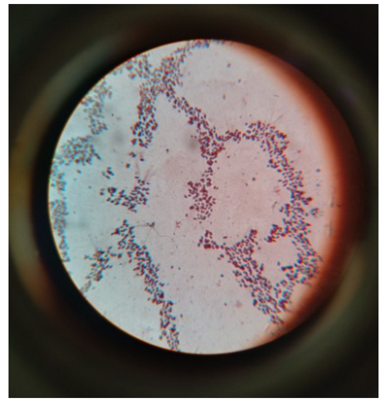

Fig. 3. Microscopic view of bacteria after gram staining

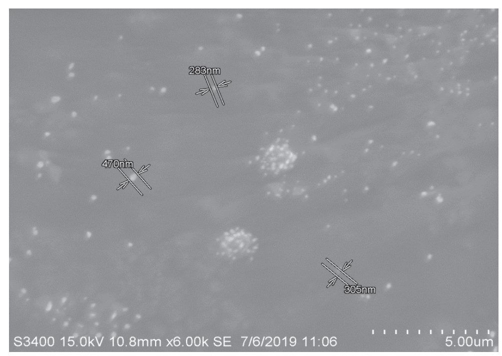

Fig. 4. Scanning electron micrograph of testbacteria

Identification of bacterial isolates based on morphology, utilization of carbohydrates, and biochemical characterization and molecular test

Morphological characterization revealed that the isolate was gram-negative encapsulated bacillus measuring 283 to 470 nm. No flagella could be detected. Whitish yellow colonies on nutrient agar were circular in shape, glistering, and convex having an entire margin. Acid production from carbohydrates revealed that the isolate showed a positive reaction against xylose, dextrose, galactose, melobiose, sucrose, mannose, and xylitol. The biochemical reaction showed positive reaction against esculin hydrolysis, citrate, malonate, lysine, and orthinine utilization, urease production, phenylalanine, demination, nitrate reduction, methyl red reaction, indole production, gelatin liquefaction, strong casein hydrolysis Growth at 27ºC was positive and negative at 47 ºC. The amplification of the 16S rRNA of this strain (accession number OQ415529) was generated. A similarity search of the 16S rRNA gene sequence against the NCBI GenBank database showed a 99 to 100% homology to known sequences of *Klebsiella pneumonia*. The isolate was identified as *Klebsiella pneumonia.* A phylogenetic tree (Fig 3) was constructed. using sequences in databases corresponding to strains of the *Klebsiella* group, in which *Klebsiella pneumonia* is found and the isolate was identified as *Klebsiella pneumonia. Pseudomonas tolaasii* is the predominant and extensively researched pathogen, given its broad range of occurrence, impacting various mushroom hosts like *A. bisporus, A. bitorquis, Pleurotus ostreatus*, and *Pleurotus eryngii* (Bradbury, 1987) and resulting in significant setbacks for mushroom cultivation. (Soler-Rivas *et al.*, 1999).

In the present study, *Klebsiella pneumonia*was found to be associated with the yellowish browning of bulbus base followed by the rotting of the sporophore of marketed samples of *Volvariella* spp. In India (Kannaiyan 1978) reported the only bacterial rot disease of *Volvariella* spp. It is to be caused by *Pseudomonas* spp. In this study, we got the association of *Klebsiella pneumonia* responsible for causing the yellowish browning and rotting of commercially cultivated paddy straw mushrooms (*Volvariella* spp.) is a new report.

Pathogenic ability of *Serratia marcescens*

Klebsiella pneumonia caused complete fruiting body rotting in 72 hours, while the control remained unaffected.

Sensitiveness of *Klebsiella pneumonia* against different plant extract

Crude extracts with water base were collected from 14 plant species selected based on their frequent use in ayurvedic medicine and tested against the bacterial species. The zone of inhibition of bacterial growth was recorded and presented in Table 1. *Laurus nobilis and Amomum sabulatum* plant extract were found to be most effective in inhibiting bacterial growth against the test bacterial species. Similarly, *Psidum gujava*, *Curcuma longa*, *Ocimum sanctum, Syzygium aromaticum*, *Aloe barbadensis*, *Tridax procumbens,* and *Piper nigrum* could not inhibit the growth of the test bacterial species. The rest of the plant extracts differed in their effectiveness against the bacterial species. Plant extract-based antibacterial biocides offer several advantages over chemical bactericides, serving as environmentally friendly alternatives with demonstrated potent antibacterial properties against pathogenic bacteria. Paddystraw mushroom is grown using paddy straw as its substrate. Therefore, mixing plant parts along with the substrate having antibacterial properties will be a better proposition for the management of bacterial diseases as they will not allow the pathogen to es-

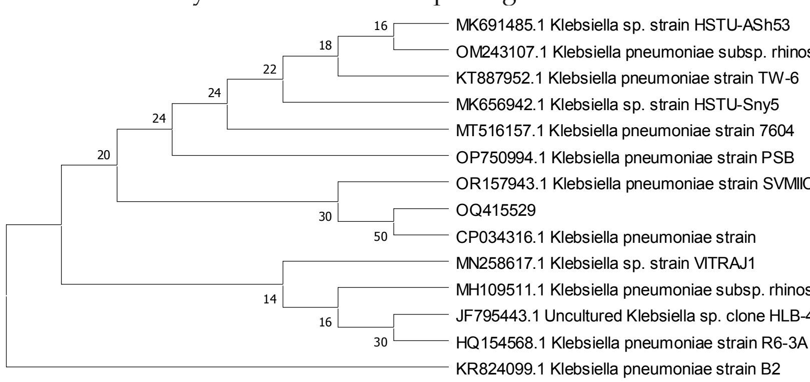

Fig. 3. Phylogenetic placement of *Klebsiella pneumonia* with other ex-type strain sequences obtained from NCBI Genbank database)

seven plant extracts out of which five plant parts contained essential oil. More studies are required about the temperature sensitiveness of the active ingredients and toxicity against the mycelial growth of mushroom fungus before any recommendation is made.

Sensitiveness of *Klebsiella pneumonia* against different antibiotics

Commercially available antibiotic discs were tested against the bacterial species by recording the growth inhibition zone formed on inoculated media plates. The result revealed in Table 2 that among different antibiotics irrespective of test bacterial species,

Cefaclor recorded the maximum zone of inhibition followed by Cefuroxime. Six of the test antibiotics could not inhibit the growth of any bacterial species. Among the twelve test antibiotics, was found sensitive to six antibiotics. The highest zone of inhibition 93.06 mm of bacterial growth by Cefachor was recorded against *Klebsiella pneumonia*. Cefotaxime recorded the lowest zone of inhibition of 22.3 mm against the bacteria. A global study revealed that bacterial diseases impact key commercially significant mushroom genera like *Agaricus*, *Pleurotus*, *Lentinus*, *Flammulina*, *Volvariella*, and *Auricularia*. Pseudomonadales are typically responsible for crop losses, especially in more intensive cultiva-

Table 1. Effect of plant extract in inhibiting the growth of *Klebsiella pneumonia*

Sl No	Scientific name of the plant	R1	R2	R3	Mean(mm)
1	*Elettaria cardamomum*	9	9.1	9.3	9.133
2	*Curcuma longa*	0	0	0	0
3	*Zingiber officinale*	11	10.9	11	10.967
4	*Trigonella foenum*	10	10.8	10.4	10.4
5	*Amomum sabulatum*	14	13.9	14.1	14
6	*Ocimum sanctum*	0	0	0	0
7	*Syzygiumaromaticum*	0	0	0	0
8	*Psidium guajava*	0	0	0	0
9	*Clitoriateunalis*	10	10.4	10	10.133
10	*Aloe barbadensis*	0	0	0	0
11	*Cinnamomum verum*	8	8.2	8.4	8.2
12	*Laurus nobilis*	16	16.1	16	16.033
13	*Tridax procumbens*	0	0	0	0
14	*Piper nigrum*	0	0	0	0
	Control	30	30	30	30
	C.D. at Pd”0.05		0.234		
	SE(m)		**0.081**		

Table 2. Effect of antibiotics on the growth of *Klebsiella pneumonia*

Sl No.	Antibiotics	R1	R2	R3	Mean(mm)
1	Ampicillin	0	0	0	0
2	Cefuroxime	40.6	40	40.2	40.267
3	Cefadroxil	0	0	0	0
4	Amoxiclav	0	0	0	0
5	Penicillin-G	31.3	31	31.2	31.167
6	Cefotaxime	22.3	22	22.4	22.233
7	Cefaclor	93.06	93	93.1	93.053
8	Azithromycin	35.3	35.4	35	35.233
9	Erythromycin	0	0	0	0
10	Cefoperazone	0	0	0	0
11	Clarithromycin	0	0	0	0
12	Ciprofloxacin	33.6	33.4	33.2	33.4
	Control	70	70	70	70
	C.D. at Pd”0.05		0.232		
	SE(m)		**0.079**		

tion methods. Fermor (1986) has recommended environmental adjustments within the growing room and the regular use of chlorinated water drenching on the casing layer as potential solutions. The use of terramycin at a rate of 9mg per square foot, along with streptomycin (200 ppm), oxytetracycline (300 ppm), kasugamycin, and kanamycin, has proven to be an effective approach for controlling bacterial diseases in mushrooms (Sharma *et al*., 2007). Detection of bacterial pathogens present in the mushroom bed and application of selected antibiotics is thought to be a difficult proposition in managing bacterial diseases of *Volvariella* spp. Therefore, more investigation is required for selecting a broad-spectrum antibiotic.

Conclusion

Mushroom samples from six different marketplaces were examined for various bacterial rot disorders. Bacterial isolates causing these disorders were identified as Klebsiella pneumonia during pathogenicity studies on healthy mushroom buds. In vitro tests assessed their sensitivity to 14 different plant extracts, with *Laurus nobilis* and *Amomum sabulatum* extracts proving the most effective. *Psidum gujava, Curcuma longa, Ocimum sanctum, Syzygium aromaticum, Aloe barbadensis, Tridax procumbens*, and *Piper nigrum* extracts were ineffective against bacterial growth. Among 12 antibiotics tested, cefachlor was highly effective, while others showed varying degrees of sensitivity. Further research is needed to develop effective measures, including sanitation, integrated cultivation, and disease management, alongside plant extracts and chemical control

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Conflict of interest: Authors do not have any conflict of interest to declare.

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