

**COMPARATIVE STUDIES OF SOME OYSTER MUSHROOMS
(*Pleurotus* spp.) AND THE EFFECT OF ENDOPHYTIC
BACTERIA ON THEIR GROWTH, DEVELOPMENT,
ANTIMICROBIAL AND BIOREMEDIATION POTENTIALITIES**

**THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY (SCIENCE)
IN MICROBIOLOGY**

**SUBMITTED BY
CHANDANA PAUL**



Postgraduate and Research Department of Microbiology
St. Xavier's College (Autonomous), Kolkata
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Year 2025

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**THIS THESIS IS DEDICATED TO
MY BELOVED PARENTS
&
SWEETEST DAUGHTER**

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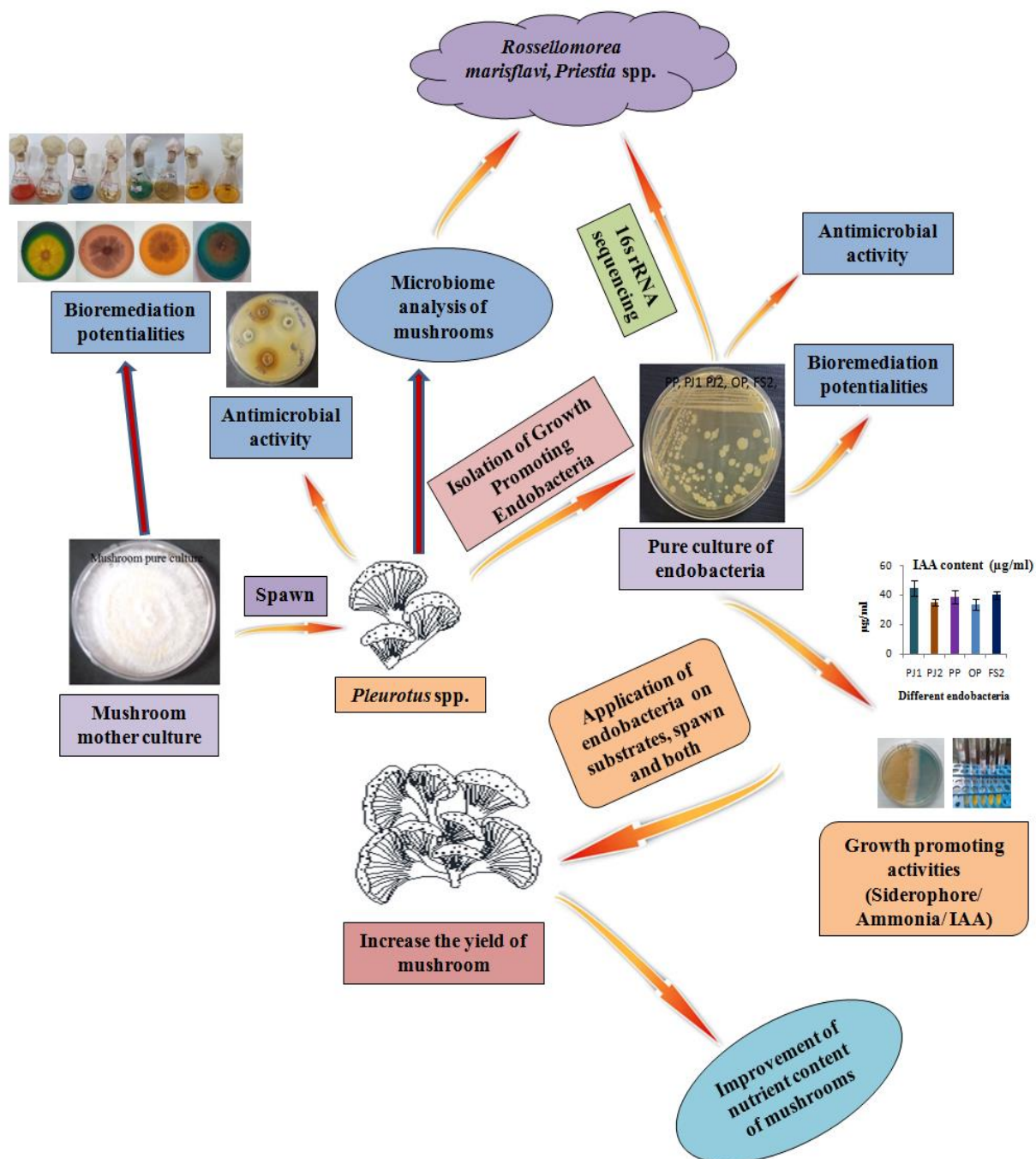
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ABSTRACT

Oyster mushrooms (*Pleurotus* species) are commercially cultivated in India owing to the suitable agro-climatic conditions and easy cultivation methods. Three *Pleurotus* spp. viz. *P. ostreatus* (MTCC 1802), *P. pulmonarius* (MTCC 1805) and *P. florida* (ITCC 3308) are used in this study. Here, rice straw showed better biological efficiency than sugarcane bagasse as the mushroom substrate. Like other living systems, mushrooms also possess an array of bacteria in their external surface and internal tissue systems. The mushroom microbiome showed *Pseudomonas* as the major bacterial genus, followed by *Paenibacillus*, *Stenotrophomonas*, *Priestia* etc. A number of growth-promoting bacteria were isolated from different parts of the sporophore of these mushrooms. Five endobacterial strains were finally selected based on growth-promoting abilities (production of Indole acetic acid, siderophores and ammonia) and studied thoroughly within which PP, PJ1 and PJ2 were isolated from *P. pulmonarius* whereas OP and FS2 were isolated from *P. ostreatus* and *P. florida*, respectively. These bacterial isolates were identified as the species of *Priestia* and *Rosellomorea* by morphological, biochemical and phylogenetic analysis using 16S rRNA sequencing. The 16S rRNA sequences of all these strains are submitted to GenBank and the strains are submitted to Culture Collections. None of the endobacteria produced laccase but they induced the production in co-culture with their respective host mushrooms. All the endobacteria significantly increased the mycelial growth rate and biomass of the mushrooms. Supplementation of bacteria with rice straw substrate/spawn /both spawn and substrates enhanced the fruiting body production of all the mushroom species. The highest BE (64.84%) was found when PJ2 was used as substrate. The endobacterial-supplemented mushroom also enhanced the nutrient content of the host mushrooms with respect to the control set. Both the mushroom species and their endobacteria tolerated 0.05% pesticides (Imidacloprid and Carbendazim) but were unable to degrade them. All the *Pleurotus* spp. also efficiently decolorize some industrial dyes (Congo red, Bromocresol green, Malachite green and Acridine orange) and showed antibacterial activity against both Gram positive and Gram negative bacteria, but the isolated endobacteria could not show these activities in the present experimental condition. Beyond their role in sporophore induction, these endobacterial isolates could be used for enhanced synthesis of bioactive compounds in a sustainable way

GRAPHICAL ABSTRACT



ABBREVIATIONS

16sF- The forward primer

16sR- The reverse primer

AO- Acridine orange

APS- Ammonium per sulphate

BCG- Bromocresol green

BE- Biological efficiency

B.O.D- Biological oxygen demand

BLAST- Basic local alignment search tool

BSA- Bovine serum albumin

CFU- Colony forming unit

CAS- Chrome azurol S

cm: Centimeter

CMC- Carboxy methyl cellulose

CR- Congo red

CV- Crystal violet

dd H₂O- Double distilled water

DMSO- Dimethylsulfoxide

DNSA- 3,5-Dinitrosalicylic acid

DPPH-2, 2-Diphenyl-1-picrylhydrazyl

EcMF-Ectomycorrhizal fungi

EDTA- Ethylene diamine tetra acetic acid

FBI- Fungal Bacterial interactions

g/l- Gram/liter

gm- Gram

GPA- Growth promoting activity

hr- Hour

HDTMA-Hexa-decyl (trimethyl) ammonium bromide

IAA- Indole acetic acid

IMTECH- Institute of Microbial Technology

ITCC- Indian Type Culture Collection

IU- International Unit.

Kg- Kilogram

lb- Pound

LB- Luria Bertani

M- Molar

MEGA - Molecular Evolutionary Genetics Analysis

mg- Milligram

min- Minute

mM- Millimolar

MG- Malachite green

MGPB- Mushroom growth promoting bacteria

MRVP- Methyl red Voges-Proskauer

MSM- Minimal salt medium

MTCC- Microbial Type Culture Collection

MTT- 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

NA- Nutrient Agar

NB-Nutrient Broth

NBRIP- National Botanical Research Institute's phosphate.

NCBI- National Center for Biotechnology Information

NCIM- National Collection of Industrial Microorganisms

nm- Nanometer

OD- Optical density

OD600- Optical density at 600nm

PCR- Polymerase chain reaction

PDA- Potato Dextrose Agar

PDB- Potato Dextrose Broth

pM- Picomolar

PO- Peroxidase

ppm- Parts per million

rpm- Revolution per minute

sq. μm - Square micrometer

TAE buffer- Tris Acetic Acid and EDTA buffer

TCP- Tri-calcium phosphate

TE buffer- Tris-EDTA buffer

TEMED- N,N,N',N'-Tetramethylethylenediamine

Trp-Tryptophan

WRF- White rot fungi

Wt- Weight

μg - Microgram

$\mu\text{g/ml}$ - Microgram per milliliter

μl - Microlitre

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CHAPTER I

INTRODUCTION

Mushrooms are defined as fleshy, spore-forming fruiting body of a fungus that is usually developed on soil, above ground or on its substrates. According to Chang and Miles (1992) mushroom has distinctive fruiting body which is big enough to be picked up by hand and seen with the unaided eye. It may be epigeous (above ground) or hypogeous (below ground) in appearance. Although, in general, all macrofungi are referred to as mushrooms, in formal definition, only edible macrofungi are called mushrooms, and non-edible or toxic macrofungi are called toadstools.

Some mushrooms have been used as a food item since ancient time, and they have been utilized for therapeutic purposes. Mushrooms were regarded by the Romans as the Food of the Gods, and they were even listed among the meals that were only offered during special events. Additionally, mushrooms were considered a gift from the deity Osiris by the Egyptians (Rahi et al. 2016; Maihara et al. 2012; Ahmed et al. 2023). Numerous mushrooms have demonstrated a variety of therapeutic activities, including antitumor, antihypertensive, anti-inflammatory, hypocholesterolaemic, antibacterial, and antiviral qualities (Weinheim, 2006; Khatun et al. 2015). The notable medicinal mushrooms include *Polyporus umbellatus*, *Ganoderma spp.* (Reishi), *Coriolus versicolour*, *Grifola frondosa* (Maitake), *Lentinus edodes* (Shiitake), *Lactarius sp.*, *Cordyceps sinensis*, *Tremella fuciformis* (Silver-Ear), *Psilocybe sp.*, *Agaricus bisporus* (Button mushroom), *Poria cocos* (Hoelen), *Pleurotus* species etc. Mushrooms can be used as food in the form of soups, pills, teas, capsules, etc. Mushrooms' contained bioactive substances including lectins, polysaccharides and terpenoids that are absent from plant and animal meals (Acharya et al.

2017, Rai et al. 2005) in addition to protein, carbohydrate, minerals, vitamins, polyphenol, flavonoids, essential amino acids etc. (Purkayastha and Nayak, 1981; Kurtzman, 1976;). They are now-a-days considered as functional foods which not only supply the energy and nutrients but also amend some specifications of the body by increasing the physiological responses as well as by decreasing the chance of disease (Bell et al. 2022).

Oyster mushrooms (*Pleurotus* spp.) are placed in second position as per human consumption throughout the globe right behind *Agaricus bisporus*, button mushrooms (Sanchez 2010). Correa et al. (2016) also reported that mushroom belonging to the genus *Pleurotus* has significant nutritional value as well as therapeutic applications. The genus *Pleurotus* (member of Basidiomycotina) has many edible species but about twenty of them are cultivated commercially around the globe. Oyster mushrooms are rich in protein and other nutrients, and they also contain a variety of vital enzymes having biotechnological importance, with laccase being one of the most significant. In addition, it produces a range of secondary metabolites having pharmaceutical values. *Pleurotus* belongs to the family Pleurotaceae, order Agaricales, Class Hymenomycetes, subdivision Basidiomycotina, Division Eumycota and kingdom Mycota. Globally, oyster mushrooms (*Pleurotus* spp.) are farmed for commercial purposes as well as in the wild in subtropical, tropical, and temperate climates (Li, 2017). Their easy cultivation methods in a broad temperature range (15°C - 30°C) on a variety of substrates, including woods, weeds, wastes, and agroforestry residues, make them one of the most widely used edible mushrooms in India (Obodai et al. 2003; Das et al. 2007; Chirinang and Intarapichet, 2004; Gregory et al. 2007). Since oyster mushrooms are abundant in vitamins, fiber, protein, and minerals and low in calories, they are regarded as a nutrient-dense diet. They produce different secondary metabolites like

polyphenols, flavonoids, tannins, carotenoids etc. which prevent variety of illnesses like diabetes, cancer, and hypertension etc., thus called nutraceuticals (Khatun et al. 2015).

Oyster mushrooms showed antimicrobial activities. The extracts of oyster mushrooms even the extracellular culture filtrate of some *Pleurotus* species showed antimicrobial activities (Benedict and Brady, 1972; Kunjadia et al. 2014; Suzuki et al, 1990). The fruiting body of oyster mushroom and mycelium both contain substances with broad-spectrum antibacterial action. Immunomodulatory compound like glucan was isolated from several mushrooms. They generate several secondary compounds that can act against the fungi, viruses, and bacteria (Suzuki et al. 1990; Benedict and Brady, 1972). Research has demonstrated that the combination of commonly used antibacterial medications with naturally occurring substances obtained from mushrooms may represent a novel approach for treating infections caused by bacteria that are resistant to several treatments (Jakubczyk & Dussart, 2020, Sutthisa and Anujakkawan 2023). The antimicrobial properties of polyphenolic compounds derived from mushrooms, like phenolic acids or flavonoids, have been demonstrated against a range of microorganisms. These substances represent a potential class of natural antimicrobial agents because they can make strains of bacteria that are resistant to several drugs receptive to bacteriostatic or bactericidal medicines (Cardoso et al. 2021; Kosanić et al. 2012; Patra, 2012). Oyster mushroom also showed antiviral activities. The extract of oyster mushroom showed anti-HIV activity as it showed anti – reverse transcriptase activity. *Pleurotus* sp. extract also showed the inhibitory activity against Human Cytomegalovirus (Roy et al. 2020).

Oyster mushrooms also have significant potential for bioremediation. As members of the fungal kingdom, the bioremediation ability of *Pleurotus* spp. is specifically known as

"mycoremediation." The term "mycoremediation" was first coined by Paul Stamets to describe the process by which fungi detoxify contaminated soil. By definition, mycoremediation involves using fungi to sequester and break down various toxic compounds in polluted soil or water (Chaudhary et al. 2018). Beyond, being a protein-rich food source, mushrooms possess enzymatic systems capable to degrade pollutants, which in turn support their growth and development. This has led to increasing interest in using mushrooms for studies on biodegradation and decolorization.

According to Elekes and Busuioc (2010), most mushrooms belong to the class Basidiomycetes, which secretes a range of extracellular enzymes necessary for their development and survival. Key enzymes include cellulase, laccase, manganese peroxidase (MnP), versatile peroxidase (VP) phenol oxidases and lignin peroxidase (LiP) (Dutta & Hyder, 2019). White-rot fungi (WRF), such as *Pleurotus* species, are particularly noted for their capability to degrade both cellulose and lignin, making them model organisms for mycoremediation. Their capacity to break down various dyes and xenobiotic compounds is particularly valuable for environmental cleanup (Singh, 2017). Due to the structural similarities between lignin and a wide range of environmental contaminants, such as PCBs, dioxins, polycyclic aromatic hydrocarbons (PAHs), dyes, pesticides, and other phenolic compounds, mushroom-based mycoremediation has garnered significant attention as an effective solution for detoxifying these contaminants (Su et al. 2018).

Every living organism like Plants, animals, humans, are associated with a large number of micro-organisms through their life cycles which can reside not only in their external surfaces but also in their internal tissues (Proctor 2011). There is a continuous interaction between these microbes with their host and environment accounting ecological balance and

sustainability. It also regulates the growth and development of the participating living beings and thus the analysis of whole microbiome is gaining interest now-a-days. The metagenomic analysis revealed the whole microbiome of the host and its associated microorganisms irrespective of their parasitic or symbiotic nature (Bahram et al. 2021). In addition to microorganisms living on the surface, a number of microorganisms reside within the internal tissue of the mushrooms. These endophytes are important for improved nutrient uptake, pathogen infection management, host development enhancement or inhibition, and bioactive chemical production (Chen et al. 2022a; Aslani et al. 2018). De Bary (1866) coined the word “endophytes” for first time to distinguish between epiphytic organisms that live on plant surfaces. The word "endophyte" is derived from the Greek words "endon," which means within, and "phyton," which means plant. Endophytic organisms are described in early definitions as asymptomatic colonies that reside inside their host plants, presumably for a portion of their life cycle (Stone et al. 2000). More recently, Hardoim et al. (2015) classified endophytes as microorganisms that colonise inside of the plants regardless of the nature of their interaction, and that belong to the bacteria, archaea, fungi, and also protists. Though mushrooms are members of fungal kingdom but the term ‘mushroom endophytes’ are often used to describe the endogenous organisms of mushrooms (Carrasco and Preston, 2020; Gautam et al. 2016). The possible reason may be due to the inclusion of fungi within ‘Thallophytes’ of the Plant kingdom (Plantae) according to old classification system. Thus the microbiome of mushrooms includes a large number of endobacteria like other living organisms (Rossouw and Korsten 2017).

Endophytic bacteria are recognized to be crucial to the development and growth of their host organisms (Proctor 2011). Endophytic bacteria are known to generate a variety of beneficial substances, including enzymes, antibiotics, and regulators of plant growth. These substances have the capacity to improve the development and well-being of their hosts. The microbiome of the species, which is primarily composed of endophytic bacteria, is crucial to the development and growth of the hosts. Their connections vary widely, ranging from parasitism to commensalism to mutualism, and they typically do not cause harm to their host. Therefore, complex relationships between hosts and endophytes are regarded as unique ecological niches (Aslani et al. 2018, Carrasco and Preston 2020, Ancheeva et al. 2020). These relationships include parasitism, commensalism, and mutualism whereas according to Schulz et al. (1999), endophytes do not harm their host plants, and their interactions with hosts are viewed as a balanced host–pathogen antagonistic relationship that produces secondary metabolites. According to Watts et al. (2023), enhancing nutrient intake, encouraging or inhibiting host development, avoiding pathogen infection, and generating bioactive substances are all critical functions of endogenous microorganisms. A unique ecological niche is defined by the complex host-endophyte interactions (Aslani et al. 2018; Ancheeva et al. 2020; Carrasco and Preston 2020). Many studies are being conducted to modify the substrate compositions (De et al. 2023) and add different supplements (Wang et al. 2010; Cunha et al. 2011; Zhou et al. 2010) in an effort to reduce the fruiting cycle and increase the production of edible mushrooms. Numerous research have focused on the role of bacteria in composting and casing as well as the fact that under sterile conditions, certain macrofungi do not develop sporophores (Pardo-Giménez et al. 2017; Noble et al. 2009). According to some recent research certain mushroom growth-

promoting bacteria (MGPB) can trigger different stages of the development process, including fruiting induction, mycelial elongation, substrate conversion and adaptation of mushroom (Suwannarach et al. 2022; Li et al. 2016). There have been reports of isolation of endobacterial isolates from several mushrooms, including *Agaricus bisporus* (Gautam et al. 2016), *Rhizopogon roseolus* (Pramoj et al. 2019), *Tuber* spp. (Le Roux et al. 2016), *Tricholoma* sp. (Oh et al. 2018) and *Hipsizygus ulmarius* (Poonga and Kaviyarasan 2015). To enhance the production of quality mushroom, the mushroom biotechnologists are continuously adopting different measures. Use of variety of compost substrates, supplementation of inorganic or organic substances as well as use of mushroom growth promoting bacteria (MGPB) were done by several workers. Despite the isolation of some MGPB from the culture beds/compost of some mushroom species, there are very scanty amount of report to use these organisms in mushroom fruiting body production possibly due to extensive laborious and time –intensive nature of the work (Carrasco and Peterson 2020; Carrasco et al. 2018; Chen et al. 2022a; Suarez et al. 2019). In higher filamentous fungus, sporophore formation is a complex process which required the involvement of a several genetic, metabolic, and environmental factors. Currently there is very few information available on the significant role of bacteria in mushroom's growth and sporophore generation. The main goal of our present study is to investigate the role of endobacteria in the growth, development and nutraceutical contents of some oyster mushrooms.

RESEARCH OBJECTIVES

1. To study the biological efficiency and nutraceutical compositions of three *Pleurotus* spp.
2. Metagenomic analysis of sporophores of *Pleurotus* spp.
3. Isolation and characterization of endophytic bacteria from *Pleurotus* spp.
4. Determination of role of endobacteria in growth, development, and nutraceutical compositions of *Pleurotus* spp.
5. To study the bioremediation potentialities and antimicrobial activities of *Pleurotus* spp. and their endobacteria.

CHAPTER II

LITERATURE REVIEW

For the last few decades, researchers have been quite interested in mushroom cultivation as an alternative protein source of human. Traditionally, mushrooms are grown on standard lignocellulosic substrates, but more recently, unconventional sources specifically, leftover biomass like weeds—have been exploited to produce mushrooms (Das & Mukherjee, 2007). Many mushrooms are valued for their potential medicinal uses in addition to being nutrient-dense foods (Gregori et al. 2007; Atila et al. 2017; Galappaththi et al. 2021).

Due to their increased biological efficiency and inexpensive cost of production, oyster mushrooms (*Pleurotus* spp.) are now commonly grown worldwide (Mane et al. 2007). In terms of global consumption oyster mushroom farming has risen to second place, behind button mushrooms (Chang, 1999; Sanchez, 2010). In contrast to button mushrooms, oyster mushrooms (*Pleurotus* spp.) are relatively easy to grow, and India's agroclimatic conditions are ideal for their production. Wood logs, straw, vegetable peels, and even weeds are among the many lignocellulosic substrates on which oyster mushrooms can be cultivated (Gregori et al. 2007). Pavlik (2005) reported that the biological efficiency (BE) of *Pleurotus ostreatus* differs depending on the substrate. Generally speaking, *Pleurotus* is produced commercially using a variety of straw varieties. Oyster mushrooms can be grown with non-pasteurized straw, even if pasteurized or composted straw is utilized for commercial purposes (Das et al. 2007). The ideal conditions for substrate length and spawn percentage were identified by Das et al. (2015), who recently reported growing five *Pleurotus* species on non-sterilized substrate (rice straw) at a warm temperature (30 °C). Although mushrooms are cultivated in solid state fermentation to produce fruiting bodies, they are now also used in submerged fermentation to produce enzymes, antibiotics, and

other secondary metabolites (Das et al. 2012). Although oyster mushrooms (*Pleurotus* spp.) are now considered to be both edible and medicinally significant due to the numerous nutraceuticals they have produced (Gregory et al. 2007).

2.1. Nutritional value and medicinal importance of *Pleurotus* species

2.1.1. Nutritional value of oyster mushrooms

Mushrooms are considered a nutritious food that is suitable for children and adults of all ages. The species, developmental stage, and environmental circumstances of mushrooms all have an impact on their nutritional content. Mushrooms include a lot of vitamins, minerals, protein and dietary fibre (Thatoi et al. 2014; Da Silva, 2012). Alam et al. (2007) reported that mushrooms are the excellent source of vitamins, protein and minerals. According to Zhang et al. 2003), *P. tuber-regium* sclerotia generated water-soluble sulphated derivatives of water-insoluble β -glucans that have antiviral properties against types 1 and 2 of herpes simplex virus. According to Stanley (2011), the antiviral effect is probably due to sodium minerals and sulphated β -glucans binding to virus particles and preventing them from infecting the host cells. Their nutritional content is determined by the substrate they are grown on (Alam et al. 2007; Chakraborty et al. 2016). In addition to being high in vitamins B, D, and K and low in fat as well as occasionally the presence of vitamins C and A, cultivated mushrooms also contain higher protein contents and minerals (Manzi et al. 2001). Isoleucine, aspartic acid, valine, methionine, serine, lysine, cysteine, glutamic acid, tyrosine, phenylalanine, arginine, tryptophan, histidine, proline, alanine and glycine are among the 18 essential amino acids present in mushrooms (Bach et al. 2017, Effiong et al. 2024). Essential unsaturated fatty acids, such as alfa-linolenic, oleic, linoleic and palmitic acids, are also

abundant in mushrooms. Table 2.1 lists the nutritional values of several types of oyster mushrooms.

Table 2.1. Nutritive values of different oyster mushrooms (%)

Sl No.	Mushroom species	Fiber (%)	Ash (%)	Protein (%)	Carbohydrate (%)	Fat (%)	References
1.	<i>Pleurotus florida</i>	7.8	6.4	2.13	5.60	2.30	Ahmed et al. 2009
2.	<i>Pleurotus pulmonarius</i>	4.94	7.07	2.68	4.60	1.80	Silva & Costa, 2002
3.	<i>Pleurotus ostreatus</i>	3.40	1.18	3.40	5.10	3.20	Alam et al. 2008; Yehia, 2012
4.	<i>Pleurotus djamor</i>	1.46	5.90	3.55	4.47	1.72	Jegadeesh et al. 2018
5.	<i>Pleurotus flabellatus</i>	1.20	3.24	2.20	6.02	0.90	Mshandete & Cuff, 2008; Khan & Tania, 2012
6.	<i>Pleurotus populinus</i>	1.40	4.30	3.50	4.55	2.31	Khan & Tania, 2012; Ramana et al. 2021
7.	<i>Pleurotus citrinopileatus</i>	2.07	7.65	3.00	4.25	3.90	Raman et al. 2021; Atila, 2017
8.	<i>Pleurotus abalones</i>	1.50	2.30	3.60	4.30	1.00	Khan & Tania, 2012; Raman et al. 2021
9.	<i>Pleurotus subcaesarius</i>	4.60	5.10	3.22	5.90	1.90	Khan & Tania, 2012; Raman et al. 2021

Sl No.	Mushroom species	Fiber (%)	Ash (%)	Protein (%)	Carbohydrate (%)	Fat (%)	References
10.	<i>Pleurotus cornucopiae</i>	2.07	7.65	3.00	4.25	3.90	Raman et al. 2021
11.	<i>Pleurotus fuscus</i>	1.80	3.30	3.60	4.20	1.99	Khan &Tania, 2012; Raman et al. 2021
12.	<i>Pleurotus tuber-regium</i>	2.07	7.65	3.00	4.25	3.90	Ijeh et al. 2009
13.	<i>Pleurotus eryngii</i>	1.60	5.40	2.90	4.50	2.90	Mehmet & Sevda, 2010
14.	<i>Pleurotus cystidiosus</i>	8.74	9.62	1.54	6.31	3.10	Yang et al. 2001
15.	<i>Pleurotus sajor-caju</i>	2.97	1.10	3.06	5.09	1.10	Alam et al. 2008

2.1.2. Medicinal / therapeutic activities of *Pleurotus* species:

Mushroom species, *Pleurotus* sp. offer a variety of medicinal properties, including anti-inflammatory, anticancer, immunomodulatory, antiviral, antioxidant, hypocholesterolemic, antiplatelets, antihypertensive, and antihyperglycemic effects. (Gregori et al, 2007). Table 2.2 displayed some therapeutic/ medicinal activities of different *Pleurotus* species.

Table 2.2. Some medicinal / therapeutic activities of *Pleurotus* species

Sl no.	Mushroom species	Compounds	Medicinal properties	References
1.	<i>Pleurotus pulmonarius</i>	Saponins	Anti-inflammatory potentiality	Jose et al. 2004
		Protein in hot water extracts	prevented the activity of HIV 1 reverse transcriptase	Wanget al. 2007
		Acetone extract	Shown tumor suppressor and anticancer properties	Akanni et al. 2010
		Polysaccharide extract	Exhibited an inhibitory impact on cancer cell proliferation	Lavi et al. 2010
		β - glucan	Prevented the development of cancer in mice linked to colitis	Lavi et al. 2012
		polysaccharide	Shown potent anti-inflammatory effects against rats' paw edema caused by formalin and carrageenan.	Jose et al. 2002 Adebayo et al. 2012
		Hot water extract	Prevented HIV-1 from expressing its reverse transcriptase	Patel et al. 2012
2.	<i>Pleurotus tuber-regium</i>	Glucans	Antiviral activity	Zhang et al. 2004
		Polysaccharide	Prevented mice from developing solid tumors. Showed antitumor properties against many human tumor cell lines	Zhang et al. 2001 Zhang et al. 2004

Sl no.	Mushroom species	Compounds	Medicinal properties	References
2.	<i>Pleurotus tuber-regium</i>	Sulphated β -Glucans	Showed antitumor and antiviral properties against Typ 1 and Type 2 Herpes Simplex Virus	Zhang et al. 2003, 2004
3.	<i>Pleurotus florida</i>	Glucan	Anti-inflammatory activity Triggered mice macrophages' in vitro phagocytic activity	Jose et al. 2004; Rout et al. 2005
		Lectin	Showed the capacity to alter the harmful effects caused by arsenic	Rana et al. 2012
		Protein fraction	Stimulated bone marrow cells, spleenocytes, and thymocytes, as well as the cytotoxicity of natural killer cells in mice. Additionally, it also showed antiproliferative properties	Maiti et al. 2008
		Pleuran	Exhibited anti-inflammatory activity	Jose et al. 2004;
		Methanolic extract	Inhibited inflammation, reduced paw edema in mice, and stopped platelet aggregation	Jose et al. 2004
4.	<i>Pleurotus flabellatus</i>	Alpha- tocopherol	Showed antioxidant activity	Vamanu, 2013
		Lycopene		Adhiraj et al. 2014
		Phenolics		Pumtes et al. 2016
		L- ergothionene		Vangkapun et al. 2011
5.	<i>Pleurotus sajor-caju</i>	Ribonuclease	Showed antibacterial and an antiproliferative impact on spleen cells in mice	Nagi and Ng, 2004

Sl no.	Mushroom species	Compounds	Medicinal properties	References
5.	<i>Pleurotus sajor-caju</i>	Xyloprotein	Exhibited antitumor activity	Rahi et al. 2016
		Xyloglucan		
		Proteins having polysaccharide		
		Lovaststine	Lower cholesterol	Gunde et al. 1999
		Protein-polysaccharide	Inhibited the solid sarcoma 180 tumor implanted in mice	Facchini et al. 2014
		Hot water extract	Inhibited HIV 1 reverse transcriptase activity	Patel et al. 2012
6.	<i>Pleurotus floridanus</i>	70% ethanolic and aqueous extract	Shown antibacterial activity that was efficient against <i>S. aureus</i> , <i>B. subtilis</i> , <i>B. megaterium</i> , and <i>E. coli</i>	Das et al. 2012
7.	<i>Pleurotus cystidiosus</i>	Protein fractions	Reduced blood pressure by the angiotensin-1 converting enzyme's action	Ching et al. 2011
		Methanolic extract	Shown reducing power, iron chelating, antioxidant, and radical scavenging properties	Yang et al. 2002
8.	<i>Pleurotus ostreatus</i>	Crude extract	Prevented <i>A. niger</i> and other Gram-positive and Gram-negative bacteria from growing.	Gerasimenya et al. 2002
		β -glucan	Exhibited strong anti-respiratory tract infection and anti-allergic effect	Sapena et al. 2015; Jesenak et al. 2013, 2014

Sl no.	Mushroom species	Compounds	Medicinal properties	References
8.	<i>Pleurotus ostreatus</i>	Protein polysaccharide	Prevented a solid sarcoma 180 tumor from growing when it was injected in mice	Facchini et al. 2014
		Dimeric lectin	Shown anti-tumor properties in mice with sarcoma and hepatoma	Wang et al. 2000
		Protein extract	Shown effectiveness against human colorectal adeno-carcinoma cells and human monocytic leukemia cells	Wu et al. 2011
		Polysaccharide	Either stimulated lymphocyte proliferation in male mice or increased concanavalin A (con A)	Sun et al. 2009
		Ribonuclease	Effective in breaking down HIV genetic material	Nomura et al. 1994
		Lovastatine	Showed atherosclerotic activity	Rahi et al. 2016
		Ribonucleases	Degrade genetic material to neutralize HIV	Nomura et al. 1994
		Methanolic extract	Showed the ability to scavenge radicals, chelate iron, reduce power, and act as an antioxidant	Yang et al. 2002
		Hexane-dichloro-methane extracts containing anisaldehyde.	Exhibited the inhibition of <i>B. subtilis</i> , <i>P. aeruginosa</i> , <i>A. niger</i> and <i>F. oxysporum</i>	Okamoto et al. 2002
		water soluble Proteoglycan fraction	Shown anticancer and immunomodulatory properties	Sarangi et al. 2006

Sl no.	Mushroom species	Compounds	Medicinal properties	References
8.	<i>Pleurotus ostreatus</i>	Pleuran	Showed anti-inflammatory activity	Jose et al. 2004
		Mevinolin	Exhibited rat antihyper-cholesterolemic action.	Hossain et al. 2003
		Acetone extract	Showed anticancer potential	Akanni et al. 2010
		Aqueous polysaccharide	Stimulated apoptosis and anti-proliferative effects	Lavi et al. 2012
9.	<i>Pleurotus eous</i>	Methanolic extract	Showed antioxidant properties	Ramkumar et al. 2010
		Lectin	Showed antiproliferative properties against tumor cell lines in humans	Mahajan et al. 2002
10.	<i>Pleurotus djamor</i>	Polysaccharide precipitate	Showed antitumor properties	Borges et al. 2013
11.	<i>Pleurotus nebrodensis</i>	Dried powder and aqueous extract	Prevented high blood pressure	Miyazawa et al. 2003
		Hemolysin	Showed potent cytotoxicity against a range of cancer cell types and anti-HIV-1 properties.	Lv et al. 2009
12.	<i>Pleurotus citrinopileatus</i>	Water	Decreased the quantity of tumor nodules that had spread. Decreased the blood glucose level in diabetic rats and shown antihyperglycemic action.	Wang et al. 2005 Hu et al. 2006
		Homodimeric Lectin	Showed antitumor activity	Li et al. 2008a
		Functional protein(PCP-3A)	Prevented human leukemia cell U 937 from proliferating	Chen et al. 2009

Sl no.	Mushroom species	Compounds	Medicinal properties	References
13.	<i>Pleurotus salmoneostramineus</i>	Mushroom powder	Decreased LDL/HDL ratio, lipid, and total phospholipids	Yoon et al. 2012
14.	<i>Pleurotus ferulae</i>	Ethanol extract	Decreased the levels of triglycerides, low density lipoprotein, and total cholesterol in plasma.	Alam et al. 2011
15.	<i>Pleurotus abalonus</i>	Polysaccharide-peptide	Increased the longevity of mice with accelerated senescence.	Li et al. 2007
16.	<i>Pleurotus cornucopiae</i>	Laccase	Decreased the HIV-1 reverse transcriptase activity and inhibited the growth of human hepatoma and murine leukemia cell lines.	Wong et al. 2010
		Acetone extract	Prevented HL60 cells from growing and caused them to undergo apoptosis.	Takei et al. 2005
17.	<i>Pleurotus geesteranus</i>	Polysaccharide	Showed cytotoxicity against the MCF-7 human breast cancer cell line.	Zhang et al. 2011

Effect of different substrates and substrate supplements on biological efficiency of different mushroom:

Throughout human history, mushrooms have drawn human interest and been utilized as food from the dawn of civilization. Unlike other crops, mushrooms are cultivated on organic substrate, either composted or raw. Materials from plantations, farms or factories

make up the majority of these substrates. According to Carrera (1998), lignocelluloses, forest byproducts, and agricultural wastes may all support the growth of certain types of mushrooms. According to Miles (1998), mushroom substrate is a kind of lignocellulose substance that aids in the growth, development, and fruiting of mushrooms. When combined with a sufficient amount of additives, the substrates' varied composition may enhance output (Miles and Chang, 2004; Hu et al. 2011; Naraian et al. 2009). For oyster mushroom cultivation, a variety of supplemented substrate has been suggested (Table 2.3). It has been discovered that biological additives such wheat bran, rice husk, soybean, chicken manure and mustard cake, may significantly increase the output of mushrooms (Rose et al. 2022). The production of the mushrooms can be increased by adding the appropriate amount of these supplements to the substrate (Salami et al. 2017).

Table 2.3. Effect of substrates and substrate supplements on BE/ yield of different *Pleurotus* spp.

Sl. No.	Mushroom strains	Substrates/ substrate combination	Supplements	Biological efficiency (%)	References
1.	<i>P. ostreatus</i>	Wheat bran	25% Apple pomace	54.23	Pathania et al. 2017
		Waste paper	25% Wheat bran	52.28	Tsfay, 2020
			25% Corn stalk	48.44	
			25% Wheat bran + 25% Corn stalk	64.44	
2.	<i>P. florida</i>	Wheat straw	5% Soyabean flour	72.50	Singhand Parsad, 2012
			5% Cow dung	40.05	
			5 % wheat bran	68	
			5 % Neem cake	75.77	Aske et al. 2020
			5 % Oil cake	70.70	

Sl. No.	Mushroom strains	Substrates/ substrate combination	Supplements	Biological efficiency (%)	References
2.	<i>P. florida</i>	Wheat straw	5% Poultry manure	69.67	Aske et al. 2020
			5% Maize bran	77.65	
3.	<i>P. sajor-caju</i>	Maize stalk	12% Wheat bran	113	Mkhize et al. 2017
			14% Maize flour	132	
		Wheat straw	5% Cow dung	45.10	Singh and Parsad, 2012
			5% wheat bran	70.40	
			0.5% Soybean flour	77.20	
4.	<i>P. pulmonarius</i>	Wheat straw	5% Cotton seed meal	77.65	Pal et al. 2017
			5 % Saw dust	60.85	
			5% Ammonium nitrate	24.15	
			5% CAN	31.85	
5.	<i>P. ostreatus var florida</i>	Rice Straw + Wheat Straw	2% Gram Flour	40.66	Sharma et al. 2023
			2% Maize Flour	43.3	
			2% Soybean Flour	40.86	
			2% Wheat Flour	39.13	
			2% Wheat bran	38.43	
		Rice Straw	2% Wheat bran	33.23	
			2% Soybean Flour	38.46	
			2% Maize Flour	37.46	
			2% Gram Flour	33.56	
		Wheat Straw	2% Maize Flour	39.36	
			2% Soybean Flour	36.23	
			2% Gram Flour	34.66	
			2% Wheat Flour	34.36	

2.2. Microbiome:

All the living system are associated with numerous microorganisms which can live parasitically, symbiotically or commensally (Proctor 2011). These organisms influence the development and growth of living systems. The organisms may live on the external surface or within the internal tissues of the hosts. Since the majority of microorganisms cannot be cultivated using conventional laboratory methods, comprehending microbial interactions in diverse settings is a huge problem. The shortcomings of conventional microbiological methods are addressed by metagenomics. It is used to search the great majority of microorganisms that are difficult or impossible to cultivate since it is culture-independent. To investigate the genomes of microbial communities in their natural habitats and inside their intricate communities, metagenomics makes use of a variety of genomic technologies and tools. It has improved our knowledge of the ecology and diversity of microorganisms. Instead than concentrating on individual species, metagenomics examines the genes and interactions within communities. The development of molecular techniques, like 16S ribosomal RNA (rRNA) sequencing, made it possible to investigate microbial populations without cultivating them. The groundwork for metagenomics was established by this. Carl Woese first suggested using rRNA as a molecular identifier in the late 1970s.

Frederick Sanger and associates developed the Sanger sequencing technology in 1977, which made studying microbes even easier. The study of the collective genomes of microorganisms in environmental samples is known as "metagenomics," a word that was first used in 1998 by Jo Handelsman and others. The advent of high-throughput sequencing technology in the early 2000s made it possible to quickly sequence entire microbial communities (Shendure et al. 2005; Margulies et al. 2005).

Various specialised databases and bioinformatics tools were created in the middle of the 2000s to handle the increasing amount of metagenomic data. Initiatives such as IMG/M (Integrated Microbial Genomes with Microbiomes) and CAMERA (Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis) offered venues for exchanging and storing metagenomic data. Metagenomics is currently developing with other omics technologies. Metagenomics is developing quickly thanks to advancements in bioinformatics tools and sequencing technologies.

Importance of metagenomics

- The conventional pure culture approach in microbiology separates individual species and examines, under controlled conditions, how they react to particular substances. Our comprehension of microbial behaviour in complex communities is constrained by this approach.
- By enabling the direct study of microbial populations in their natural surroundings, metagenomics overcomes these drawbacks of conventional microbiology. This offers insights into the ecological functions and interactions of microorganisms within these communities.
- Information about the makeup, roles, and interactions of microbial communities can be found in metagenomics.
- Without the need to cultivate specific species, metagenomics enables the analysis of genetic material from vast microbial populations.

Applications of Metagenomics

- The human microbiome is studied using metagenomics. This covers studies on skin and gut bacteria and how they affect health and illness.
- Pathogens in clinical samples can be found using metagenomics, which aids in the diagnosis and management of infectious disorders.
- By examining the microbial communities in water samples, metagenomics can be used to monitor water quality and identify contamination.
- It also aids in understanding the diversity and roles of microorganisms in soil.
- By discovering microbes that can break down contaminants, metagenomics can also be utilised in bioremediation initiatives.
- By comprehending the microbial populations connected to plants, agricultural yield and health can be enhanced. In order to produce biofuels and other renewable energy sources, metagenomics aids in the identification of microorganisms with the capacity to produce energy.
- The discovery of novel enzymes, bioactive substances, antibiotics, medications, and other advantageous microbial products is another use for metagenomics.

The existence of bacteria-like organisms in the cytoplasm of Glomeromycotina spores was initially documented by Barbara Mosse in 1970 (Mosse, 1970). This is the first glimpse into a profound and intimate inter-domain interaction.

In recent years, numerous investigations have discovered endobacteria from a variety of fungal lineages (Olsson et al., 2017). For instance, Gammaproteobacteria have been detected in Ascomycota (Arendt et al., 2016), while Alphaproteobacteria have been found in Basidiomycota (Glaeser et al., 2016; Sharma et al., 2008). However, the phylum

Mucoromycota has been linked to the majority of fungal endobacteria that have been identified (Spatafora et al., 2016). Within this group, endobacteria predominantly include Mollicutes (*Mycoplasma*-related) and Betaproteobacteria (*Burkholderia*-related). A noteworthy exception is the cyanobacterium *Nostoc punctiforme*, which is found inside *Geosiphon pyriformis* (Schüßler and Kluge, 2001).

Rhizopus microsporus (Mucoromycotina), the rice pathogenic fungus that causes rice seedling blight, is among the best-known instances. According to Partida-Martinez and Hertweck (2005), the endosymbiotic bacteria *Burkholderia rhizoxinica* is responsible for its pathogenicity. This bacterium generates rhizoxin, a powerful virulence factor that the fungal host uses (Partida-Martinez and Hertweck, 2007). Furthermore, this facultative endobacterium controls the development of sporangia and spores, which influences the vegetative reproduction of fungus (Partida-Martinez et al., 2007).

The study of the microbiome connected to macrofungi has gained increased attention as a result of endobacteria found in the fruiting bodies. The endobacteria from Agaricomycetes fruiting bodies were examined and sequenced by Pent and Bahram (2017), who discovered that the bacterial populations in fruit bodies were influenced by both fungal species and soils. According to their theory, the bacteria in fungal fruit bodies might be chosen according to their environmental needs or symbiotic roles. Similar conclusions were reached by Benucci and Bonito (2016), who suggested that bacterial diversity linked to Pezizale fruit bodies may be influenced by fungal species and their regional distribution. Consequently, the development of the fruiting bodies of macrofungi may be significantly influenced by the bacteria that reside within them.

Due to the fungi's selection and suitable development conditions, the bacteria that live inside macrofungi are associated with their hosts (Kumari et al. 2013). When Rangel-Castro et al. (2017) used ^{13}C -NMR to investigate the growth media of *Cantharellus cibarius*, they discovered trehalose and mannitol exudation. This may provide an explanation for how millions of bacteria may grow inside the chanterelle's long-lasting fruiting bodies without harming the hyphae. As demonstrated by isolation studies of endobacteria in fruiting bodies, the dominant bacteria may occupy the niche rapidly and help keep other bacteria or diseases from entering (Hildebrandt et al. 2002).

The results of a study by Dahm et al. (2005) showed that the majority of the bacteria isolated from ectomycorrhizal fungi (EcMF) fruiting bodies were Gram-positive. According to Rangel-Castro et al. (2017), the great abundance of common bacteria may be explained by their similar physical environments and nutritional needs. Moreover, the proliferation of macrofungi may potentially be influenced by the large relative abundance of common bacteria (Kumari et al. 2013). Tsukamoto et al. (2002) reported that bacteria that were isolated from wild Agaricales, including *Acinetobacter* sp., *Bacillus pumilus*, and *Sphingobacterium multivorum*, were able to detoxify the tolaasin that *Pseudomonas tolaasii* produces. According to Hildebrandt et al. (2002), arbuscular mycorrhizal fungi's internal structures, hyphal surface, and associated bacteria can all support hyphal growth and help arbuscular mycorrhizal fungi to sporulate more quickly (Hildebrandt et al. 2006). Likewise, microorganisms linked to EcMF may be crucial for the development of sporocarps (Sbrana et al. 2000) and the encouragement of mycorrhizal symbiosis (Garbaye, 1994; Frey-Klett et al. 2005; Poole et al. 2010; Bending et al. 2002). Additionally, some endophytic bacteria found in plants show a process known as vertical transmission,

whereby the bacteria are transferred from parent to child through seeds that help the progeny to survive (Truyens et al. 2015). Fruiting bodies of distinct fungal taxa, in particular, produce a variety of unique circumstances that filter specific bacteria from the surrounding bulk soil (Antony-Babu et al. 2014; Barbieri et al. 2010). The growth of fruit bodies and the generation of their spores may be influenced by the endophytic bacteria that reside within them.

2.3. Endophytes:

According to Stone et al. (2000), endophytes are organisms that reside as symptomless colonies inside their host plants, maybe for a part of their life cycle. The word "endophytes" was coined to distinguish between epiphytic organisms that reside on the surface of plants (De Bary, 1866). Endophytes benefitted from the host plant's protection, nourishment, and ability to absorb nutrients while shielding the plant from abiotic, biotic stresses and as well as from pests. They have developed all means of survival and have improved their relationship with the plants as a result of their evolution alongside the plant itself. Almost all plant tissues may have endophytic microbes that live there asymptotically. Multiple plant species may be linked to a single endophyte species, while a single plant species may contain multiple endophyte species. In the host plant, some endophytic organisms stay dormant, while others may interact with other endophytes as either non-pathogens or pathogens. Endophytes belong to a number of taxa, including protistic, bacterial, fungal, and archaeal, and are categorized as mutualistic. Without producing any outward signs of illness, they colonise within healthy plant tissue intracellularly or intercellularly (Wilson et al. 1995). They are common to every plant, and

have been isolated from nearly every plant that has been studied so far. They do not harm the host plants and can form facultative or obligatory associations. However, recent studies have revealed that they engage in complicated interactions with their hosts that include antagonism and mutualism (Carroll et al. 1988).

Furthermore, several bacteria are being studied as plant endophytes, and they usually live with endophytic fungi. The existence of endophytes has been recognized for over a century. Often living as imperfect fungi, they have been characterized as real symbionts or harmless parasites. Their ability to affect the host plants' distribution, ecology, physiology, and biochemistry has been proposed. They provide/assist in absorption of nutrient and shield the plant from abiotic and biotic challenges and pests, while also receiving protection and nourishment from their host plants. Evidence suggests that endophytes may affect population dynamics, plant community diversity, and ecosystem functioning in addition to plant growth, development, fitness, and diversity (Saikkonen et al. 1998)

2.3.1. Origin and evolution of endophytes

The fossil record indicates that about 400 million years ago, endophytes were associated with terrestrial plants (Krings et al. 2009). Plants transition from aquatic to terrestrial habitats during the evolutionary process, encountered high atmospheric CO₂ levels, nutrient-poor soil, changes of temperature and water availability. Through genetic changes, including the acquisition of some plant DNA, endophytes have adapted to the plant microenvironment within the same evolutionary time. Endophytes began generating plant metabolites or their precursors as a result of this adaptation and the uptake of genetic material (Stierle et al. 1993). According to Sun and Guo (2012), endophytes may be found

in many different types of plants and ecosystems such as ferns, mosses, grasses, shrubs and coniferous and deciduous trees. According to Sturz and Nowak (2000), bacterial endophytes can enter the xylem tissue by roots and emerge from rhizosphere and phyllosphere microflora. They can enter the apical root zone through incisions, wounds and other natural openings, or they can enter by breaking down the cell wall with the help of enzymes like cellulase and pectinase. Cellulase aids in the breakdown of cellulose's β -1-4 linkage bond. In addition to cellulase, endophytes also manufacture lignin catabolic enzymes, pectinase, protease, and phenoloxidase in order to establish themselves (Wang and Dai 2011).

By creating root nodules, nitrogen-fixing bacteria (Rhizobia) typically alter the morphology of the roots; otherwise, endophytes stay silent and the system does not change morphologically. Few microorganisms are able to penetrate the xylem tissues through the endodermal barrier. Bacteria enter developing seeds through the xylem and travel to all tissues and organs, including the reproductive organs. As one gets farther away from roots, the abundant supply of nutrients, the density of endophytic bacteria drops. The mycelium of fungal endophytes often grows along the organ's longitudinal axis. Endophytes can be passed from one generation to the next through seeds (vertical transmission) or by soil or plant component breakdown (horizontal transmission) to allied species (Zabalgoitia 2008, Herrea et al. 2016).

2.3.2. Endophyte diversity

The existence of asymptomatic endophytic fungi in plants has been known since the 19th century (Guerin, 1898). Approximately one million endophytic fungal species are thought

to exist, with a ratio of 1:4–5 fungus per plant compared to the number of vascular plant species (Sun and Guo 2012). Bacterial endophytes have been found to be present in over 200 bacterial genera from 16 phyla of both culturable and unculturable bacteria, including Aquificae, cyanobacteria, bacteroidetes, firmicutes, fusobacteria, nitrospira, gemmatimonadetes, proteobacteria, planctomycetes, verrucomicrobiae, spirochaetes, and deinococcus-thermus (Hallman et al. 2011). However, members of *Burkholderia*, *Stenotrophomonas*, *Azoarcus*, *Bacillus*, *Pseudomonas*, *Streptomyces*, *Enterobacter*, *Herbaspirillum*, *Serratia*, and *Acetobacter* (now known as *Gluconobacter*), are among the most prominent endophytes, which are classified into three major phyla: proteobacteria, actinobacteria, and firmicutes (Malvonava et al. 2013). However, the actual identified numbers of endophytes are very less.

2.3.3. Functions of Endophytes

Increase availability of nutrients:

Various microorganisms can fix, solubilize, mobilize, and make macro and micronutrients accessible to plants. By adding these microorganisms to the plant, the amount of chemical fertilizers used can be reduced. Kuklinsky-Sobral et al. (2004) identified phosphate-solubilizing endophytic bacteria from soybeans. Numerous nitrogen-fixing bacteria associated with sugarcane have been shown to fix between 30 and 80 kg N/ha/year of atmospheric nitrogen (Boddey et al., 1995). Various nitrogen-fixing endophytic bacteria, including *Stenotrophomonas*, *Pseudomonas* and *Burkholderia*, are found in several grasses growing in nitrogen-deficient soil. These bacteria may convert atmospheric nitrogen to ammonia.

Nitrogen fixation of Endophytic bacteria:

Applying endophytic bacterial inoculants that supply nitrogen requirements has garnered attention in recent years as a means of efficiently and sustainably enhancing plant production across a range of crop plants. The members of *Azoarcus*, *Achromobacter*, *Burkholderia*, *Herbaspirillum*, *Gluconoacetobacter*, *Serratia* and *Klebsiella* are among the promising endophytic biofertilizers (Franché et al. 2009; Rothballer et al. 2008). According to reports, the primary contributor to endophytic biological nitrogen fixation in sugarcane is *Gluconoacetobacter diazotrophicus* (*Acetobacter diazotrophicus*), which has an annual capacity of around 150 kg of nitrogen per hectare (Muthukumarasamy et al. 2005). Another possible N₂-fixing obligate endophytic diazotroph is *Azoarcus*. Without the use of nitrogen fertilizer, it can increase hay production by 20 to 40 t ha⁻¹ year⁻¹ in salty, sodic, alkaline soils by living in the roots of kallar grass (Hurek and Reinhold-Hurek 2003). Furthermore, it has been discovered that a bacterial population is present in several energy plants (C₄ plants), such as *Spartina pectinata*, *Penisetum purpureum* and *Miscanthus sacchariflorus*, which may help the plant's Nitrogen nutrition (Kirchhof et al. 1997). A research found that Rice seedlings infected with *Herbaspirillum* sp. and maintained in N₂-free Hoagland solution containing ¹⁵N-labeled N showed a ¹⁵N dilution, increasing the plant's total N₂ content by up to 40% (Baldani et al. 2000). In a pot experiment, inoculation of seed with a strain of *Rhizobium leguminosarum* bv *trifolii* has also been shown to promote the development of mustard, corn, wheat, radish and several types of rice shoots (Webster et al. 1997). According to these studies, endophytic diazotrophs have a significant chance of raising the yield of non-legumes, including significant cash crop plants.

Phyto-hormone production:

Studies on the connection between plants and microbes have extensively established the role that various microorganisms play in the production of phytohormones. Gibberellins and IAA, two substances that promote plant growth, can be produced by endophytic bacteria. Many microorganisms have ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, which reduces the quantity of the plant growth regulator ethylene by breaking down ACC into α -ketobutyrate and ammonia. High amounts of IAA and low levels of ethylene increase plant weight and the length of the roots and shoots. Numerous bacteria, including *Azospirillum*, *Pseudomonas*, and *Enterobacter* species, are linked to ACC deaminase and actions that promote plant development. (Spaepen et al. 2007)

Degradation of toxic molecules:

Numerous endophytic microbes have the genetic machinery necessary to break down harmful and resistant compounds found in the rhizosphere and other polluted areas. In contaminated environments, endophytic populations have a higher abundance of bacteria that break down resistant chemicals than the rhizosphere of plants grown in non-contaminated environment suggesting that endophytes play a part in the bioremediation process (Siciliano, et al. 2001). By using genetically engineered *Burkholderia cepacia*, phytoremediation was enhanced and toluene tolerance was increased in plants (Barac et al. 2004)

Draught tolerance:

The ability of plant tissues to tolerate water deficiencies is known as drought tolerance (Kirchhof et al. 1997). Draught tolerance is a mechanism that varies among plants. Three main categories have been established for mechanisms-

1. Accumulation and translocation of assimilates
2. Maintenance of cell wall elasticity
3. Osmotic adjustment

The endophytic microbes have an impact on all three processes. Evidence of the first process was found in the leaf blades of endophyte-infected plants, which produced more soluble carbohydrates including fructose and glucose (Baldani et al. 2000). The release of soluble sugars, amino acids like proline, alkaloids, polyols etc. that provides osmotic adjustment and wall elasticity during drought conditions may also be fuelled by endophytes. Numerous microorganisms can release the enzyme ACC deaminase, which lowers the amount of ethylene, a hormone that inhibits plant growth and is more prevalent during drought.

Suppression of plant pathogens and insects:

Plant diseases and pests are thought to be the main factors impeding the advancement of agriculture. Traditionally, hazardous chemical pesticides are used to control illnesses and pests. Excessive use of such chemicals in agriculture can contaminate the environment and affect the health of people and animals. Biocontrol organisms can better manage such risky species in the field by reducing or eliminating the use of toxic pesticides in agriculture, thereby resolving health and environmental concerns (Azevedo et al. 2000). Various studies have suggested various endophyte mechanisms of action. However, our understanding of the mechanism behind the inhibition of endophytic plant pathogens is still in its infancy. Direct, indirect, and ecological consequences are among the potential

mechanisms. By releasing lytic enzymes, endophytes directly lower infections through antibiosis. Nevertheless, the direct interactions between viruses and fungal endophytes are intricate and susceptible to antagonistic interactions that vary depending on the species. Applying many endophytic bacterial isolates to banana seedlings early in life might reduce the incidence of banana bunchy top viruses by 60% when compared to control. During the time of plant connection, a number of endophytic fungus (Ascomycetes, Clavicipitaceae) can synthesize organic compounds like alkaloids. In gramineae and cyperaceae, this action significantly decreased plant infection, which in turn decreased *Spodoptera frugiperda* survival and development. When flour beetles *Tribolium castaneum* were infected with endophytic fungus, the number of contaminated seeds and insect development decreased (Cheplick et al. 1989). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two different pathways for induced resistances in plant immunology. Salicylic acid (SA) and Pathogenesis-related (PR) proteins mediate SAR, which is brought on by pathogen infection. ISR is caused by certain Plant Growth Promoting Rhizobacteria (PGPR) and is mediated by ethylene or jasmonic acid (JA) PR protein accumulation is not a factor in this process.

Endophyte and pathogenicity:

The most bacterial and fungal endophytes are considered to be advantageous companions for plants. Microbes will receive sustenance from the host plant as a result of endophytes' direct or indirect promotion of plant growth. Microbes must contend with constantly shifting development conditions as living things, which might cause them to transform from endophytes to pathogens. It has been shown that, especially with fungi, the organism

may evolve into a plant pathogen based on the the host's defensive mechanisms, developmental phases of the host and the fungus and the environmental factors (Lund et al. 1997). Several studies have noted that endophytes can become latent pathogens as a result of environmental changes like CO₂ buildup or O₂ depletion (Guo et al. 2002). Human diseases have also been linked to a number of endophytes. Endophytic *Salmonella* sp can lead to serious epidemics and health problems when it is found in raw fruits and vegetables (Lund et al. 1997). Similarly, human nocardiosis can result from the presence of the bacteria *Burkholderia* which can cause lung illness. It's unclear if *Mycobacterium* has a pathogenicity gene, however it is present as an endophyte in many plants.

Bioremediation and phytoremediation

Research on endophytic bacteria is essential for knowledge of their ecological function in plant interaction as well as for possible biotechnological uses such as phytoremediation and bioremediation.

This is due to the fact that endophytic bacterial genetic engineering is easier than that of plants, and endophyte gene expression might be a valuable site-monitoring tool. Thus, research has shown an intriguing relationship between the glyphosate herbicide use at pre-planting and the soybean endophytic bacterial population (Kuklinsky-Sobral, 2005). In this study, *Burkholderia gladioli* and *Pseudomonas oryzihabitans* were found to be the dominant species through isolation in culture media that contained glyphosate as the only carbon source (Lee et al. 2010). The study's findings suggested that these endophytic bacteria could be applied to bioremediation initiatives. Hexahydro-1,3,5-trinitro-1,3,5-triazine (HMX), 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-

triazine (RDX) are among the energetic chemicals that are broken down by the genus *Methylobacterium*, a group of bacteria that lives as plant endophytes (Aken et al. 2004). According to these scientists, these endophytes may be used in the bioremediation and phytoremediation processes, where these microbes could be crucial to the degradation of environmental contaminants. This hypothesis was corroborated by Siciliano and colleagues (2001), who discovered that the amount of endophytes with catabolic genotypes varied depending on the concentration and presence of pollutants. They discovered that the number of endophytes in *Scirpus pungens* that were naphthalene dioxygenase (ndoB)-positive quadrupled when petroleum hydrocarbon sediment was added. Likewise, the quantity of endophytes in *Festuca arundinacea* that were alkane monooxygenase (alkB)- or (ndoB)-positive was associated with the soil's creosote content, but not with the quantity of alkB- or ndoB-positive bacteria in the bulk soil. This indicates that the enrichment of catabolic genotypes in the root interior depends on both the plant and the contaminant. Engineered endophytic bacteria have been utilized in phytoremediation programs to enhance the breakdown of organic, volatile, and water-soluble contaminants (Siciliano et al. 2001). In certain situations, it would seem logical to speculate that certain endophytes might break down a pollutant if it passes through the plant vasculature (Taghavi et al. 2005). On the other hand, recombinant endophytes that have been altered to possess the proper breakdown pathway might be used to enhance the phytoremediation process (Barac, 2004). The concept of bacteria and phytoremediation as a novel application for endophytic bacteria in plants is also supported by certain authors (Newman et al. 2005; Van der Lelie, 2005).

Systemic resistance and endophytes:

In microbial ecology, the role of endophytes in resistance induction triggering is a novel insight that has not yet been thoroughly investigated. According to previous descriptions, these bacteria form inside tissues and interact intimately with their host plants, interfering with plant physiology and causing plant-bacterium co-evolution (Mishaghi et al. 1990; Aiub et al. 2001). The potential function of endophytes in controlling pests and diseases is one of their many potential functions in plant health and development. It has been investigated how endophytes mediate biological regulation, and the genera *Pseudomonas* and *Bacillus* are the primary bacterial group participants. At this stage, it is crucial to note that both of these genera are also mentioned as causing plants to develop systemic resistance. Thus, the ability of endophytes to induce systemic resistance is described in rice against *Rhizoctonia solani* (Nandakumar et al. 2001) and *Xanthomonas oryzae* pv. *oryzae* (Vidyasekharan, 2001), as well as in tomato, where *Pseudomonas* sp. PsJN induced resistance against *Verticillium dahliae* (Sharma et al. 1998).

2.3.4. Mushroom –Bacterial interaction: Role in growth promotion

Throughout their whole life cycle, both wild and farmed mushrooms interact with different microbes (Deveau et al. 2018). According to Frey-Klett et al. (2011), these interactions affect the interacting organisms and their surroundings through a variety of combinations of molecular communication through chemotaxis, signaling molecules, exchange, and metabolite conversion, as well as physical touches (adhesion) (Frey-Klett et al. 2011). Bacteria generally have a significant impact on the various stages of mushroom growth, such as substrate conversion and adaption, mycelial hyphal extension during substrate

mycelial colonization, and initiating the formation of fruiting bodies (Vaario et al. 2011; Li et al. 2015).

P. ostreatus is the most widely grown edible mushroom in the world, according to Oei (2016). According to some studies this fast-growing WRF may grow on a variety of substrates with varying C/N ratios, including maize stalks, bagasse, rice straw, discarded cotton, wheat stalks, sawdust, and banana leaves (López et al. 2008; Miles and Chang, 2004). Most of the time, these substrates are semi-composted, enriched with nitrogen source materials, and then pasteurized or sterilized before being injected with grain spawn (Oei, 2016). Numerous research have investigated the microbial communities and bacterial diversity in *Agaricus* spp., especially during the different compost stages (Rossouw and Korsten, 2017; Kertesz and Thai, 2018). Members of the genera *Paenibacillus*, *Bacillus*, and *Pseudomonas* comprise the bacterial isolates that were found during different phases of the *Pleurotus* species culture. It has been demonstrated that mycelial growth-promoting bacteria have advantageous to fungal strains of *A. bitorquis*, *Hypsizygus ulmarius*, *A. bisporus* and *P. ostreatus* by stimulating mycelial growth, inducing fruiting bodies, and bio-controlling fungal pathogenic bacteria (Colauto et al. 2016; Cho et al. 2003; Poonga and Kaviyarasan, 2015; Torres-Ruiz et al. 2016). On the other hand, it has been shown that bacteria can also negatively impact the cultivation of *P. ostreatus*, *A. bisporus* and *P. eryngii* by causing discoloration or decomposition of fruiting bodies, partially or completely blocking substrate colonization, influencing the development of fruiting bodies, and/or lowering the production yield (Lee et al. 2010; Lincoln et al. 1999; Lim et al. 2008). A very few studies have examined the effects of bacteria that promote and inhibit fungal growth throughout the mushroom-making process.

Due to their adaptation to this microhabitat situation, endobacteria that can grow at the inner tissue of fruiting bodies often had a beneficial influence on mycelial development. This notion is supported by the results of Oh et al. (2018), who also isolated bacteria that promote mycelial development of *T. matsutake* sporophore. However, because of their role in sporophore breakdown as it happens in nature, it is fairly uncommon to identify bacteria from fruiting bodies that adversely impact mycelial development (Oh et al. 2018). It's interesting to note that bacteria that had a favourable impact on mycelial development had generally lower hydrolytic activity than bacteria that had a negative impact. In particular, decreased lipase and peptidase activity was seen. Instead of hydrolyzing the fungal cell wall, fungus-associated bacteria obtained nutrients such sugars and amino acids that were created by fungi (Boer et al. 2005; Rangel-Castro et al. 2002). In comparison to *M. peucetia* (FMIC01000002) and *Micromonospora lupini* (AJ783996), the limited chitinase activity seen in positive interacting bacteria is most likely insufficient to stop mycelial development. Nonetheless, it might be beneficial to engage with the fungus in a different manner. For instance, plant bacterial endophytes release enzymes that break down cell walls in order to enter, settle, and proliferate inside plant cells (Compant et al. 2010). IAA has been shown to have a growth-promoting impact in addition to an enzymatic effect on fungal growth. *P. fluorescens*'s production of IAA in casing soil has been proposed as a possible way to encourage *A. bisporus* development (Xiang et al. 2017). Similarly, it has been observed that IAA at 0.5 and 1.0 µg ml⁻¹ stimulates mycelial development in *Phellinus linteus* (Guo et al. 2009).

Microbes have the ability to settle in a particular environment and engage with the creatures there. It is well recognized that secondary metabolites mediate interactions

between bacteria to control their defense systems and coexistence. Examples of interactions seen in nature are those between bacteria and fungus and between bacteria and plants (Scherlach et al. 2013). Plant development is known to be influenced by soil bacteria, which also positively interact with nearby plants. These microbes are referred to as plant growth-promoting bacteria (PGPB) (Glick, 2012). In the growing media used to cultivate mushrooms (baglog), interactions between bacteria and fungus might occur. In order to promote mushroom development, it is anticipated that the presence of bacteria in mushroom growth media will act as mushroom growth-promoting bacteria (Frey-Klett et al. 2011). Metabolites like IAA, which are produced by bacteria, can promote the development of plants and mushrooms. Although IAA is known to control plant growth and development, nothing is known about how it affects the productivity and growth of mushrooms (Spaepen et al. 2007). Recent years have seen a rise in research on MGPB, particularly in the area of beneficial bacteria that generate IAA and use its supernatant to boost the growth and production of mushrooms. Numerous species of bacteria that are known to generate IAA and have the positive impact of boosting the development of plants and mushrooms are investigated within the genera *Bacillus* and *Pseudomonas* (Bharucha et al. 2013). There are still some indications that IAA plays a bigger part in fungal proliferation than in the growth and development of plants.

To investigate the impact of IAA generated by these bacterial supplements on *Pleurotus ostreatus* growth; Hannah et al. (2020) investigated the influence of bacterial isolates *L. boronitolerans*, *B. aryabhattai* and *P. putida* on *P. ostreatus*. *L. boronitolerans* generated the highest concentration of bacterial IAA (7.51 ± 1.38 ppm), followed by *B. aryabhattai* (1.30 ± 0.12 ppm) and *P. putida* (1.02 ± 1.38 ppm respectively), according to the findings

of their experiment. Following eight days of incubation, the addition of bacterial IAA to PDA led to a notable increase in *P. ostreatus* mycelia growth between the fourth and sixth days of incubation. On the sixth day of incubation, the *L. boronitolerans* supernatant showed maximum diameter of mycelial growth (84.86 ± 5.45 mm) followed by *B. aryabhatai* and *P. putida* (81.99 ± 2.89 mm and 80.35 ± 5.12 mm, respectively)

A little more variety can be found in the makeup of the microbes that promote oyster mushroom growth. It comprises Bacilli, Actinomycetes, Gammaproteobacteria, and Alphaproteobacteria. Only fluorescent Pseudomonads that firmly attached to the mushroom mycelium promoted fungal growth among the 35 bacterial isolates from *P. ostreatus* (Cho et al. 2003). In addition to aiding in mycelium growth (Tsivileva et al. 2022; Chen et al. 2022a; Kim et al. 2008), beneficial microorganisms also boost yield, shorten production times, and improve biological efficiency of substrate usage in *Pleurotus* species (Suarez et al. 2019; Angelis et al. 2012; Kim et al. 2008; Torres-Ruiz et al. 2016; Kumari & Naraian, 2020). Fruiting bodies were shown to be the most effective source for isolating bacteria that promote mycelial growth in *P. ostreatus* (Suarez et al. 2019)

Bacterial IAA supplementation promotes *P. ostreatus* growth, according to an experiment. The plant growth hormone IAA has been found to be produced by the bacteria *Bacillus* and *Pseudomonas*. (Suarez et al. 2019; Kang & Cho, 2014), and mushrooms have been reported to employ microbes as a source of nourishment when nutritional deficiencies occur (Baat et al. 2022, Zarenejad et al. 2012). These microbes were also found to have contributed to the rise in amylase, cellulase and laccase activities by *Pleurotus* spp. (Kang & Cho, 2014, Chen et al. 2022b). Bacteria that have growth promoting activities of *Pleurotus* spp are listed in Table 2.4.

Table 2.4. Growth-promoting bacteria and their role in growth promotion of *Pleurotus* spp.

Sl. No	Mushroom strains	Microbes	Influence	References
1.	<i>P. ostreatus</i>	<i>Bacillus cereus</i> , <i>Bacillus aryabhattai</i> , and <i>Acinetobacter pittii</i>	Mycelial growth promotion	Febriansyah et al. 2018
2.	<i>P. ostreatus</i>	<i>Azospirillum brasilense</i>	Mycelial growth promotion	Tsivileva et al. 2022
3.	<i>P. ostreatus</i>	<i>Micromonospora lupini</i> from fruiting bodies	Mycelial growth promotion, reduction of growing period	Suarez et al. 2019
4.	<i>P. ostreatus</i>	<i>Bacillus</i> , <i>Paenibacillus</i> , and <i>Micromonospora</i>	Mycelial growth promotion, growth promotion	Suarez et al. 2019
5.	<i>P. eryngii</i>	<i>Pseudomonas</i> sp.	Reduction of growing period, Mycelial and Mushroom growth promotion ability,	Kim et al. 2008
6.	<i>P. ostreatus</i>	Seven fluorescent <i>Pseudomonas</i> spp.	Growth promotion,	Cho et al. 2003
7.	<i>P. eryngii</i>	<i>Pseudomonas</i> sp.	Mycelial growth promotion	Kang & Cho, 2014
8.	<i>P. florida</i>	<i>Glutamicibacter arilaitensis</i> from rhizosphere	Mycelial growth promotion, Increase of biological efficiency	Kumari & Naraian, 2021
9.	<i>P. ostreatus</i> .	<i>Bacillus megaterium</i> , <i>B.cereus</i> , <i>Kurthia gibsonii</i> , <i>Enterobacter cloacae</i> , <i>E. asburiae</i> , <i>Saccharomycetes</i> , <i>Pseudomonas pseudoalcaligene</i> .	Mycelial growth promotion	Torres-Ruiz et al. 2016
10.	<i>P. eous</i>	<i>Azotobacter</i>	Mycelial growth promotion	Eyini et al. (2005)
12.	<i>P.eryngii</i> .	<i>B.cereus</i>	Mycelial growth promotion	Chen et al. (2022a)

Mushroom endobacteria:

Like All other organisms mushroom also contained a large number of bacteria in their internal tissue. Endobacteria have recently been isolated from a number of edible mushroom species such as *Agaricus bisporus*, *Pleurotus eryngii*, *Rhizopogon roseolus*, *Tricholoma mustake* (Gautam et al. 2016; Chen et al. 2022a; Oh et al. 2018; Pramoj et al. 2019). Gautam et al. (2016) isolated an endobacterial isolate VIT-CMJ2 (*Enterobacter* sp.) from *Agaricus bisporus* that is capable of antibacterial activity. *Bacillus aryabhatai*, an endobacteria isolated from *P. ostreatus*, demonstrated mycelial growth promotion, according to Febriansyah et al. (2018). According to Chen et al. (2022a), the mycelial growth rate was accelerated by growth-promoting *Bacillus cereus* that was isolated from *P. eryngii*. Additionally, Oh et al. (2018) identified mycelial-development-promoting bacteria from *Tricholoma matsutake* fruiting bodies.

2.4. Bioremediation potentialities of oyster mushroom:

Mycoremediation is known as fungal-based remediation. Because of their capacity to break down a wide range of environmentally persistent contaminants and convert industrial and agro-industrial wastes into products, mushrooms and their enzymes are referred to as mycoremediation tools. To clean up contaminated areas and improve the environment, mushrooms employ a variety of techniques. These methods are - (i) Biodegradation (ii) Biosorption (iii) Bioconversion.

Biodegradation

"Biodegradation" is the term used to describe how complex compounds are finally broken down and recycled into their mineral components by living organisms. Living organisms use this process to fully mineralize the original chemical into simpler forms such as NO_3 , CO_2 , H_2O and other inorganic molecules. The decomposition capabilities of mushrooms and associated enzymes have been extensively studied. Numerous studies on the chemicals created by the breakdown of different wastes and the variables influencing the processes have been published. According to Nyanhongo et al. (2007), mushrooms are capable of producing ligninases (laccase, manganese peroxidase and lignin peroxidase), pectinases, cellulases, xylanases and other oxidases. These can oxidise pollutants that are difficult to remove in vitro. Usually, these enzymes are triggered by their substrates. In vitro tests have also shown that these enzymes can break nonpolymeric, resistant pollutants like pentachlorophenol (Lin et al. 1990), nitrotoluenes (VanAcken et al. 1999), synthetic and organic dyes (Heinfling et al. 1998; Ollikka et al. 1993;), and polycyclic aromatic hydrocarbons (PAHs) (Johannes et al. 1996; Hammel et al. 1991). According to da Luz et al. (2013), several kinds of mushrooms have the capacity to degrade polymers, including plastics. The process of biodegradation is quite intricate. This is due to the influence of various biochemical systems, the way ligninolytic enzymes interact with hydroxyl radicals, the monooxygenase system, cytochrome P450, and the quantity of H_2O_2 produced by the mushroom.

Biosorption

The second important way that mushrooms eliminate metals and other pollutants from the environment is by biosorption. Both the recovery of metals from effluent and the remediation of industrial effluents are seen to be alternatives to biosorption. Biosorption is the process by which pollutants, xenobiotics, and metallic ions are absorbed from wastewater by living or dried biomass, which often exhibits a strong resistance to metals and other unfavorable circumstances (Gavrilescu, 2004). Spent mushroom compost as well as mycelium can be used to make biosorbents.

In order for mushrooms to absorb pollutants or xenobiotics, they must first undergo (i) bioaccumulation, which is a process that depends on active metabolism and includes both transport into the cell and partitioning into intracellular components; and then (ii) biosorption, which is the binding of pollutants to the biomass without the need for metabolic energy. A number of chemical reactions, including as covalent binding, ion exchange, and adsorption, may be involved in biosorption. Mar'in et al. (1997) reported that biosorption process may involve the polar groups of amino acids, lipids, structural polysaccharides such as glucans, chitin, chitosan and also proteins

According to reports, dead biomass may have a higher, comparable, or lower capacity for biosorption than living cells (Mar'in et al. 1997). Dead mushroom biomass has several advantages over active cells in the biosorption process. Industry can supply dead mushroom biomass, which is a byproduct of fermentation processes. Additionally, unlike living mushroom biomass, this is not affected by the levels of toxicants, their harmful effects, or adverse working conditions (nutrition supply, temperature, pH, cell

concentration, starting metal ion concentration etc.). Both fungus species and contact duration affect the absorption of xenobiotics by living cells. Pollutant removal via biosorption methods is currently highly prevalent. Because of its high absorption capacity and low cost of raw materials, biosorption is a successful technique.

Bioconversion

Research is currently being conducted on the conversion of industrial or agro-industrial waste into several other beneficial forms. Mushrooms are the most significant product of bioconversion. It is possible to grow mushrooms from any lignocellulosic waste produced by business enterprises, which can then be turned into a product. Mushrooms are grown using agro-industrial and industrial waste. The substrate's regional availability typically dictates the choice of material for mushroom production.

Several industrial wastes have also been successfully used for cultivation of mushroom (Kulshreshtha et al. 2013b; Dulay et al. 2012; Kulshreshtha et al. 2010 and Singhal et al. 2005). When mushrooms are used as a mycoremediation method to bio-convert these industrial wastes into protein-rich mushroom sporophores, they both produce mushrooms and help reduce pollution that could otherwise result from their disposal. Although there are several benefits to mycoremediation of environmental pollutants by mushrooms, researchers and engineers face difficulties. Both in situ and ex situ mycoremediation of wastes are possible. When the hazardous compounds are handled on-site, there is no need to transport them to treatment facilities. It is a low-cost, low-skilled method that requires little space and is simple to use in the field. It is also environmentally friendly. Compared to the foregoing, using this mycoremediation technique has certain drawbacks. It takes time

for mushrooms to clean up garbage and adjust to their surroundings. It is possible for mushrooms grown on industrial waste to be poisonous or genotoxic. The genotoxins found in the waste used to grow mushrooms have an impact on their genotoxicity. Therefore, the toxicity and genotoxicity of mushrooms must be assessed if they are used for bioremediation.

Biodegradation and biosorption are the two facts that determine the degree of toxicity in the sporophore. The proper enzymatic machinery for biodegradation is present in mushrooms, which causes contaminants to be broken down from the substrate and transformed into less hazardous compounds. Because of this, the sporophores are safe for humans to consume. A number of recent studies have documented that mushrooms have the capacity to both digest contaminants and lessen their mutagenicity or toxicity (Malachová et al. 2006; Choi et al. 2013; Kulshreshtha et al. 2013b). According to several researches, the reduction of mutagenicity caused by mushrooms mostly depends on the species. Kulshreshtha et al. (2011 & 2013b) did not find *Pleurotus florida* to be genotoxic, but *P. citrinopileatus* shown genotoxicity in its fruiting bodies when both species were grown on industrial wastes and a mixture of industrial wastes and wheat straw under the same growth conditions.

Additionally, toxicity reduction depends on the substrate. Similar fungi may have different capacities for decomposing different toxic pollutants because of the enzymes present in mushrooms, which not only aid in degradation but also minimize the impacts of toxic and genotoxic pollutants (Choi et al. 2013). Numerous investigations have shown that mushrooms possess antimutagenic and antigenotoxic properties (Taira et al. 2005; Mendez-

Espinoza et al. 2013; Mlinaric et al. 2004; Menoli et al. 2001; Filipic et al. 2002), which could be utilised to lessen the genotoxicity of these contaminants. Because mushrooms have antimutagenic and antigenotoxic properties, it has been demonstrated that they can also decrease genotoxicants and other harmful pollutants in addition to their degrading power. These kinds of mushrooms are suitable for both human consumption and animal feed. This concept provides a natural framework for further study, which should focus on the necessity of decomposing pollutants so that they may be disposed of without creating further problems and fruiting bodies can be safely eaten. On the other hand, mushrooms that absorb contaminants are unfit for human consumption.

According to Lambrood and Ralegankar (2013), biosorption can be an effective method for cleaning up hazardous metals that pose a concern to the environment. However, it also produces non-consumable biomass, which creates a new disposal issue. Researchers have focused on using mushroom mycelium for biosorption and have compared the sorption capabilities of biomass. The reasons why different types of mushrooms have varying capacities for biosorption have not been well studied (Das, 2005; Kumhomkul and Panichpat, 2013).

2.4.1. Pesticide degradation by *Pleurotus* species:

Pleurotus species are well-known fungi that have been studied in bioremediation because of their ability to break down complex chemical compounds. Their ability to secrete oxido-reductases, including manganese peroxidase, laccases and lignin peroxidase makes them particularly capable of detoxifying a wide range of environmental contaminants. The impact of crude oil on the yield and chemical makeup of *P. tuber-regium* was examined by

Ogbo and Okhuoya (2009) in soils supplemented with NPK fertilizer, shredded banana leaves, sawdust and chicken litter. The results of the investigation demonstrated that the general health of fungus is enhanced by crude oil pollution. The nutritional status of the mushroom is impacted by the substrate composition, which results in variations in the amount of protein, ash, fat, crude fiber and carbohydrate. While shredded banana leaf blades and NPK fertilizer lessen the fertilizer impact, sawdust and chicken litter increase the fungus's response to crude oil fertilizer. The fruit bodies of the mushrooms grown on the site affected by crude oil were of a similar quality to those grown on the optimum substrate composition.

Zebulun et al. (2011) reported the use of *P. ostreatus*-induced biodegradation to decontaminate soil contaminated with anthracene. They found that, in comparison to control soil (33 to 51%), the rate of decomposition of all levels of anthracene was influenced by time, contamination level, and fungal treatment (76 to 89%). It has been observed that the breakdown of anthracene is linked to the release of lignolytic enzymes by *P. ostreatus*, including laccase, and manganese peroxidase and lignin peroxidase,. Additionally, some of the soil samples showed an increase in anthracene concentration at various sampling periods.

Sadiq et al. (2015) reported biodegradation potentiality of *Pleurotus ostreatus* (OST-1), *P. pulmonarius* (WC-537), *P. ostreatus* WC-522, A-6 and R-22 against organochlorine pesticides like HCH, DDT, Aldrin, Dieldrin, Endosulfan, Endrin, and Heptachlor. 84% of HCH was degraded by OST-1 followed by 55% by A-6, 45% by WC-522, 29% by OST-Belgium and 4% WC-537 strains. They also reported

that maximum 2,4-DDT and 4,4-DDT were degraded by OST-1 (65%&67%) respectively. Endrin's mean highest reduction was seen in WC-537 (32%), followed by WC-522 (29%) and OST-Belgium (14%). A-6 and OST-1 were found to be the most efficient and comparable to the others in terms of lowering the amount of α - and β -endosulfan in bioassay, however the isolates' efficacy varied significantly for α -endosulfan and β -endosulfan.

2.4.2. Dye decolorization:

Nowadays, synthetic dyes are used in textiles because they are better stable. In the textile sector alone, over a million tonnes of dyes are produced each year, generating 93% of liquid waste that comprises organic compounds, heavy metals, and colouring agents. If not adequately managed, this waste will have negative consequences. One of the issues is coloured organic waste, which complicates decomposition, lowers wastewater quality, and increases the risk of turbidity and toxicity (Abioye et al. 2015).

Traditional waste treatment methods based on dyes have the potential to create intricate aromatic compounds and poison nearby microbes. According to Zouari-Mechichi et al. (2006), conventional biological waste treatment turns trash into nitrogen-containing colours and aromatic amines, which are more hazardous than the original waste. Waste issues can be reduced by using bio-decolorization to process colour waste. Bio-decolorization is the process of treating waste by using the color-degrading properties of fungi, bacteria, algae and yeast in both anaerobic and aerobic environments (Singh et al. 2015). Waste can be treated with bio-decolorization, which is economical, environmentally safe, and produces

less sludge. Oyster mushrooms (*Pleurotus* spp.) are decaying fungi that can grow and decolorize waste in the textile industry (Table 5).

According to a study, *Pleurotus ostreatus* exhibited enzymatic decolorization of up to 100 % for the heterocyclic dye indigo carmine (indigoid dye) and azure B and 74.5% for the triphenylmethane dye malachite green (MG) (Papinutti & Forchiassin, 2010). According to Ilyas et al. (2012), *P. ostreatus* decolorized synazol red HF6BN, an azo dye, by 40, 66, 82, and 96% from the medium after 6, 12, 18, and 24 days, respectively. Some textile dye decolorization using *Pleurotus* spp. are summarized in table-2.5

Table 2.5. Summarization of some textile dye decolorization by *Pleurotus* spp.

Sl. No	Mushroom strains	Dyes	Result/Decolorization	Reference
1.	<i>P. ostreatus</i>	Basic Red 46	69.3% after 25 days of incubation	Zuleta-Correa et al. 2016
2.	<i>P. ostreatus</i> BWPH and K4	Mixtures of Congo Red + Brilliant green & Congo Red + Remazol brilliant blue R	CK + ZB was better decolorized	Przystas and Zabocka-Godlewska, 2019
3.	<i>P. ostreatus</i> BWPH	Evans blue, Brilliant green	0.06g/L or above for Brilliant green 0.08g/L or above for Evans blue	Przystas et al. 2018
4.	<i>P. ostreatus</i> (PLO9)	Remazol brilliant blue R	while compare to free enzyme, PLO9 enzyme extracts exhibited 18 -fold increasing of LiP specific activity	de Oliveira Rodrigues et al. 2018

Sl. No	Mushroom strains	Dyes	Result/Decolorization	Reference
6.	<i>P. ostreatus</i>	Remazol Brilliant Blue R	70.42% after only 8 hours of incubation.	Afiya et al. 2019
7.	<i>P. ostreatus</i> BWPH	Anthanthrone Red	94.31% after 168 hours	Upadhyay and Przysaś, 2022
8.	<i>P. pulmonarius</i>	Basic Red 46	63.1% after 25 days	Zuleta-Correa et al. 2016
9.	<i>P. pulmonarius</i> CCB-20	Remazol brilliant blue, Methyl green, Bromophenol blue, Green bromocresol, Brilliant Blue, Reafix BFNG, Methylene blue, Methyl violet Poly R-478, Congo red, Ethyl violet, Methyl red.		Contato et al. 2020
10	<i>P. pulmonarius</i>	Tartrazine & Allura red mixture	Above 90% in pure cultures & above 60% by the fungal consortium.	Jiménez et al. 2019
11.	<i>P. pulmonarius</i>	Textile effluent & Methylene Blue (MB)	above 46% of textile effluent & 80% of methylene Blue (400mg/L)	Duran-Rivera et al. 2018

2.5 Antimicrobial activities

Pleurotus spp have also been shown to exhibit antibacterial and antifungal properties. According to Nagi and Ng (2004), a ribonuclease that was isolated from *P. sajor-caju* prevented the development of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Fusarium oxysporum*, and *Mycosphaerella arachidicola*. Antimicrobial potentiality against *Bacillus subtilis*, *Escherichia coli*, *S. aureus* and *B. megaterium* was demonstrated by an ethanolic extract of *P. florida* sporophore. According to Gerasimenya et al. (2002), *P. ostreatus* crude extracts have antibacterial activity against *Aspergillus niger* as well as both gram-positive and gram-negative bacteria. The growth of *P. aeruginosa*, *F. oxysporum*, *A. niger* and *B. subtilis* was suppressed by hexane-dichloromethane extracts from *P. ostreatus* that included anisaldehyde (Okamoto et al. 2002). The growth of *M. arachidicola* and *F. oxysporum* is inhibited by eryngin, an antifungal peptide that was isolated from *P. eryngii* (Wang and Ng, 2004). According to Nagi and Ng (2006), eryngeolysin, a hemolysin that was isolated from *P. eryngii*, inhibits the development of *Bacillus* sp. Ethanolic and aqueous extracts of *P. floridanus* mycelium and fruiting bodies have been shown by Das et al. (2012) to suppress the development of *S. aureus*, *B. subtilis* and *E. coli*. Additionally, endobacteria also has antibacterial qualities that can aid in defending both humans and plants against infections. An endobacteria, *Enterobacter* sp. that Gautam et al. (2016) isolated from *Agaricus bisporus* exhibited antibacterial activity against *E. coli* (MTCC No. 9721), *Salmonella typhi* (MTCC No. 8587), *Staphylococcus aureus* (MTCC No. 3160) and *Bacillus subtilis* (MTCC No. 2547).

CHAPTER III

MATERIALS AND METHODS

Mushroom species:

Pleurotus florida (ITCC 3308) was acquired from Society of Rural Industrialization, Ranchi, India whereas *Pleurotus pulmonarius* (MTCC 1805) and *Pleurotus ostreatus* (MTCC 1802) were acquired from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Bacterial species:

Bacillus safensis KY094643, *B. cereus* KY094642, *Pseudomonas donghuensis* MH145358 strains were previously isolated from soil in our laboratory and identified using NCBI (National Centre for Biotechnology Information) BLAST and the 16s rDNA sequences and submitted to Genbank. *B. cereus* and *B. safensis* strains were deposited to National Collection of Industrial Microorganisms (NCIM) having accession no NCIM 5557 and 5558, respectively. *Escherichia coli* (NCIM 2687) was collected from NCIM, NCL Pune. *Klebsiella pneumoniae* (MTCC 4030) was collected from MTCC, IMTECH, Chandigarh, India.

Fungal species:

Alternaria sp.MH087096 was previously isolated from diseased lentil seed in our laboratory and identified using NCBI BLAST. *Rhizopus solanii* (NCIM 1348) and *Fusarium oxysporum* (NCIM 1072) were collected from NCIM, NCL Pune.

Cultivation of Oyster Mushroom**Spawn preparation:**

Spawn was made following the method of Das and Mukherjee (2007) with slight modifications. Thousand gram of wheat grains were taken for spawn preparation. The grain was boiled for thirty minutes. After draining off excess water, the grains were spread out on sanitized blotting paper and air dried. The grains were blended with 10 gm of calcium carbonate and 15 gm of calcium sulphate. After filling a 250 ml conical flask with around 100 gm of grain, it was decontaminated by autoclaving for 15 minutes at 121°C. Once cooled, grains were inoculated with the appropriate strains of mushrooms and stored at 30 °C $\pm$ 2 °C for 15 days.

Substrates preparation:**Rice straw:**

Dried chopped rice straw was acquired from Barasat, 24 Parganas (N), WB, India. About 5 cm cut rice straws are immersed in water for overnight. Extra water of straw was drained off and then dried in air for fifteen minutes. The straws were placed in polythene bags (30 cm $\times$ 42 cm). Then the bags were sterilized by autoclaving for 20 mins at 15 lb pressure and 121°C temperature. After sterilization once the substrate was cooled then mixed with 10% (wet wt. /wet wt.) of spawn. It was then tightly closed and kept in 30 °C $\pm$ 2 °C after making some pin holes on the surface of the packet (Das et al. 2015).

Sugarcane bagasse:

From a local farm at Barasat sugarcane bagasse was acquired. It was dried for 6-8 hr in hot air oven at 65 °C and cut into small pieces. The subsequent steps are alike to preparation of rice straw.

Biological efficiency (BE)

BE was measured following the formula of Das et al. (2015).

$$\text{BE (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Determination of moisture content and dry matter of mushroom:

The moisture content of *Pleurotus* spp. was calculated according to Khan et al. (2013).

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

The dry matter was calculated by the following formula-

$$\text{Dry matter (\%)} = \frac{\text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Determination of ash content

The furnace gravimetric method was used to determine the total ash content. Oven-dried sample weighing 1 gm was placed in a crucible that had been cleaned and weighed beforehand. After that the crucible was kept at 550 °C ± 50 °C for three hours in a muffle furnace. Following cooling in a desiccator, the crucible was weighed. The ash contents were computed using the formula of Khan et al. (2013). The formula is as follows-

$$\text{Ash (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Where W1= weight of crucible, W2= weight of crucible + mushroom

W3= weight of oven-dried mushroom (here 1 gm)

Determination of crude fiber content

Crude fiber was determined following the method of Khan et al. (2013).

10 g of sample was heated with 200 ml H₂SO₄ to a simmering temperature (80 °C) and left for approximately 30 minutes. The volume was maintained by adding hot water frequently. The boiling was stopped after addition of 500 ml of cold water. The content was filtered under vacuum. The residues were digested after being repeatedly cleaned with hot water. Following the addition of acetone, digested material was filtered until it became neutral. After being thoroughly cleaned, the leftovers were put in a crucible. After that, the sample was dried at a constant weight. The following formula was used to compute crude fiber-

$$\text{Crude fiber (\%)} = \frac{A-B}{W} \times 100$$

Where A=Dry wt. after digestion, B=Wt. of ash, W=Wt. of sample

Preparation of fruiting body extract

Mushroom sporophores (fresh) were dried for overnight at 60°C to gain the constant weight. Then the dry mushroom fruiting bodies were crushed using mortar and pestle for preparation of mushroom powder. Oven dried powder (0.5 gm) of fruiting bodies were crushed with 5 ml imidazole buffer (20 mM; pH 7.8) having EDTA (1mM), PMSF(2mM) for estimation of protein & carbohydrate. 70% methanolic extract was prepared for other biochemical tests. Oven dried sporophore powder of mushroom was extracted with methanol (70%) and centrifugation was carried out for 10 min at 10,000 rpm followed by collection of supernatant and biochemical analysis. The amount of all the biochemicals was expressed as per mg dry mushroom powder.

Determination of Protein

With minor modifications, the method of Lowry et al. (1951) was used to estimate the protein concentration. The Lowry A and B reagents were initially made with Na_2CO_3 (2%), NaOH (0.1 N) solution and copper sulphate (1.56%) solution and sodium potassium tartarate (2.37%), respectively. Lowry B (2 ml) and Lowry A (98 ml) were combined to create the Lowry C reagent. 2 ml of Lowry C reagent, 980 μl of distilled water and 20 μl of mushroom extract were combined, and for 15 minutes the mixture was incubated. Following incubation, 1 ml of 1N Folin-Ciocalteu reagent was added and incubated in a dark place for 30 min. Measurement of absorbance was done at 660 nm, and the protein content of the fruiting bodies was ascertained in comparison to the BSA standard curve.

Total reducing sugar assay

The method for measurement of total reducing sugar content was determined by Miller (1959). Clean test tubes containing 50 μl sample (aqueous extract) was mixed with 450 μl of distilled water. Next, added 1.5 ml DNSA and shake thoroughly. Every test tube was incubated for 5 min in boiling water bath. The DNSA's yellow hue turned red. After reaching in ambient temperature they are placed in cold water bath. The OD values were measured at a wavelength of 540 nm. By comparing the Dextrose standard curve, the total sugar content was ascertained.

Determination of Polyphenol

With few modifications, the polyphenol content of the sample was calculated using the methodology of Mukhia et al. (2014). Initially, each test tube contained 50 μl of a methanolic extract of the fruit bodies of mushrooms. It was then mixed with 50 μl of 70% methanol. 400 μl of distilled water was then added. After that, 500 μl of Na_2CO_3 was

added and thoroughly mixed. After keeping 2 mins at room temperature 0.5 ml of Folin-ciocalteau reagent was added. The volume was made up to 2.5 ml with distilled water. The mixture was kept in the dark for half an hour. Following the period of incubation, the reaction mixtures turn to green in color. The OD measurements were made at 720 nm wavelength. The polyphenol concentration was ultimately measured in comparison to gallic acid standard curve.

Determination of Total Flavonoid

Quercetin was used as a standard to estimate the flavonoid concentration in accordance with Sultana et al. (2009). Initially, 50 μ l of the sample's methanolic extract were transferred to each test tube. To it, 50 μ l of 70% methanol was added. After that, the reaction mixture was mixed with 75 μ l of NaNO₂ solution. After that, it was incubated for 6 minutes. 150 μ l of 10% AlCl₃ solution was then thoroughly mixed, and incubated for 5 minutes. 500 μ l of 1 M NaOH was then added to it. Finally, by adding distilled water, the volume was adjusted to 2.5 ml. The reaction mixture was incubated for 15 mins. The colour of the reaction mixture changed to pink after the incubation period. A wavelength of 510 nm was used to measure absorbance. The flavonoid concentration was ultimately measured in comparison to Quercetin standard curve.

Determination of Antioxidant Activity

According to Sharma and Bhat (2009), reduction of DPPH (2, 2-diphenyl-1 picrylhydrazyl) radical was quantified. To start the reaction, 200 μ l of the sample was mixed with 2 ml of DPPH, and DPPH without sample was used as a reference. The OD values were taken at 517 nm following a 20-minute dark incubation period. Scavenging activity of the sample

was calculated based on percentage decolorization of the sample. The following formula according to following equation

$$\text{Percentage (\%) inhibition of DPPH activity} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Here, A_1 = Absorbance value of tested sample,

A_0 = Absorbance value of the control.

Measurement of sodium and potassium concentration

500 mg of dried mushroom powder was mixed with 5 ml of ternary acid, (mixture of nitric acid, H_2SO_4 , and perchloric acid in 9:4:1 ratio) in a 50 ml volumetric flask. Mixture was heated on a hot plate until it became colourless and transparent. Following that, distilled water was added to the volumetric flask until the content reached 50 ml. Next, compare the flame photometer's Na/K value to the standard solution (Das et al. 2015).

Metagenome analysis:

- **Overview of Sequencing Protocol:**

DNA was extracted using Xploreagen kit. The purified DNA was subjected to amplification using the primers (16sF: - 5' AGAGTTTGATGTTGGCTCAG3'; 16sR: - 5' TTACCGCGGCMGCSGGCAC3'). 40 ng of Extracted DNA is used for amplification along with 10pM of each primer. Extracted DNA was subjected to Nano Drop and GEL Check. The Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality and allowed for PCR amplification. The amplified 16s PCR Product is purified and subjected to GEL Check and Nanodrop QC. The Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality. The amplicons from each sample were purified with Ampure beads to remove unused primers and an additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the

sequencing libraries. Libraries were purified using Ampure beads and quantified using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using Illumina Miseq with 2x300PE v3-v4 sequencing kit. The bcl data received from the sequencer is de-multiplexed into fastq raw data.

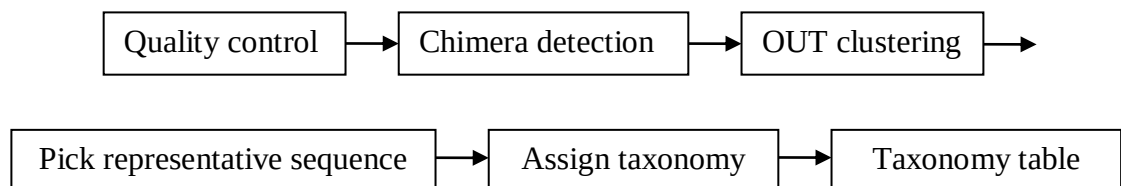
- **Bioinformatic analysis:**

The database used for 16s V3-V4 region is NCBI.

Step 1: The bcl data received from the sequencer is de-multiplexed into fastq raw data.

Step 2: The de-multiplexed data quality will be checked using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools.

Step 3: The QC passed samples are qualified for further analysis. The pipeline for metagenomics was conducted following to the methods of some previous studies (Jang et al. 2021; Kim et al. 2017; Kim et al. 2022). The pipeline for metagenomics is as follows-



Step 4: Once the run is over we get the final raw OTU table from which we start visualization of the analysis.

Step 5: The abundance feature tables and the top ten organisms in each samples are constructed using Microsoft excel (2024).

Step 6: The other analyses like Heatmap, core microbiome, Dendrogram, Alpha diversity, Beta diversity, PCOA plot, Rarefaction curve, KEGG pathway etc. were built using Microbiome analyst (online tool: <https://www.microbiomeanalyst.ca/>).

Isolation of Endobacteria

Endobacteria were isolated according to the method of Gautam et al. 2016. At first, the surface of fruiting bodies were sterilized with sterile distilled water for three times followed by 4% NaOCl solution and 70% ethanol. Then again washed with sterile distilled water. Blocks of different parts of fruiting bodies were transferred on NA plates and incubated at $28\pm 2^{\circ}$ C for 24 hours. The endobacterial isolates were screened and purified by repetitive sub-culturing and preserved on nutrient agar slants.

Characterization of endobacteria:

Gram staining, endospore staining were done by using standard methods. Biochemical tests like sugar utilization and fermentation, Indole production, MRVP tests, Nitrate reduction, utilization of Citrate and presence of Oxidase, Gelatinase, Catalase, Urea and Starch hydrolysis etc. All these experiment were performed according to Dubey and Maheswari (2006).

Indole production test:

Tryptone broth media was prepared for this test and inoculated with each of the bacterial strains. The incubation was allowed at 37° C for 1-2 days. Following incubation, 500 μ l of the Kovac's reagent was added to the culture tubes slowly along the walls, without shaking. A reddish ring forming on the uppermost layer was interpreted as a positive result.

Citrate utilization test:

Culture tubes containing Simmon's citrate agar medium (HiMedia), pH 6.9, were sterilised. The slants were placed in an incubator for 24 hours at 37° C after inoculation with the respective bacterial strains. The colour of media changed from green to deep blue after

incubation was seen as a positive sign that citrate was being used effectively. No change in colour would show a negative result.

Nitrate reduction test:

Nitrate broth media was prepared and inoculated with respective bacterial cultures. Then the culture tube was allowed to incubate at 37°C for 24 hr. After incubation 1 ml of reagent A and 1 ml reagent B was mixed to the culture tube respectively. The development of a red colour was interpreted as a positive result that the nitrate to nitrite reduction was successful. If no color change takes place, then zinc dust was added. The development of a red colour with the addition of zinc dust shows presence of nitrate in the media that is converted to nitrite after addition of zinc dust. This is now considered a definite negative result.

Methyl red (MR) & Voges-Proskauer (VP) test:

After sterilizing the culture tubes with MRVP broth (two for each bacterium and one as a control), bacterial cultures were inoculated in respective tubes at 35°C for 48 hours incubation. Five drops of methyl red were mixed to only one tube of each bacterium, and the colour change was observed. 10 drops of VPI and two to 3 drops of VPII reagent were added to the remaining tubes of each bacteria and control. The tubes were gently shaken, the plugs were taken out, and the tubes were left to wait for half an hour. The colour change was noticed. Yellow color pointed out negative result where as red color pointed out positive result for methyl red test. For Voges-Proskauer test pink-red color pointed out positive result and no color change or faint brown color signified the negative result.

Glucose Fermentation test:

The glucose broth was prepared and transferred it with in culture tube. Durham's tube containing the same broth placed invertedly in each culture tube, each medium was inoculated with bacterial cultures and incubated for 48 hr at 37 °C. Turbidity in the media was taken as an indicator of bacterial growth, and thus their ability to utilize that particular sugar. If the colour of the media changed from red to yellow, it used as an indicator of acid production during fermentation. If bubbles were present in Durham's tube after incubation, it was taken as an indicator of gas production during fermentation.

Sucrose Fermentation test

The sucrose broth was prepared and transferred it with in culture tube. Durham's tube containing the same broth placed invertedly in each culture tube, each medium was inoculated with bacterial cultures and incubated for 48 hr at 37 °C. The color change and bubble/gas formation was observed in the Durham's tube.

Lactose Fermentation test:

Lactose broth was prepared and transferred it in to the culture tubes at an amount and the inverted the Durham's tube filled with same broth in each culture tubes, inoculated with bacterial cultures, incubated for 48 hr at 37 °C. The color change and bubble/gas formation was observed in the Durham's tube.

Sugar mannitol fermentation:

In this test Mannitol broth was inoculated with respective bacterial culture and the tubes were incubated at 37 °C for 48 hours. If the result was positive the color of media changed from purple to grayish yellow as acid was produced in the media. A negative result for

sugar mannitol fermentation was shown the medium remain purple that means no acid has been produced.

Catalase test:

Drops of 3% H₂O₂ were added to the fresh bacterial culture, which had been put on a glass slide in the shape of a water drop. Within 30 seconds, several bubbles formed, indicating positive catalase activity; no bubbles formed on isolates that were catalase negative.

Oxidase test

The test organism colony was pierced onto filter paper that had been wet with sterile distilled water and steeped in a 1.5% solution of tetramethyl paraphenylene diamine dihydrochloride. Within 10 to 30 seconds, the colour of filter paper changed to deep blue or purple, indicating a positive result.

Urea hydrolysis:

Urea broth media were inoculated with each bacterial isolates and incubated at 37° C for 48 hours. For positive result the media turn to pink from yellow. A negative result showed the no change of color.

Starch hydrolysis:

Starch agar plates were prepared and streaked with cultures, incubated for 48 hr at 37 °C. Then flooded with iodine solution and blue–black color appeared due to formation of starch-iodine complex. If clear zone was found around streaked culture it indicated positive result.

Gelatin hydrolysis:

Stab of Nutrient Gelatin medium (Peptone-0.5%, Beef extract-0.3%, NaCl- 0.5%, Gelatin-1.5 %, pH- 7.0) was prepared for this test. Stabs were then inoculated with the respective bacterial strains. Inoculated and uninoculated control tubes were removed after 48 hours of incubation at 37 °C and left in an ice bath for at least half an hour. A negative result for gelatin liquefaction was indicated by the media solidifying after 30 minutes in an ice bath; on the other hand, a positive result for gelatinase activity was shown if the medium remained liquid.

Gram staining:

The bacterial smear on glass slide was dried in air followed by heat fixing. Crystal violet solution was added on the smear for 1-2 mins & washed it by using sterile distilled water. After flooding the slide with iodine solution for 30 seconds again washing with water was done for removal of excess stains. Then 95% alcohol was used drop wise. Then safranin solution was added drop wise. Then again washed with water. Finally the slide was observed under high power objective (40x and 100x) lens. Presence of the violet color bacteria were Gram-positive whereas the reddish bacteria were gram-negative in nature.

Endospore staining:

5-7 days old culture was used for spore staining. The culture was smeared on a grease free slide and heat fixed. Then malachite green solution was flooded on it and incubated it in steam for 5 mins. The excess stain was removed by washing with sterile distilled water and again flooded with safranin solution and left for 1 min. Finally, it was washed with tap water, air dried and observed under microscope at high power 40x and 100x objective lens.

Determination of cytotoxic effect of the isolates:**MTT Assay:**

A colorimetric MTT assay was used to estimate the cytotoxicity of bacterial lysate in a standard protocol described by Mosmann 1983. Bacterial lysates (2.5 μ L, 5 μ L, 7.5 μ L, and 10 μ L) were added to a polystyrene-coated plate containing about 10,000 Hek 293 cells (non-malignant cell line) and 7500 Caco-2 (human carcinoma cell line) cells in 100 μ L of medium and incubated for 24 hrs at 37 °C in a CO₂ incubator. 20 μ L of MTT reagent was added to each well and incubated for an additional 3.5 hours after the initial 24 hours of incubation. When MTT convert to purple formazan crystals in each well after the incubation, 150 μ L of MTT solvent was added in each well and the plates were scanned in a microplate reader VARIOSKAN LUX (Thermo Scientific) at 590 nm. Wells with medium, bacterial lysate and MTT reagent but no cells were enrolled as blanks, while positive controls were treated as wells with all the components mentioned above except the bacterial lysate.

Hemolytic activity of the isolates:

Isolates were streaked on blood agar plate containing 5% sterile sheep blood. At room temperature the plates were incubated for 18 hr (Mogrovejo et al. 2020). After incubation growth was observed.

Identification of endobacteria:

The isolated bacteria were sequenced using morphological, biochemical and 16S rRNA techniques.

Morphological characterization of the isolated bacteria

All the isolated bacterial strains were studied for their colony morphology on NA plates and Gram staining character and cellular morphology under compound light microscope. The features observed in colonies were: shape, size, colour, texture, elevation and margin.

16S rRNA technique:

16S rRNA sequencing was used to identify the bacterial strain. The cetyltrimethyl ammonium bromide method (Wu et al. 2001) was used to extract and purify the genomic DNA of bacteria from a single colony that had been cultured on Luria-Bertani agar medium for 24 hours. The Bio-Rad™ C-1000 PCR System (California, USA) was used to run the polymerase chain reaction (PCR) using the primer sets fD1 5'-GAG TTT GAT CCT GGC TCA G-3' and rP2 5'-CGG CTA CCT TGT TAC GAC TT-3' (Weisburg et al. 1991). Following the manufacturer's instructions, the PCR products were purified using the SIGMA GenElute™ PCR Cleanup Kit (St. Louis, MO, USA) and cloned in Escherichia coli (DH5α) using the pGEM®-TEasy Vector Cloning Kit (Promega, Madison, WI, USA). Plasmid isolation was performed using the FavorPrep™ Plasmid Extraction Mini Kit (Favorgen Biotech Corporation, Ping-Tung, Taiwan), and PCR sequencing was performed using the M13 forward and reverse primer sets 5'-GTA AAA CGA CGG CCA GTG -3' and R 5'-GGA AAC AGC TAT GAC CAT G-3', respectively. Finally, this primer set was used for PCR. Excess dye was removed using the Montage™ SEQ96 Sequencing Reaction Cleanup Kit (Merck Millipore, Darmstadt, Germany), and a DNA sequencer (Hitachi, Tokyo, Japan) was used for sequencing. BLAST was run using the National Centre for Biotechnology Information database once the sequences had been aligned. Use of the

minimal evolution approach was used to phylogenetic analysis. The construction of phylogenetic trees was done using MEGA version 6 (Saitou and Nei 1987; Tamura et al. 2013).

Antibiotic sensitivity test:

Sterile Mueller-Hinton agar (HiMedia) plates were used to spread the actively growing cultures of the bacterial strains. After, the plates were dried; commercially available antibiotic discs were placed on the plates having the bacterial cultures. The antibiotics used are- Amoxy/ Clav (Augmentin), Tetracycline, Ciprofloxacin, Streptomycin, Nitrofuranton, Imipenem, Meropenem, Co-trimoxazole, Vancomycin, Cefoperazone/Sulbactam, Chloramphenicol and Linezolid. The discs were pressed gently using sterile forceps and incubated at 37°C for 18 hours (Barry et. al. 1969). Then, the Inhibition zones around the antibiotic discs were measured. After comparing with standard reference, the response classified as sensitive, intermediate or resistant.

Testing the efficacy of endobacteria in growth and development of *Pleurotus* spp.**Co- culture of endobacteria with respective host mycelium**

Mushroom mycelial lawn were cut using a cork borer (0.6 cm in diameter) and placed at the middle of the Petri plates containing solidified mixed media(PDA:NA at 1:1) and endobacteria were streaked around the blocks. It was incubated at 30 ±2 °C for 7-14 days. Finally, the growth of both mushroom and respective endobacteria were observed and noted.

Quantitative determination of Indole acetic acid (IAA) of endobacteria:

Salkowski's reagent (2% 0.5 FeCl₃ in 35% Perchloric acid) was used to determine the IAA production of bacterial isolates using a colorimetric technique. The bacterial isolates were cultured for 72 hours at 32 °C on NB medium in presence of 0.05 M L-tryptophan. After centrifugation, the supernatant was collected for additional examination. 1 ml of supernatant was added with 2 ml of Salkowski's reagent and left in the dark for 30 mins. At 530 nm, the OD was determined in a spectrophotometer (Roy et al. 2018).

Solubilization of inorganic phosphate

Isolates were allowed to grow on NBRIP Agar plate after point inoculation. Discoloration around the colony indicated acid production by isolates (Roy et al. 2018).

Ammonia Production

Production of ammonia was determined according to the method of Lata and Saxena (2003). In culture tubes having 5 ml of peptone-water, endobacterial isolates were inoculated. Inoculated tubes were then incubated at 30° C for 4 days. 1 ml of Nessler's reagent was added to each tube.

Qualitative detection of Siderophore production

Siderophore production was assessed using the method described by Milagres et al. A Petri plate was divided into two halves. One half of the Petri plates contained Nutrient Agar, while the other half contained Chrome Azurol S- agar. Bacterial culture was streaked on the Nutrient Agar, and was incubated for a period of 5 days at 37°C. The distance of discharge of the blue colour on Chrome Azurol S- agar was measured (Milagres et al. 1999).

***In vitro* Assay for mushroom growth on agar plate:**

To assess the role of endobacterial culture on the growth of mushroom, an *in vitro* experiment was conducted. In a 90 mm Petri dish, 15 ml of mixed medium (PDA:NA at 1:1) and 1 ml of each of the five endobacterial culture (4×10^6 CFU) were mixed and pour plated, respectively. A control Petri dish containing only media was made. Mycelia from respective host mushrooms were placed on the centre of the Petri dish, which was then incubated at 27 ± 2 °C for 2 weeks. Finally, the diameter of the mycelia was measured that gives us the growth of the mushrooms.

***In vitro* Assay for mushroom growth in liquid submerged fermentation:**

Bacterial isolates were individually co-cultured with their host mycelium in a mixed medium (PDA:NA at 1:1) at 30 ± 2 °C. Host mycelial mushroom grown on mixed media were cut using a cork borer (6 mm in diameter) and two of these mycelial blocks were placed in flasks containing the mixed medium. After 5 days of incubation, respective endobacteria (4×10^6 CFU) were added into the flasks, which were then incubated for 7 days. Subsequently, the mycelial mass was filtered and the wet weight was recorded. Mycelial mass were kept in hot air oven at 70 °C for overnight and dry weight was recorded.

Production of Lytic Enzymes by bacterial isolates

Pectinase, Cellulase, and Chitinase enzyme production of endobacterial isolates were evaluated by the methods of (Aneja, 2003) with a few modifications.

Chitinase (EC 3.2.1.14)

Endobacterial culture were inoculated in basal medium complemented with 0.2% peptone (without peptone, too) and 2.5% colloidal purified chitin (Sigma, USA) and incubated at

30°C for 2–5 days. The production of chitinase was indicated by the formation of a clear zone surrounding the colony after addition of iodine solution in the medium

Cellulases (CMCase or Endoglucanase)

Peptone (0.2%) and Carboxymethyl Cellulose (1%) (Himedia) were supplemented with MS medium to cultivate endobacterial strains, which were then incubated for two to five days at 30 °C. Following the development of the bacterial colony, a 1% aq. solution of hexadecyl (trimethyl) ammonium bromide was added to the Petri plates. The enzyme production was measured by the creation of a transparent zone surrounding the line of growth of the tested organisms.

Pectinases (EC 3.3.1.15)

In order to prevent pectin breakdown, endobacterial isolates were cultivated in sterilized MS medium complemented with (0.2%) peptone and pectin (1%) (Himedia) and then incubated at 30 °C for two to five days. Following the bacterial colony's growth, a 1% aqueous solution of Hexadecyl (trimethyl) ammonium bromide was added to the Petri plates. The production of pectin enzymes was measured by the formation of a clear zone surrounding the test organism colonies' growth line.

Laccase (EC 1.10.3.2):

Extracellular laccase activity of *Pleurotus* spp. with and without isolated endobacteria was measured according the experiment by Das et al. (2011) using guaiacol as substrate with slight modification. At first, 20 mM guaiacol was prepared in 20% acetone. The final reaction mixture has 10 mM guaiacol and 0.1M acetate buffer (pH 5.0) in 2 ml volume. 200 µl of culture filtrate was used as source of enzyme. The OD₄₇₀ values were taken for Guaiacol ($\epsilon=6740 \text{ M}^{-1}\text{cm}^{-1}$). Enzyme activity was expressed in International Unit.

Polyacrylamide gel electrophoresis (PAGE)

PAGE was performed at pH 8.8 on 10% (w/v) polyacrylamide gel using gel plate (8 x 8 0.15 cm³). A mini gel system (Bangalore Genei) electrophoresis unit was used (Ladig et al. 2011). Gel slabs were made as follows:

A) Separating gel: 10% (w/v) Polyacrylamide, 0.255% (w/v) N,N'-Methylene-Bisacrylamide in 0.375 M Tris-HCl, pH 8.8, TEMED 0.025% (v/v), Ammonium Per Sulphate, 0.033% (w/v) .

B) Stacking gel: 4% (w/v) polyacrylamide and 0.1% (w/v) N,N'-Methylene-Bisacrylamide in 0.125M Tris-HCl, pH 6.8, TEMED 0.05% (v/v), Ammonium Per Sulphate 0.1% (w/v) .

C) Sample buffer: 10 mM Tris-HCL, pH 8.0, EDTA 1.0 mM, Glycerol 20% (v/v), Bromophenol blue 0.01% (w/v).

D) Electrophoresis buffer: Tris, 6 gm; Glycine, 28.8 gm, in a final volume of 1000 ml, pH- 8.5. Around 10 µg of samples was loaded to each lane and the gel was run at a constant current of 150 volt for 3-4 hours till the tracking dye had reached the lower edge. Thereafter, the gel was removed for laccase zymogram development.

Zymogram for laccase enzyme:

Extracellular culture filtrate was loaded in native Polyacrylamide Gel Electrophoresis (PAGE). The zymogram was developed after two hours incubation with 10mM Guaiacol in 0.1M acetate buffer (pH 5.0).

Study of fruiting body production in presence of endobacteria

In this experiment, spawn and substrates were inoculated with respective endobacteria to observe the growth and development of fruiting body. Endobacteria were grown in Nutrient Broth media. Different concentrations of endobacteria were supplemented in Rice straw substrate and finally the optimum concentrations of endobacteria were added in following 4 sets:

1. Control set: In control sets no endobacteria was added with spawn or substrates.
2. Substrate + (Spawn+ endobacteria): In these sets endobacteria was supplemented during spawn preparation but substrate was not supplemented with endobacteria.
3. (Substrate+ endobacteria) + Spawn: In these sets sterilized substrates were supplemented with different endobacteria but spawn was remained untreated with endobacteria.
4. (Substrate+ endobacteria) + (Spawn+ endobacteria): In these sets endobacteria were supplemented in both, during preparation of spawn as well as with sterilized substrates.

Pesticide tolerance by *Pleurotus* spp. :

Mycelia of different mushrooms were inoculated separately into the Petri plate containing PDA media in presence of different pesticides (Imidacloprid and Carbendazim) at two different concentrations (0.01% and 0.05%). Control set was also prepared (Roy et al. 2017). Then the Petri plates were incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Then the growth of mycelia was observed and measured the diameter.

***In vitro* testing of pesticide degrading capacity:**

Mushroom mycelia were cultured separately in flask containing MS media with different concentration (0.01% and 0.05%) of pesticide (Imidacloprid and Carbendazim) without adding any carbon or nitrogen source. Control set without pesticide were also prepared. Then the Petri plates were incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Growth of mycelia on Petri plate were observed and measured the diameter.

Pesticide tolerance test of endobacteria:

The isolated endobacteria were inoculated in Nephelometric flask containing NB medium supplemented with different concentration (0.01% & 0.05%) of pesticides. Then incubated at 30°C . After incubation the growth were observed in terms of OD at 600 nm (Roy et al. 2017).

***In vitro* testing of pesticide degrading capacity:**

Endobacteria were cultured separately in Nephelometric flask containing MS media with different concentration (0.01% & 0.05%) of pesticide (Imidacloprid and Carbendazim) without adding any carbon or nitrogen source. Control set without pesticide were also prepared. Growth was observed in terms of OD at 600 nm (Roy et al. 2017).

Dye decolorization capabilities:

Dye decolorization test was done according the methods of Si et al. (2013) with slight modification. The decolorization capacities of the three mushrooms and their respective endobacteria were tested in both solid and liquid submerged fermentation. Four industrial dyes- Congo Red (CR), Bromocresol Green (BCG), Malachite Green (MG) and Acridine

Orange (AO) were used for this test. For this assay, Yeast Glucose Media (5 gm Yeast extract, 20 gm Glucose, 1.0 gm KH_2PO_4 , 0.5 gm $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 gm $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 gm vitamin B1.) was spiked with 50 mg/ lit of different dyes. The pH of the medium was 5.8. Mushroom mycelia as well as endobacteria were inoculated separately in this media containing different dyes. Initial OD was taken (λ_{max} of CR- 498 nm, BGC- 423, MG- 618 nm and AO-490 nm). After 7 days of incubation, the cultures were centrifuged at 10,000 rpm for 10 min to remove mycelial debris. Then the supernatant was used for determination of the decolorization capacity by using spectrophotometer. The decolorization rate was expressed as a percentage and calculated as follows:

$$\text{Decolorization rate (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 = Initial value of absorbance,

A_1 = Final value of absorbance

Antimicrobial Activity of *Pleurotus* spp. and endobacterial isolates:

The antimicrobial activity of endobacterial isolates and *Pleurotus* species was assessed using a slightly modified version of the agar-well diffusion technique (Iwalokun et al. 2007). For anti-bacterial activity assay an overnight grown bacterial culture (test organisms) was added to NA plate and for anti-fungal activity assay fungal spore (test organisms) was added to PDA plate. Then well were created using sterile cork borer. 25 μl Crude extract of fresh fruiting bodies and 25 μl of cell free endobacterial culture filtrate were placed into each well for testing the antimicrobial activity of mushroom extract and endobacteria. The Petri plates were then incubated at 37 °C. As positive control, Amoxicillin and Fluconazole, at 20 μg per well, were present in the bacterial and fungal sets, respectively.

CHAPTER IV

RESULTS

4.1. Studies on biological efficiency and nutraceutical compositions of three *Pleurotus* spp.

4.1.1 Biological efficiency of three *Pleurotus* spp. grown on two distinct substrates viz.

Rice straw and sugarcane bagasse

Pleurotus pulmonarius, *Pleurotus florida* and *Pleurotus ostreatus* were successfully cultivated in our laboratory on both rice straw and sugarcane bagasse substrates (Figure 4.1). The morphometric information of three distinct *Pleurotus* - fruiting bodies was examined. It was observed that mushrooms grown from rice straw had a higher average length and diameter, but sugarcane-derived mushrooms had a higher number of fruiting bodies (Figure 4.2). The rice straw substrate showed better biological efficiency (BE) in both the first flush and second flush. For rice-straw substrates maximum BE was recorded in rice straw-derived *P. florida* (63 %) followed by *P. ostreatus* (54.5%) and least in *P. pulmonarius* (48 %). In sugarcane bagasse highest BE was found in *P. florida* (49%) followed by *P. pulmonarius* (42%) and least in *P. ostreatus* (41%) (Figure 4.3).



Figure 4.1. Cultivation of three different *Pleurotus* spp on rice straw and sugarcane bagasse substrates. A- *P. pulmonarius* (MTCC 1805), B. *P.ostreatus* (MTCC 1802), *P. florida* (IICC 3308) grown on Rice-straw; D- *P. pulmonarius* (MTCC 1805), E- *P.ostreatus* (MTCC 1802), F- *P. florida* (IICC 3308) grown on sugarcane-bagasse.

In figure, 1805 denotes *Pleurotus pulmonarius* (MTCC 1805), 1802 denotes *Pleurotus ostreatus* (MTCC 1802), 3308 denotes *Pleurotus florida* (ITCC 3308)

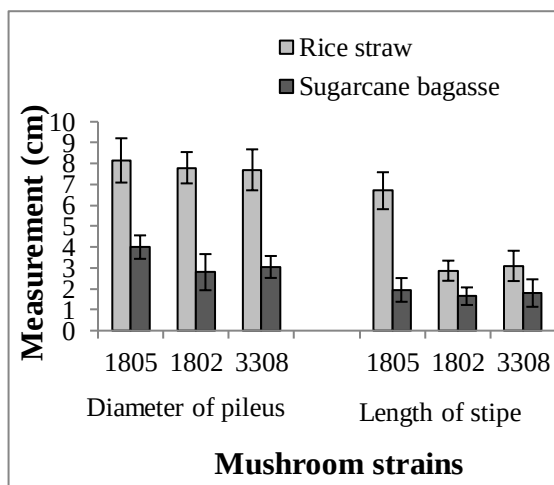


Figure 4.2. Graphical representation displaying the morphometric informations of fruiting bodies of *Pleurotus* spp. cultivated on two distinct substrates.

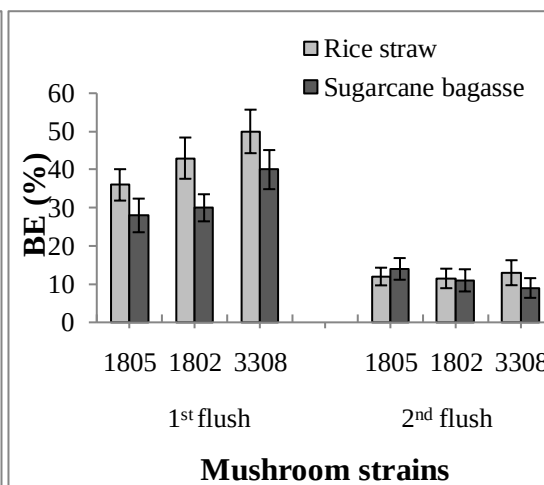


Figure 4.3. Graphical illustration showing the Biological Efficiency (%) of three *Pleurotus* spp. grown on two distinct substrates.

Table 4.1. Determination of Moisture content and Dry matter of *Pleurotus* spp.

Mushroom strains	Rice-Straw substrates		Sugarcane-bagasse substrates	
	Moisture content (%)	Dry matter (%)	Moisture content (%)	Dry matter (%)
MTCC1805	86.0±0.92	14±0.12	84.5.0±1.9	15.5±0.13
MTCC 1802	89.2±0.87	10.8±0.09	88.6±1.37	11.4±0.10
ITCC 3308	88.5±1.2	11.5±0.14	87.2.5±1.4	12.8±0.07

4.1.2. Study of reproductive structures (basidia and basidiospores) of three *Pleurotus* species

The length of basidium was maximum in *P. florida* and minimum in *P. ostreatus*. Breadth of basidium was maximum in *P. pulmonarius* and minimum in *P. ostreatus*. Average area of basidiospore was maximum in *P. florida* and minimum in *P. pulmonarius*. The number of basidium present per unit area (1000 sq. μm) in *P. pulmonarius* was 6 but 4 in *P. florida*. (Table 4.2 and Figure 4.4)

Table 4.2. Measurement of basidia and basidiospores of three *Pleurotus* species

Name of the strains	Length of basidium (μm)	Breadth of basidium (μm)	Number of basidium per 1000 sq. μm	Area of basidiospore (sq. μm)
<i>P. pulmonarius</i> (MTCC 1805)	22.30 $\pm$ 2.7	6.24 $\pm$ 1.2	6	6.20 $\pm$ 1.1
<i>P. ostreatus</i> (MTCC 1802)	20.33 $\pm$ 2.2	3.34 $\pm$ 0.7	5	7.64 $\pm$ 1.3
<i>P. florida</i> (ITCC 3308)	29.20 $\pm$ 3.2	4.06 $\pm$ 0.9	4	15.68 $\pm$ 2.1

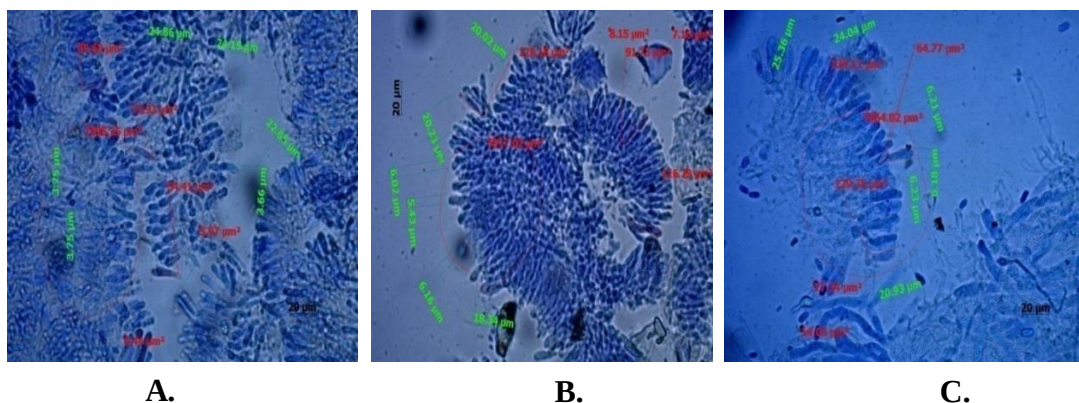


Figure 4.4 Section of Fruiting bodies showing basidia and basidiospores (A. MTCC 1805; B. MTCC 1802, C. ITCC 3308)

4.1.3.1 Nutraceutical compositions of fruiting bodies of *Pleurotus* spp.

The three different species of *Pleurotus* were grown on two substrates namely, rice straw and sugarcane bagasse, in order to study comparative nutraceutical compositions of the fruiting bodies of *Pleurotus*. The rice straw derived *P. ostreatus* showed maximum protein (18 µg/mg) followed by *P. pulmonarius* (16 µg/mg) and *P. florida* (15 µg/mg). In sugarcane bagasse highest protein content was recorded in *P. ostreatus* (16.5 µg/mg) and lowest in *P. pulmonarius* (14 µg/mg) (Figure 4.5). With regard to the carbohydrate content, it was found that all fruiting bodies of *Pleurotus* derived on sugarcane bagasse exhibits more carbohydrate content than those grown on the Rice straw. Maximum carbohydrate content was recorded in sugarcane bagasse- derived *P. ostreatus* (57 µg /mg) whereas least in *P. florida* (43.5 µg/mg). In rice straw derived mushroom highest carbohydrate content was found in *P. ostreatus* (53.6 µg/mg) followed by *P. pulmonarius* (50.5 µg/mg) (Figure 4.6). Highest ash content was recorded in rice straw derived *P. florida* (8.7 %) and lowest in *P. pulmonarius* (5.6 %) whereas in sugarcane bagasse derived fruiting bodies highest result was also recorded in *P. florida* (8.1 %) followed by *P. ostreatus* (7 %) (Figure 4.7). Maximum fiber content was recorded in *P. pulmonarius* for both rice straw and sugarcane bagasse -derived fruiting bodies among all the strains. The highest fiber content was recorded in rice straw -derived *P. pulmonarius* (9.5 %) whereas the recorded value for sugarcane bagasse - derived fruiting bodies was 9.1 % (Figure 4.8). Highest polyphenol content was found in rice straw derived *P. ostreatus* (6.5 µg/mg) followed by sugarcane bagasse-derived *P. florida* (5.9 µg/mg) and lowest in sugarcane bagasse-derived *P. pulmonarius* (4.9µg/mg) (Figure 4.9). Highest flavonoid content was recorded in sugarcane bagasse-derived *P. florida* (0.38 µg/mg) followed by rice straw-derived *P. florida* (0.34

µg/mg) and lowest in sugarcane bagasse-derived *P. pulmonarius* (0.22 µg/mg) (Figure 4.10). Highest antioxidant activity was recorded in rice straw-derived *P. ostreatus* (60%) followed by rice straw-derived *P. florida* (55.5%) and lowest in sugarcane bagasse-derived *P. florida* (Figure 4.11).

4.1.3.2 Mineral (sodium and potassium) content of fruiting bodies of *Pleurotus* spp.

Sodium and potassium contents in three different *Pleurotus* spp. were measured using dried mushroom powder (Table 4.3). All the mushroom strains shown better potassium content than sodium content for both substrates. Highest potassium content was recorded in rice-straw derived *P. pulmonarius* (23.42±1.9 ppm) followed by *P. florida* (22.38±1.7 ppm) and *P. ostreatus* (22.38±1.7 ppm). Rice-straw derived *P. pulmonarius* also shown highest sodium content (21.5±2.3 ppm) and lowest in *P. florida* (5.5±1.3 ppm). All the mushroom derived from sugarcane bagasse substrates shown more or less similar result.

Table 4.3. Determination of quantity of Na and K in three *Pleurotus* strains

Mushroom strains	Rice-straw substrates- derived mushrooms		Sugarcane bagasse substrates- derived mushrooms	
	Average quantity of Potassium (ppm)	Average quantity of Sodium (ppm)	Average quantity of Potassium (ppm)	Average quantity of Sodium (ppm)
MTCC 1805	23.42±1.9	20.89±2.1	21.5±2.3	18.2±1.7
MTCC 1802	22.38±1.7	6.31±0.7	20.25±1.9	5.9±1.1
ITCC 3308	23.35±2.2	5.78±1.8	23.10±2.1	5.5±1.3

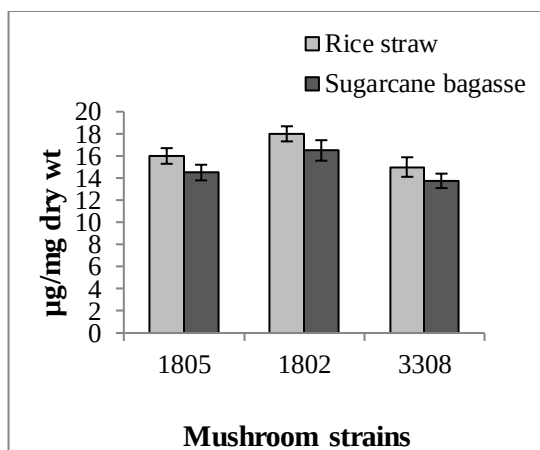


Figure 4.5. Graphical representation displaying the Protein content ($\mu\text{g}/\text{mg}$ dry weight) of fruiting bodies grown on two distinct substrates.

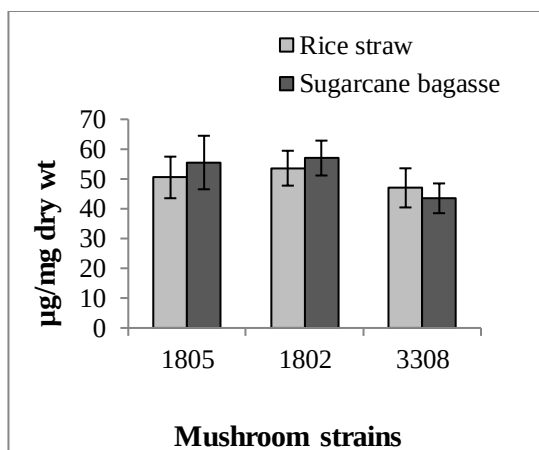


Figure 4.6. Graphical representation displaying the Carbohydrate content ($\mu\text{g}/\text{mg}$ dry weight) of fruiting bodies grown on two distinct substrates.

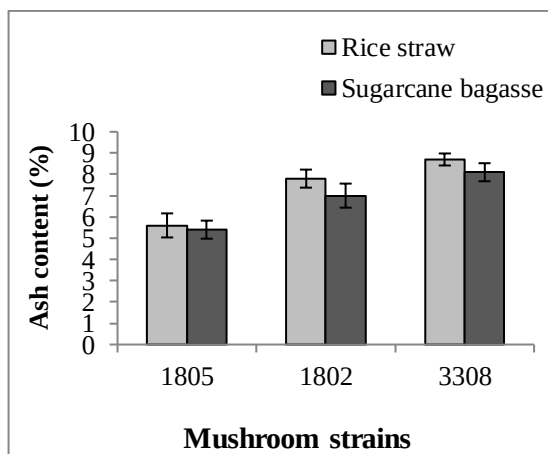


Figure 4.7 Graphical representation displaying the Ash content (%) of fruiting bodies grown on two distinct substrates.

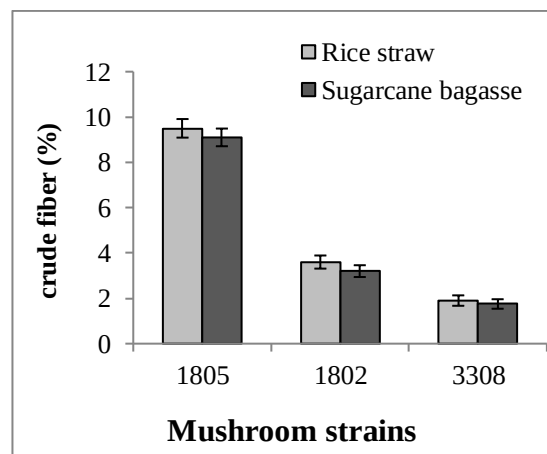


Figure 4.8 Graphical representation displaying the Fiber content (%) of fruiting bodies grown on two distinct substrates.

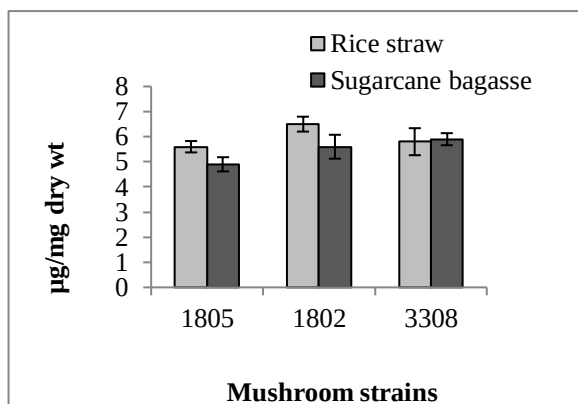


Figure 4.9 Graphical representation displaying the Polyphenol content (µg/mg dry weight) of fruiting bodies grown on two distinct substrates.

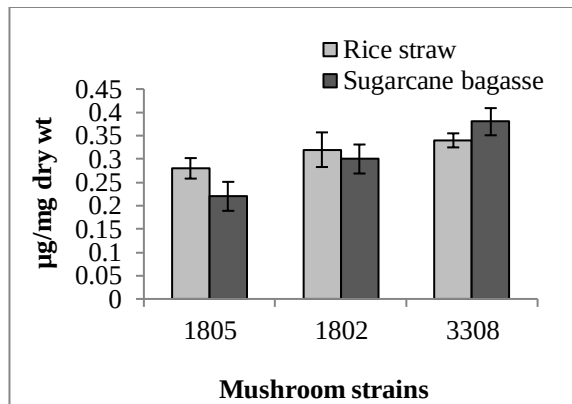


Figure 4.10 Graphical representation displaying the Flavonoid content (µg/mg dry weight) of fruiting bodies grown on two distinct substrates.

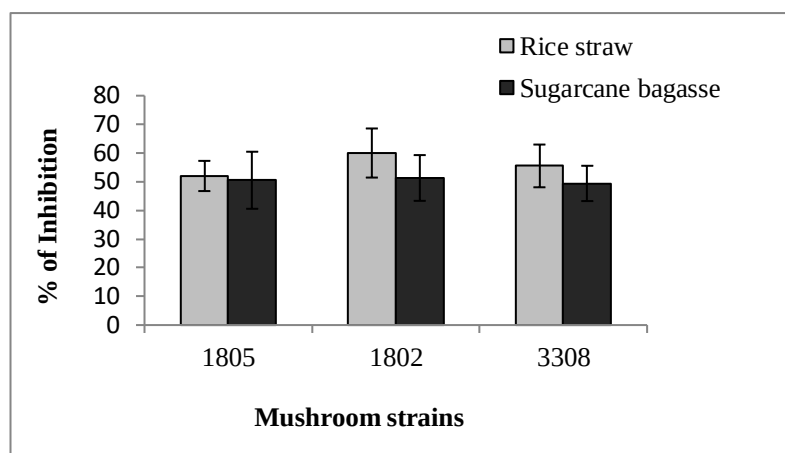


Figure 4.11 Graphical representation displaying the Antioxidant activity (using DPPH) (% of inhibition) of fruiting bodies grown on two distinct substrates.

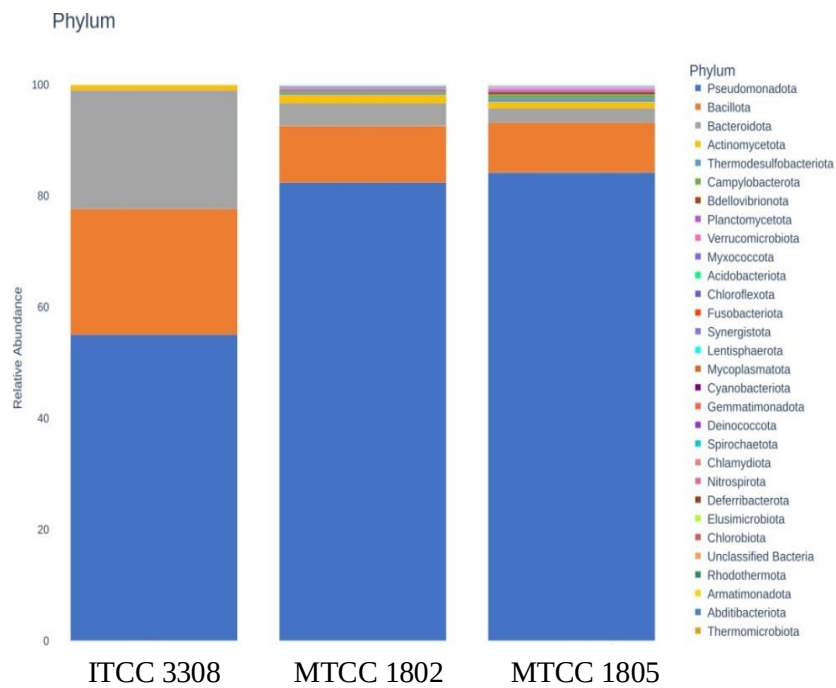
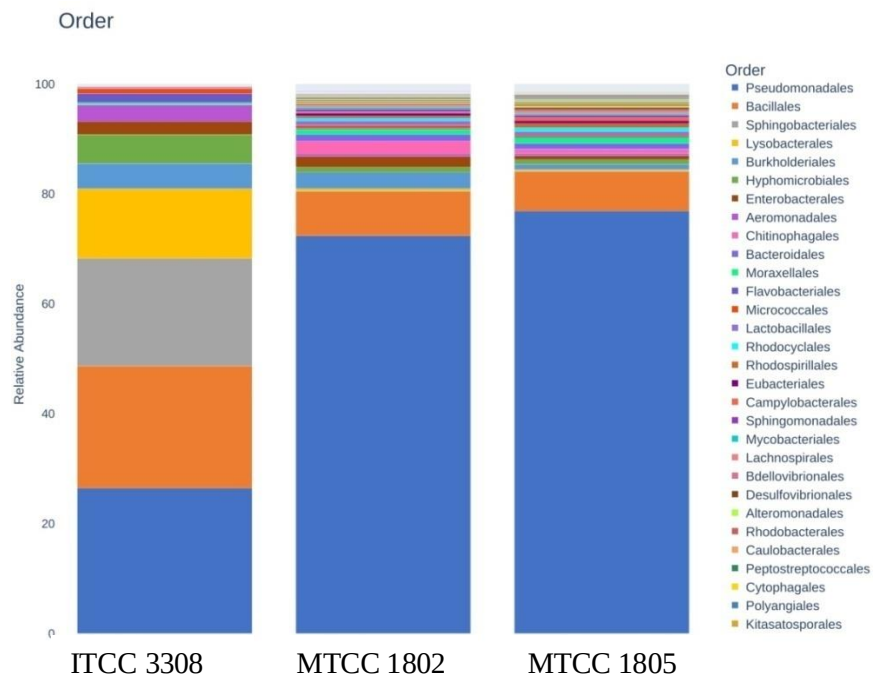
Conclusion:

- Recycling of agro-waste products like Rice straw and sugarcane bagasse as mushroom substrates is both environmentally sustainable and beneficial.
- So far the yield is concerned Rice straw is better substrate than sugarcane bagasse.

4. 2. Metagenomic analysis of sporophores of *Pleurotus* spp.

All the three mushroom species showed the abundance of microbiota. Comparative microbiome diversity at the different levels (Phylum, Order, Class, Family, Genus and Species), along with the average relative abundance of microbes from *Pleurotus florida* (ITCC 3308), *Pleurotus ostreatus* (MTCC 1802) and *Pleurotus pulmonarius* (MTCC 1805) showed in Figure 4.12.A- 4.12.F. The results indicated that common microorganisms belonged to the genera are *Pseudomonas*, *Paenibacillus*, *Stenotrophomonas*, *Priestia*, *Sphingobacterium*, *Pantoea*, *Bacillus*, *Rosellomorea* etc. (Figure 4.12.E). A high Chao1 index value indicated the presence of rare species in samples *P. ostreatus* and *P. pulmonarius* (Figure 4.13.A.). Value of simpson's index for *P. florida* is 0.821 which is closer to "1" whereas the value for *P. pulmonarius* is 0.393 which is closer to "0" (Figure 4.13.B.). The venn diagram showed that 13.1 % genus are common to all the three mushroom species (Figure 4.14). It was found that *P. pulmonarius* contained 27.5% of unique species followed by *P. ostreatus* (17.4%) and least in *P. florida* (3.5%). The major bacterial genus in all the mushrooms was *Pseudomonas* which was present in highest amount in *P. pulmonarius* (88.9%) followed by *P. ostreatus* (84.2%) and least in *P. florida* (28.2%) (Figure 4.15 A, B, & C). In *P. florida* except *Pseudomonas* other predominant genus were *Paenibacillus* (23.5%), *Sphingobacterium* (20.9%), *Stenotrophomonas* (13.2%), *Rhizobium* (13.2%) (Figure 4.15.A). Unit variance scaling with the top 50 organisms (genus level) was used for heatmap construction (Figure 4.16).

Taxonomic Plot Analysis

Figure 4.12.A Taxonomic Plot Analysis of *Pleurotus* spp. (Phylum Level)Figure 4.12.B Taxonomic Plot Analysis of *Pleurotus* spp. (Order Level)

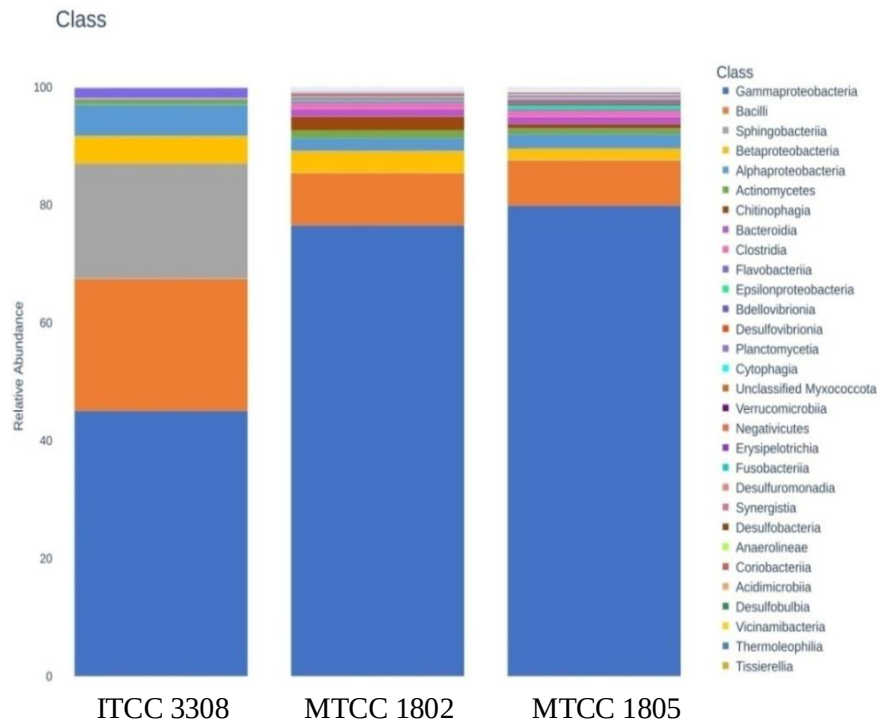


Figure 4.12.C Taxonomic Plot Analysis of *Pleurotus* spp. (Class Level)

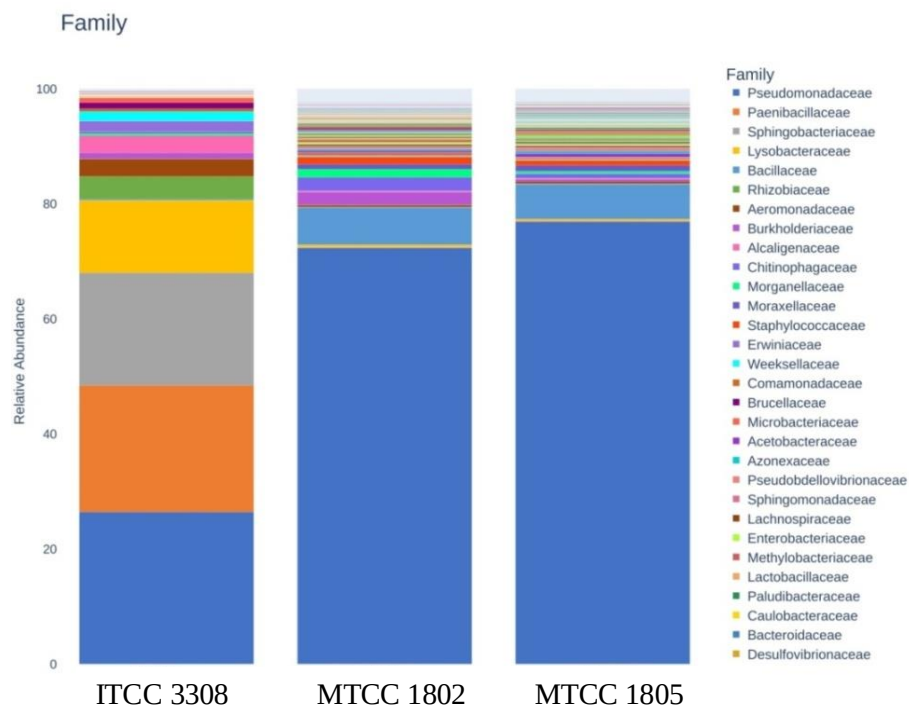


Figure 4.12.D Taxonomic Plot Analysis of *Pleurotus* spp. (Family Level)

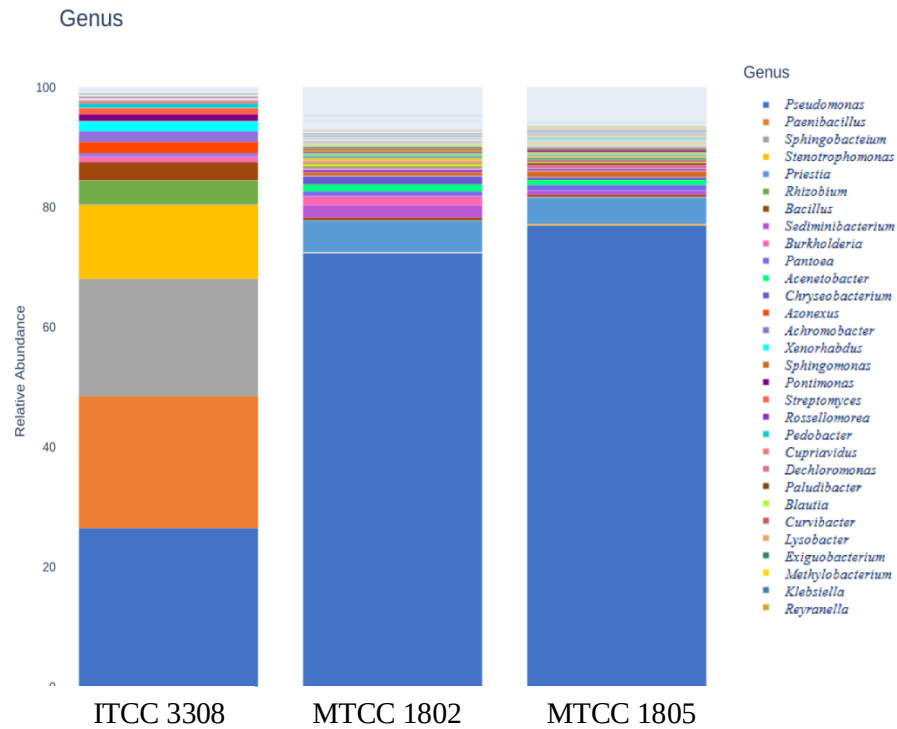


Figure 4.12.E Taxonomic Plot Analysis of *Pleurotus* spp. (Genus Level)

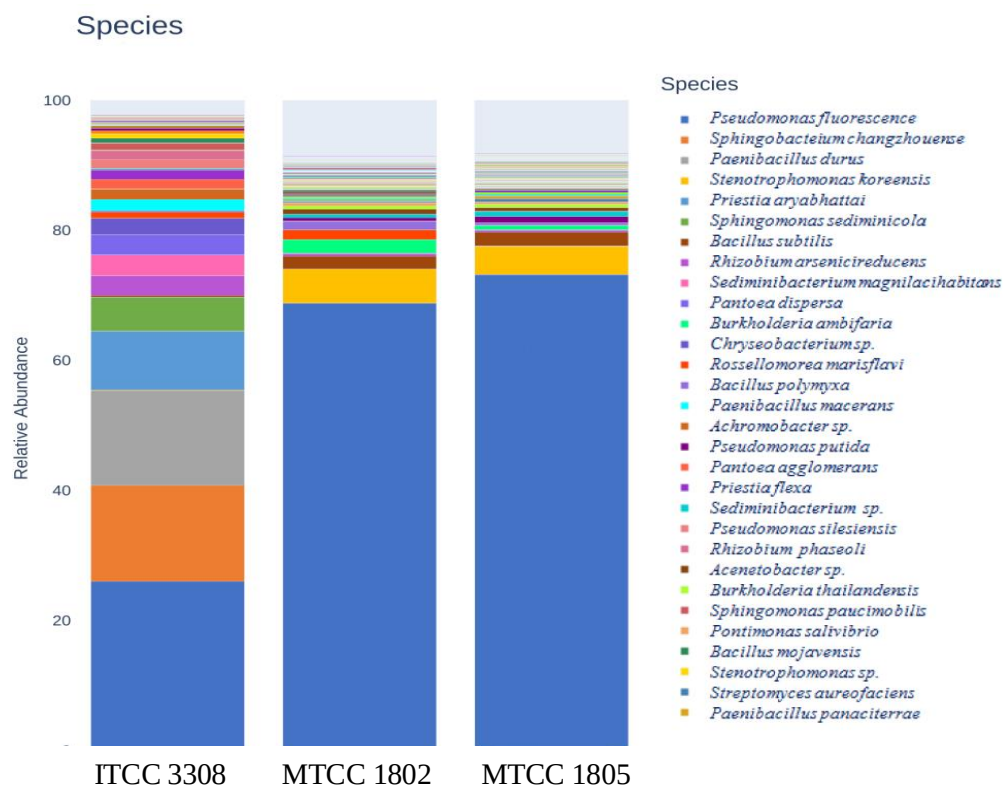


Figure 4.12.F Taxonomic Plot Analysis of *Pleurotus* spp. (Species Level)

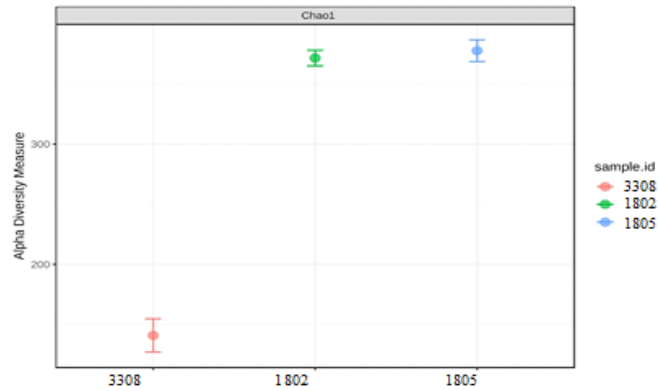


Figure 4.13.A Chao 1 index of *Pleurotus* spp.

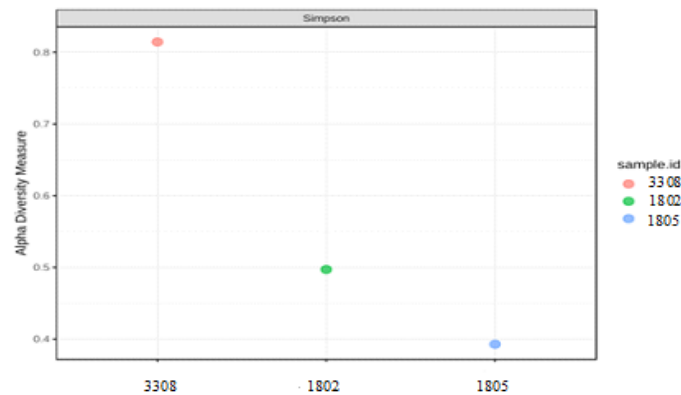


Figure 4.13.B Simpson's index of *Pleurotus* spp.

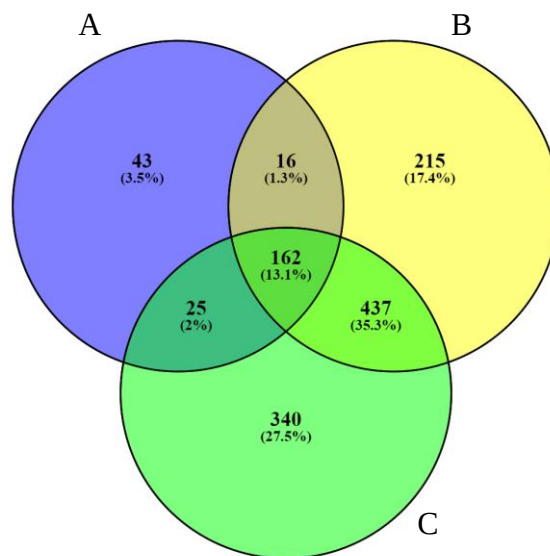


Figure 4.14 Venn analyses of *Pleurotus* spp. (A. *Pleurotus florida*, B. *Pleurotus ostreatus*, C. *Pleurotus pulmonarius*)

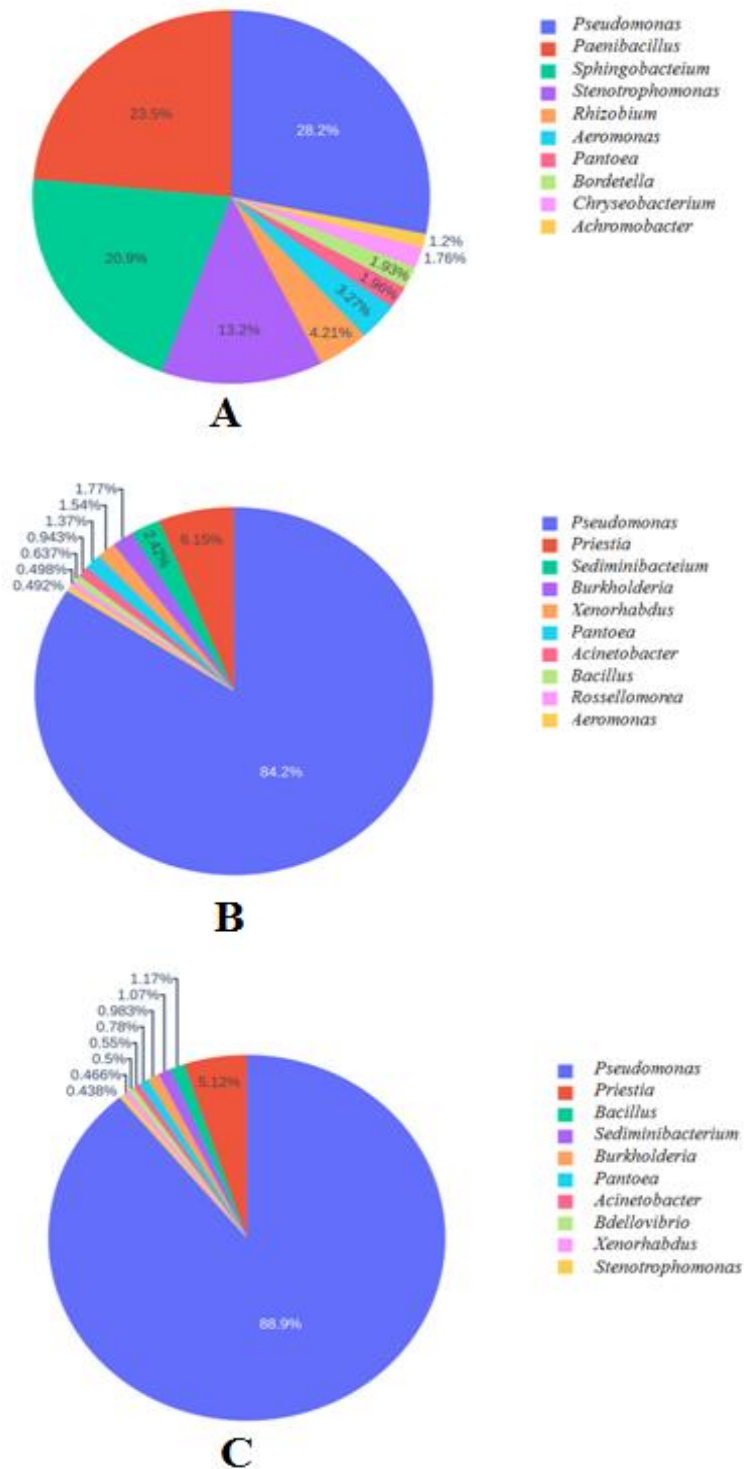


Figure 4.15. Bacterial abundance of *Pleurotus* spp. (A. *Pleurotus florida*, B. *Pleurotus ostreatus*, C. *Pleurotus pulmonarius*)

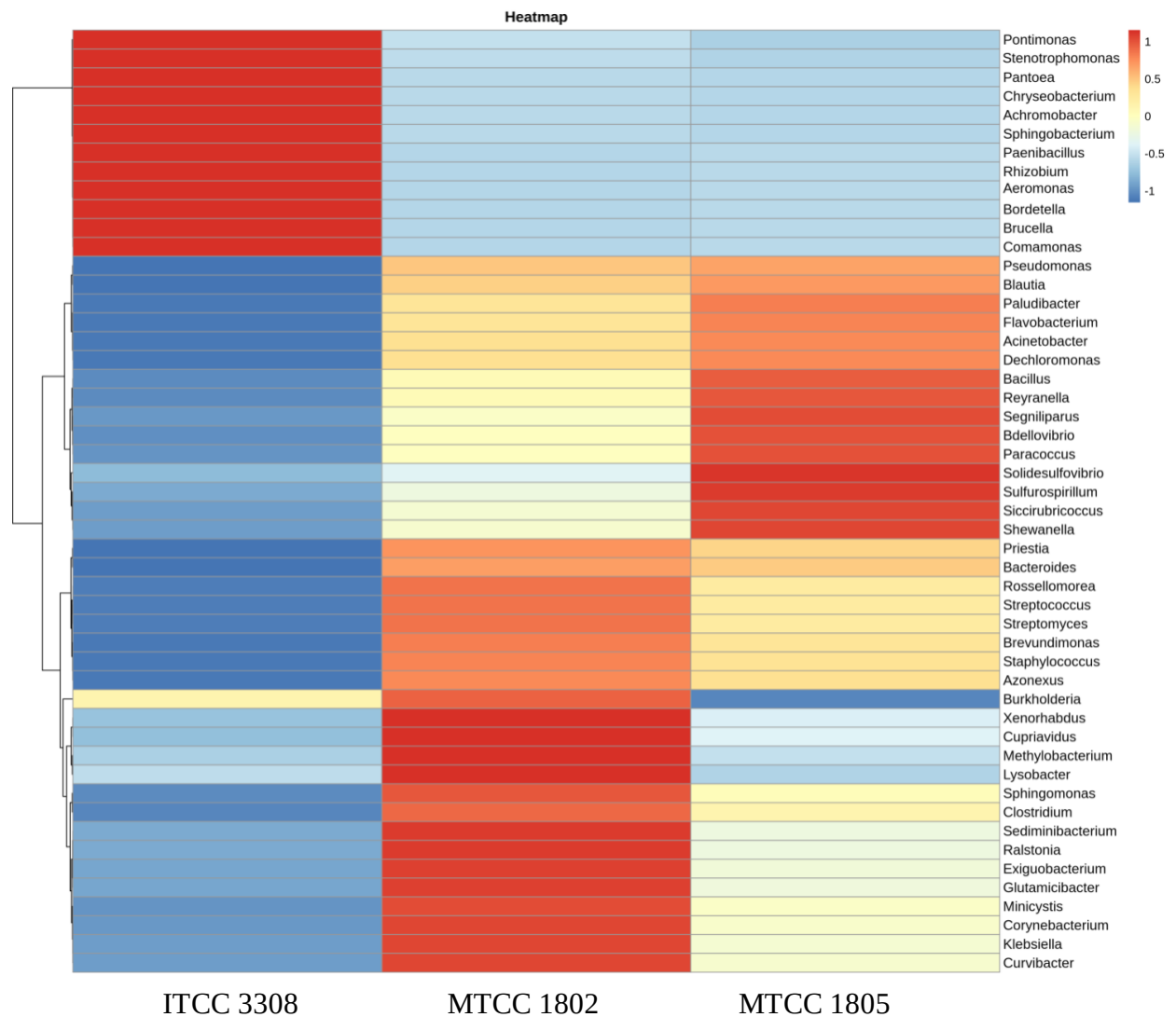


Figure 4.16. Heat map representation of core taxa across the members.

Conclusion:

- The results of Metagenomic analysis indicated an array of microorganisms in the fruiting bodies of Oyster mushrooms. The most abundant genera is *Pseudomonas* (proteobacteria) followed by *Paenibacillus*, *Stenotrophomonas*, *Priestia* etc.

- In *P. ostreatus* and *P. pulmonarius*, bacterial population was dominated by a single species i.e. *Pseudomonas* whereas *P. florida* showed more or less equal distribution of *Pseudomonas*, *Paenibacillus* and *Stenotrophomonas*
- The abundance of beneficial bacteria like *Stenotrophomonas*, *Pseudomonas*, *Priestia*, *Rhizobium*, *Burkholderia*, *Rosellomorea* etc. were found in all three tested oyster mushrooms.

4.3. Isolation and characterization of endophytic bacteria from *Pleurotus* spp.

4.3.1. Isolation of Endobacteria from the fresh sporophore of *Pleurotus* spp.

Endobacteria were isolated from different parts of the sporophores of all the tested *Pleurotus* species such as stipe, pileus and junction of pileus and stipe. 130, 48 and 53 endobacteria were isolated from the sporophores of *P. pulmonarius*, *P. ostreatus* & *P. florida*, respectively on the basis of colony. Finally on the basis of growth promoting activities (GPA) three strains (PP, PJ1 & PJ2) were finally selected from *P. pulmonarius* (Table - 4.4), one isolate named OP was selected from *P. ostreatus* (Table- 4.5) and one isolate (FS2) was selected from *P. florida* (Table - 4.6).

Table 4.4. Growth promoting traits of endobacteria isolated from *P. pulmonarius*

Sl. No	Endobacterial isolates	IAA production	Siderophore production	Ammonia production	Phosphate solubilization
1.	PP	+++	+++	++	-
2.	PP3	-	-	+	+
3.	PP7	-	+	-	-
4.	PP10	+	-	+	-
5.	PJ1	+++	+++	++	-
6.	PJ2	+++	+++	++	-
7.	PJ13	+	-	-	+
8.	PJ19	-	+	+	-
9.	PS2	+	-	+	+
10.	PS5	+	-	+	-
11.	PS6	-	+	+	-
12.	PS12	-	+	+	-

“+++” indicates excellent, “++” indicates very good amount, “+” indicates positive, “-” indicates negative

Table 4.5. Growth promoting traits of endobacteria isolated from *P. ostreatus*

Sl. No	Endobacterial isolates	IAA production	Siderophore production	Ammonia production	Phosphate solubilization
1.	OP	+++	+++	++	-
2.	OP4	-	-	+	+
3.	OP8	-	+	-	-
4.	OJ3	+	-	+	-
5.	OJ9	+	-	-	+
6.	OS6	+	-	+	-
7.	OS17	-	-	+	-

“+++” indicates excellent, “++” indicates very good amount, “+” indicates positive, “-” indicates negative

Table 4.6. Growth promoting traits of endobacteria isolated from *P. florida*

Sl. No	Endobacterial isolates	IAA production	Siderophore production	Ammonia production	Phosphate solubilization
1.	FP4	+	-	+	-
2.	FP7	+	-	-	+
3.	FJ7	-	+	-	-
4.	FJ13	+	-	+	-
5.	FS2	+++	+++	++	-
6.	FS9	+	-	+	-

“+++” indicates excellent, “++” indicates very good amount, “+” indicates positive, “-” indicates negative

4.3.2. Co-culture of isolated endobacteria with host mushroom mycelia in solid media:

Both host mycelia and their respective endobacteria were grown in same plate using a mixed media (PDA and NA at 1:1 ratio). Endobacteria and their host mycelia were found to grow on the same plate without affecting one another (Figure 4.17).

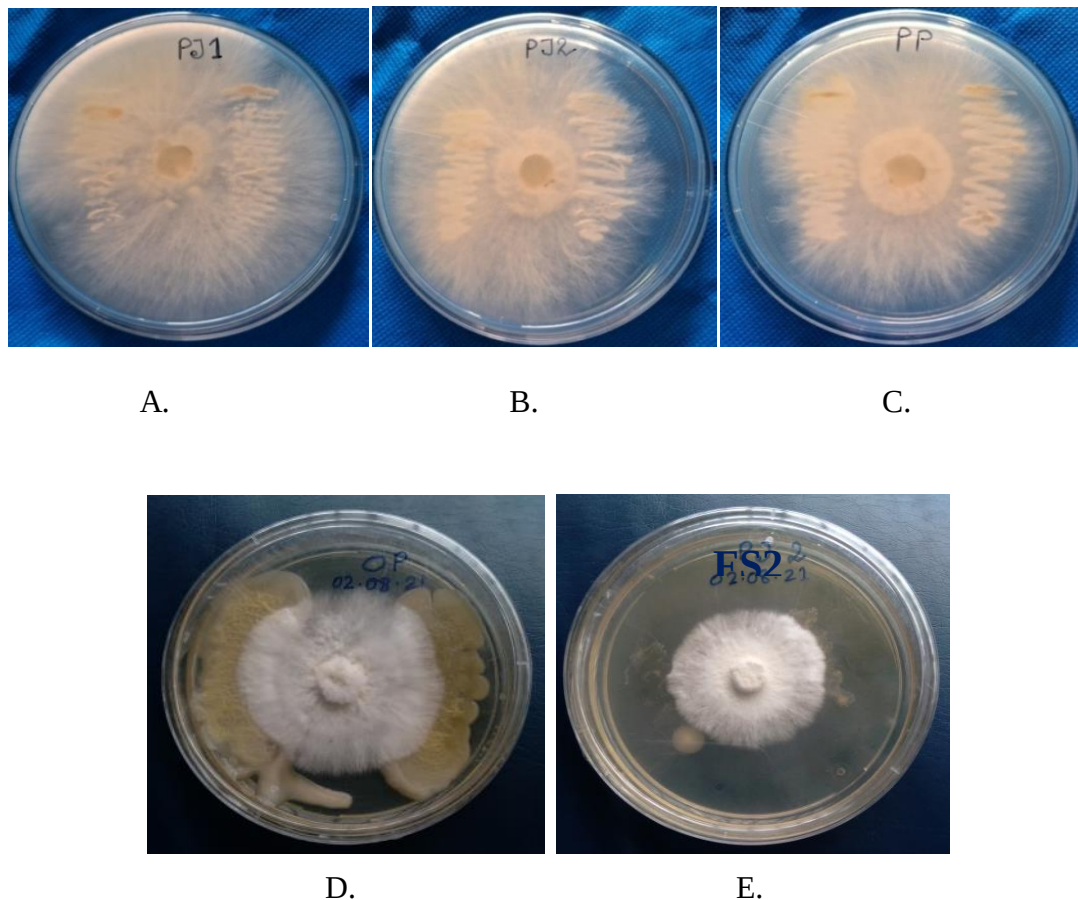


Figure 4.17. Co- culture of bacterial isolates with respective host mycelium. Co-culture of PJ1, PJ2 and PP with their host mycelium *P. pulmonarius* (Figure A, B &C); OP and FS2 with their host mycelium *P. ostreatus* and *P. florida* respectively (Figure D & E)

4.3.3. Characterization of five selected endobacteria

All of the five endobacteria isolated from three species of *Pleurotus* were found to be Gram positive, rod shaped, endospore formers (Figure 4.18). Morphological and colony characters of each of the bacterial isolates were observed on nutrient agar plates and under light as well as electron microscopes (Table 4.7 & Figure 4.19). Scanning Electron microscopic images (Figure 4.20) shown that the largest endobacteria is OP which is 4.549 μm long and the smallest one is PJ1 which is 1.264 μm in length. The length of PJ2, PP and FS2 were found to be 1.557 μm , 2.843 μm and 1.709, was respectively. All the five endobacteria showed positive results for Indole, Catalase and Oxidase tests and negative for nitrate reduction, Methyl red, Voges-Proskauer, Citrate and Gelatin hydrolysis activity. PP, PJ1 and OP hydrolyzed Starch and only PP showed the Urea hydrolysis activity. The bacterial isolates PP and OP were able to ferment sugar alcohol, mannitol. Biochemical characterization of all the endobacteria are given in the Table- 4.8.

Table 4.7. Morphological characterization of the five endobacterial strains

Bacterial strains	Colony morphology	Microscopic morphology	Spore former or not
PP	Whitish, irregular, undulate margin, flat, opaque	Gram positive, Rod	Spore Former
PJ1	Yellowish, circular, entire margin, flat, opaque	Gram positive, Rod	Spore Former
PJ2	Yellowish, circular, entire margin, flat, transparent	Gram positive, Rod	Spore Former
OP	Whitish, irregular, undulate margin, flat, opaque	Gram positive, Rod	Spore Former
FS2	Yellowish, circular, entire margin, flat, opaque	Gram positive, Rod	Spore Former

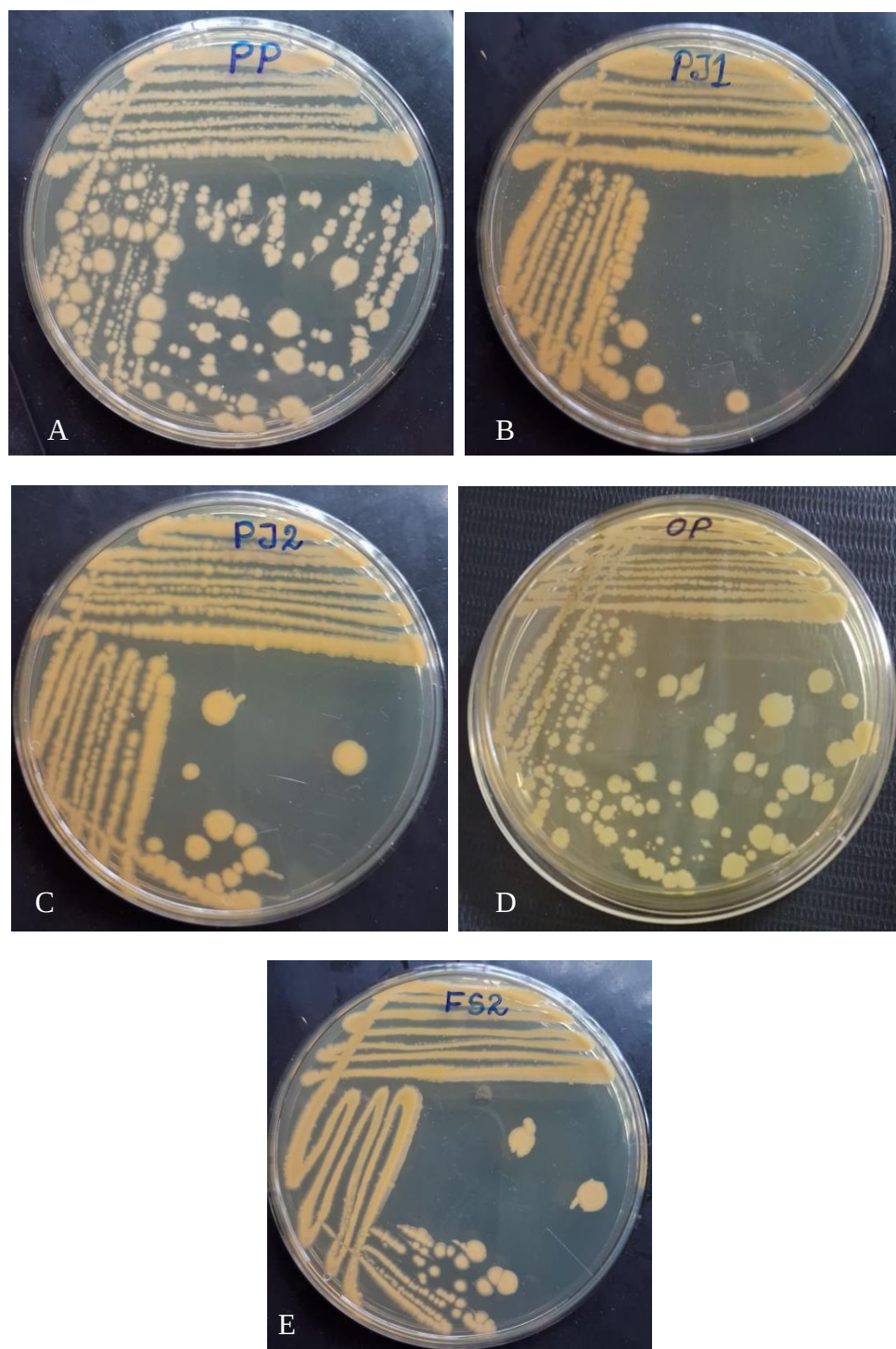


Figure 4.18. Colony morphology of endobacterial isolates on NA plates (A- PP, B- PJ1, C-PJ2, D-OP, E-FS2)

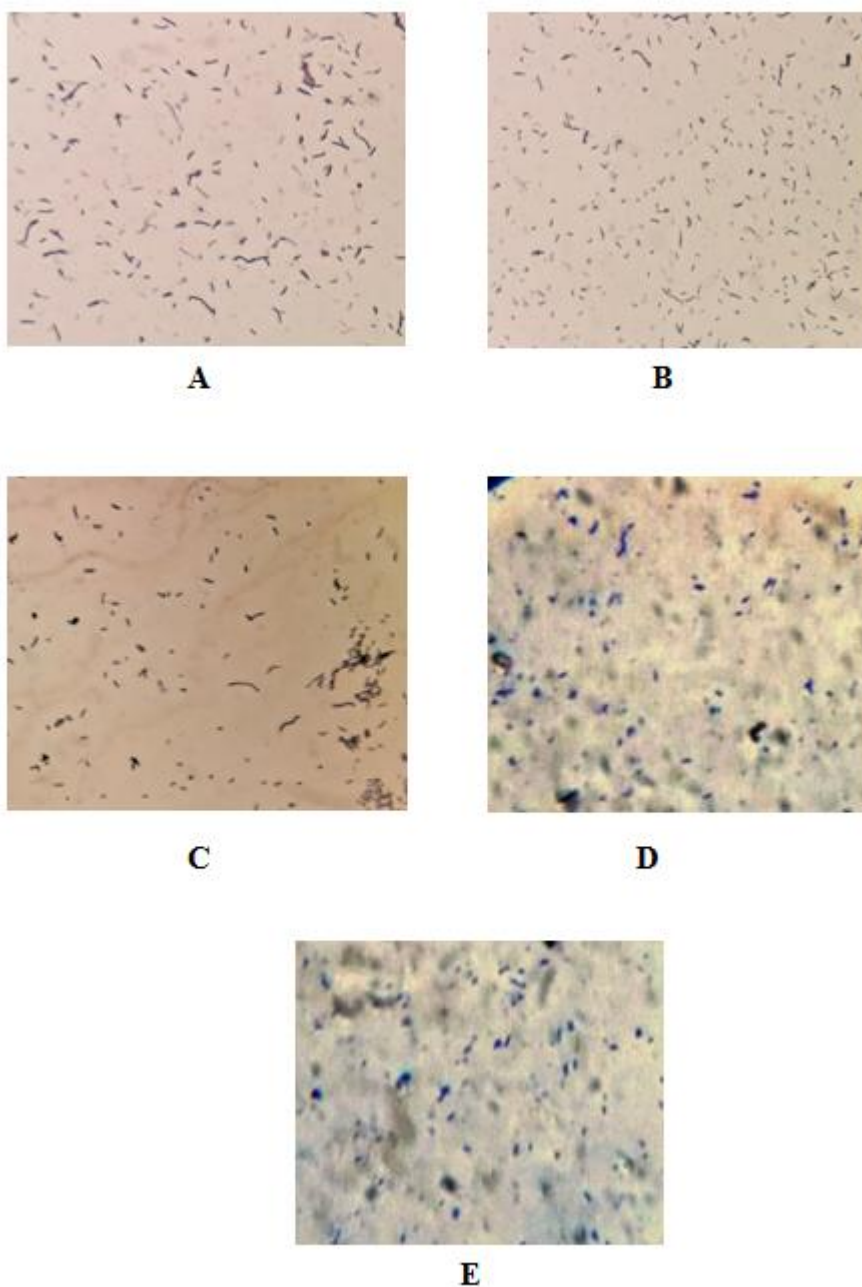


Figure 4.19. Gram staining of endobacterial isolates (A- PP, B- PJ1, C- PJ2, D- OP, E- FS2)

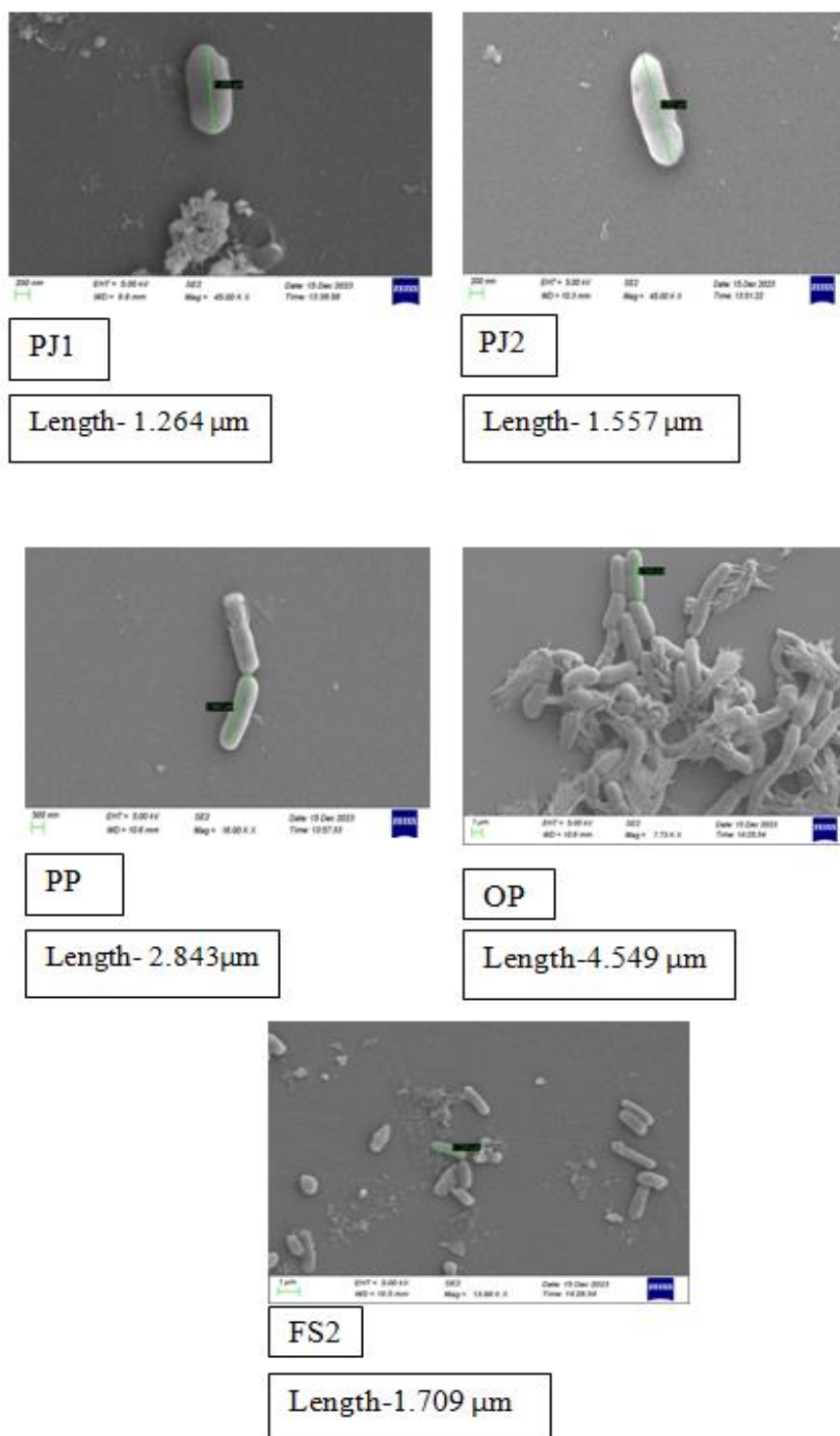


Figure 4.20. Scanning electron microscopic images of endobacterial isolates (PJ1, PJ2, PP, OP & FS2)

Table 4.8. Biochemical characterization of endobacteria

SL No.	Biochemical tests			Endobacterial isolates				
				PP	PJ1	PJ2	OP	FS2
1.	Nitrate reduction			-	-	-	-	-
2.	Oxidase			+	+	+	+	+
3.	Gelatin hydrolysis			-	-	-	-	-
4.	Catalase			+	+	+	+	+
5.	Citrate			-	-	-	-	-
6.	Starch hydrolysis			+	+	-	+	-
7.	Methyl red			-	-	-	-	-
8.	Voges-Proskauer			-	-	-	-	-
9.	Urea hydrolysis			+	-	-	-	-
10.	Indole			+	+	+	+	+
11.	Oxidation - fermentation and acid production from different carbohydrates	Glucose	Turbidity	+	+	+	+	+
			Acid production	+	+	+	+	+
			Gas production	-	-	-	-	-
		Lactose	Turbidity	+	+	+	+	+
			Acid production	-	-	-	-	-
			Gas production	-	-	-	-	-
		Sucrose	Turbidity	+	+	+	+	+
			Acid production	+	+	+	+	+
			Gas production	-	-	-	-	-
12.	Sugar mannitol fermentation			+	-	-	+	-

“+” indicates positive, “-” indicates negative

4.3.4. Growth curve of endobacterial isolates:

Endobacterial isolates were grown in both Nutrient Broth (NB) and Luria Bertani Broth (LB) media. The growth patterns are more or less similar in both NB and LB media. All the endobacterial isolates showed optimum growth at 30 °C temperature and 7.0 pH. Generation time of PP is 6 hrs, PJ1 is 8 hrs, PJ2 is 11 hrs, and OP and FS2 is 4 hrs approximately. The growth patterns of endobacteria are given below (Figure 4.21.A & Figure 4.21.B)

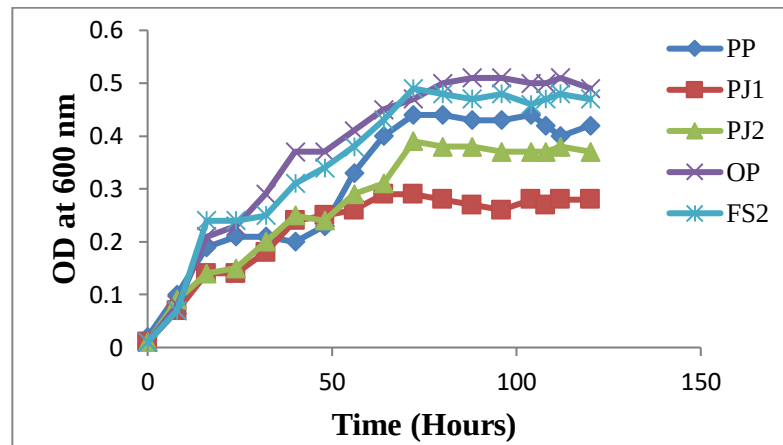


Figure 4.21.A Growth curve of endobacterial isolates in NB

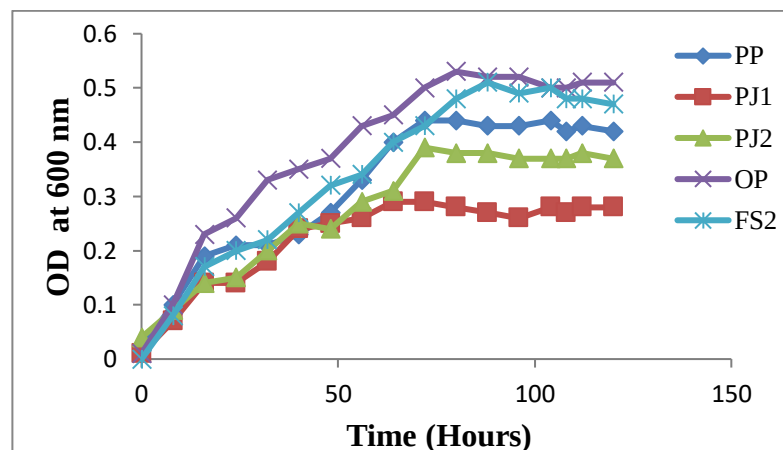


Figure 4.21.B Growth curve of endobacterial isolates in LB

4.3.5.A. Identification of endobacteria based on 16S rRNA sequencing

All the endobacterial isolates were identified by 16S rRNA sequencing. Bacterial genomic DNA was extracted and purified using the CTAB method. BLAST analyses were performed with the contig sequences and then submitted to NCBI to get accession numbers. According to the BLAST description list, every isolate exhibited 97%–99% similarity with the corresponding strains. For PJ1, PJ2, PP, OP, and FS2, their sequences have been assigned accession numbers ON851052, ON870959, ON912004, ON912006, and ON872222 to Gen-Bank, accordingly (Table 4.9). According to 16S rRNA sequencing, *Rossellomorea marisflavi* is closely related to PJ1, PJ2, and FS2; PP is close to *Priestia paraflexa* while OP is close to *Priestia aryabhattai*. The phylogenetic tree was created using the Minimum Evolution approach; the bootstrap test (1000 repetitions) showed that the optimal tree had a sum of branch lengths of 0.65128663 and a percentage of replicate trees where the related taxa clustered together (Figure 4.22).

Table 4.9. 16S rRNA sequencing related information of the isolates

Strain Code	Identified as	Query cover	bp sequenced	Percent identity (%)	E-value	Accession no of most similar strain
PP	<i>Priestia paraflexa</i>	99%	1454	99.24%	0	NR_113800.1
PJ1	<i>Rossellomorea marisflavi</i>	99%	1389	99.78%	0	NR_118437.1
PJ2	<i>Rossellomorea marisflavi</i>	97%	1458	99.65%	0	NR_118437.1
OP	<i>Priestia aryabhattai</i>	95%	1496	99.37%	0	NR_115953.1
FS2	<i>Rossellomorea marisflavi</i>	98%	1463	98.82%	0	NR_118437.1

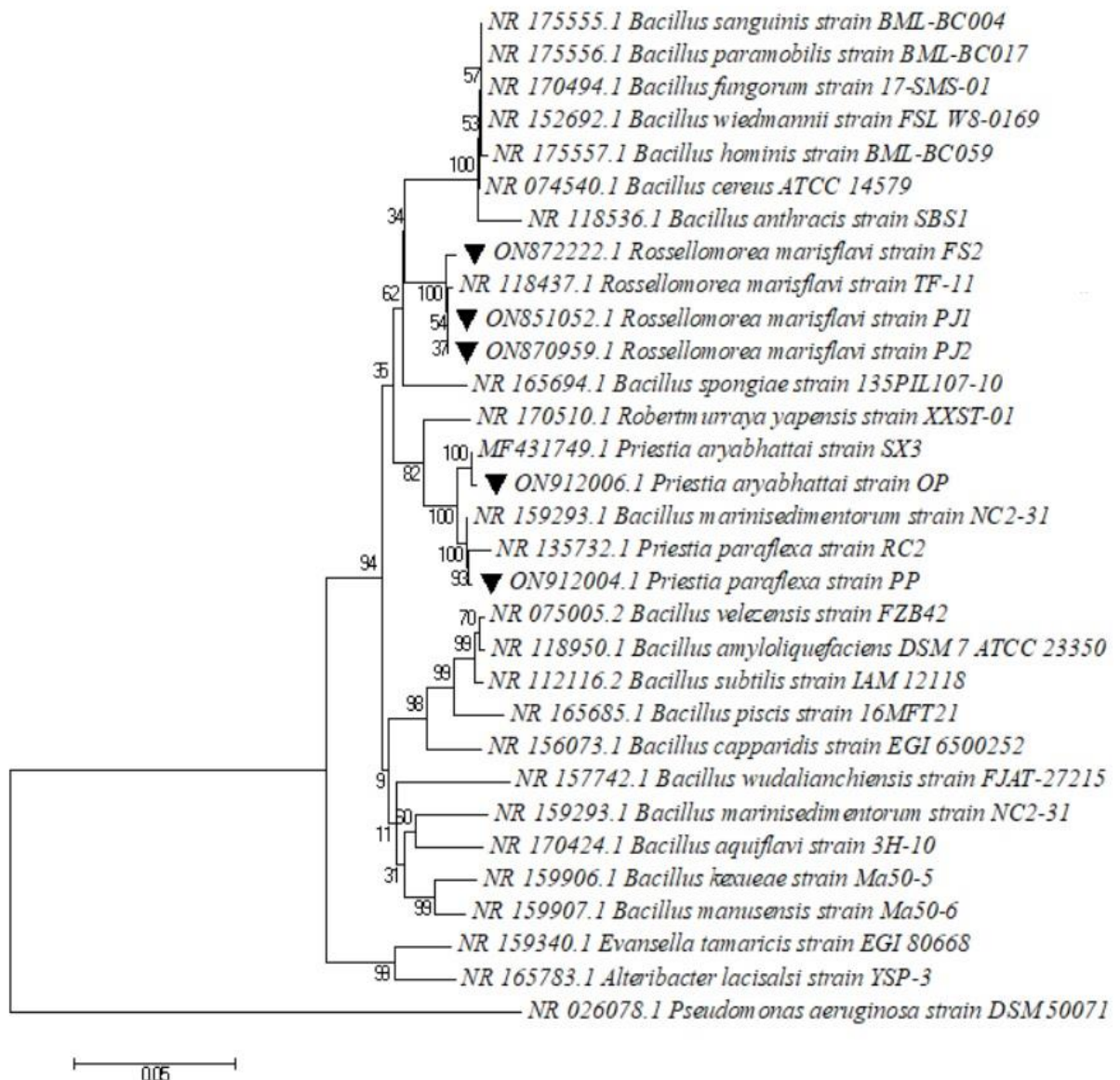


Figure 4.22. Phylogenetic tree of five endobacteria generated using the maximum composite likelihood method with the homologs identified using BLAST analysis. A neighbor-joining phylogenetic tree was constructed to establish the taxonomic placement and evolutionary associations of endobacterial isolates of *Pleurotus* spp. (indicated by black triangles ▼). The study was based on partial 16S rRNA gene sequences, and the tree comprises closely related reference sequences retrieved from the NCBI GenBank database. Bootstrap values (expressed as percentages of 1,000 replicates) are shown at the branch nodes and designate the robustness of each clade; values below 50% are not shown.

4.3.5.B. Submission of endobacterial cultures and sequences

All the five endobacterial strains were deposited in Culture Collections and assigned the strain numbers (Table 4.10). PP, PJ1 and PJ2 were deposited in National Centre for Industrial Microorganisms, NCL, Pune, India and obtained the accession numbers as *Priestia* sp. NCIM 5828, *Rossellomorea marisflavi* NCIM 5829 and *Rossellomorea marisflavi* NCIM 5830, respectively whereas FS2 and OP were deposited to Microbial Type Culture Collections, IMTECH, Chandigarh, India as *Rossellomorea marisflavi* MTCC 13776 and *Priestia aryabhattai* MTCC 13777, respectively.

The 16S rRNA sequences of all these strains are submitted to GenBank and accession numbers are obtained as ON912004, ON851052, ON870959, ON912006 and ON872222 for PP, PJ1, PJ2, OP and FS2, respectively.

Table 4.10. Accession numbers of endobacterial isolates

Strain code	Identified as	GenBank accession number	Name of culture collection	Culture collection accession number
PP	<i>Priestia sp</i>	ON912004	National Centre for Industrial Microorganisms	NCIM 5828
PJ1	<i>Rossellomorea marisflavi</i>	ON851052	National Centre for Industrial Microorganisms	NCIM 5829
PJ2	<i>Rossellomorea marisflavi</i>	ON870959	National Centre for Industrial Microorganisms	NCIM 5830
OP	<i>Priestia aryabhattai</i>	ON912006	Microbial Type Culture Collections	MTCC 13777
FS2	<i>Rossellomorea marisflavi</i>	ON872222	Microbial Type Culture Collections	MTCC 13776

4.3.7. Production of lytic enzymes by endobacteria:

Cellulase, chitinase and pectinase enzyme activities were tested in all the endobacteria. Isolates PP and OP showed cellulase, chitinase and pectinase activities but other strains didn't show the activity of any of these three enzymes (Figure 4.23 and Table 4.11).

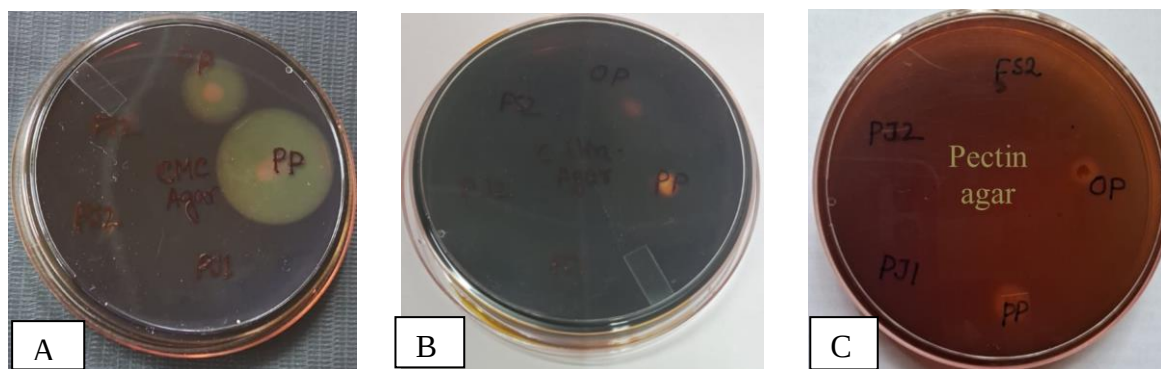


Figure 4.23 Different enzyme activity of five endobacterial isolates (A. Cellulase, B. Chitinase, and C. Pectinase)

Table 4.11. Different enzyme activity of endobacterial isolates

Endobacterial strains	Enzyme activity		
	Cellulase	Chitinase	Pectinase
PP	+++	+	+
PJ1	-	-	-
PJ2	-	-	-
OP	++	Weak	+
FS2	-	-	-

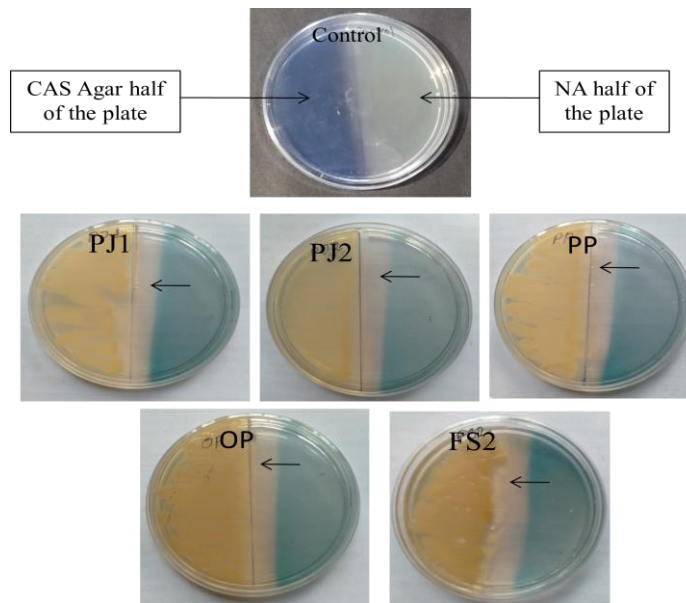
“+++” indicates excellent activity, “++” indicates very good activity, “+” indicates good activity, “-” indicates no activity.

4.3.8. Comparisons of growth promoting traits of the endobacteria

All of the tested five isolates showed *in vitro* growth promoting activities. All the isolates produced good amount of IAA, siderophore and ammonia but didn't show phosphate solubilizing activity (Table 4.12 and Figure 4.24). IAA was produced in presence of precursor molecule tryptophan (Trp). Both qualitative and quantitative tests were done. Highest IAA produced by PJ1 (45 µg/ml) followed by FS2 (40 µg/ml) (Figure 4.24.C). All the strains also showed the ability to produce IAA in a Trp-deficient medium. For siderophore production assay CAS agar-NA plates were used where petriplates divided into two halves; one half contains nutrient agar and other half contain CAS agar. Each bacterial strain was grown on nutrient agar portion and the discharge of blue colouration on the CAS half of the plate was regarded a positive response to siderophore production. Highest siderophore activity was found in FS2 (4.1 mm/day) followed by PJ2 (4 mm/day) and least in PJ1 (2.9 mm/day) (Fig 4.24.A). For ammonia production test qualitative assay was done. All the strains also produced ammonia as the reaction mixture turned to deep yellow (4.24.B).

Table 4.12. Some growth promoting traits of the endobacteria

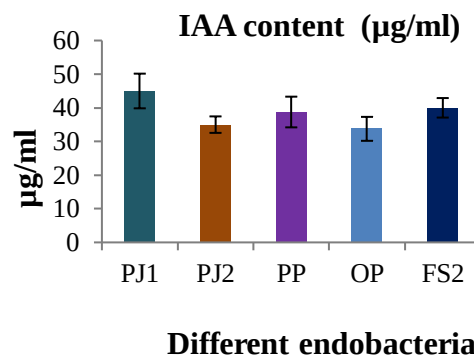
Growth promoting traits	Endobacteria				
	PJ1	PJ2	PP	OP	FS2
IAA production (µg/ml)	45	35	38.75	33.75	40
Siderophore production (mm/Day)	2.9	4	3.1	3	4.1
NH ₃ production	+	+	+	+	+
Phosphate solubilization test	-	-	-	-	-



4.24.A. Siderophore production assay by observing the discharge of blue colour on the CAS-agar half of the plate. The black arrow in the pictures indicate discharge of blue colouration in the CAS-agar (blue coloured) half of the plate. No such discharge is seen in the control plate.



4.24.B. Ammonia production



4.24.C Graphical representation showing the IAA content (µg/ml) of different endobacteria

Figure 4.24. Some growth promoting activities of endobacteria (4.24.A. Siderophore production ; 4.24.B. Ammonia production; 4.24.C. Graphical representation showing the IAA content (µg/ml) of different endobacteria)

4.3.9. Cytotoxic activity of endobacteria

All the five endobacteria didn't show hemolysis on blood agar plate (Figure 4.25. A) but when MTT assay was done using endobacterial cell lysates on Caco-2 human cancer cell line, only the PJ1 cell lysate decreased cell viability at high concentration (Figure 4.25.B).

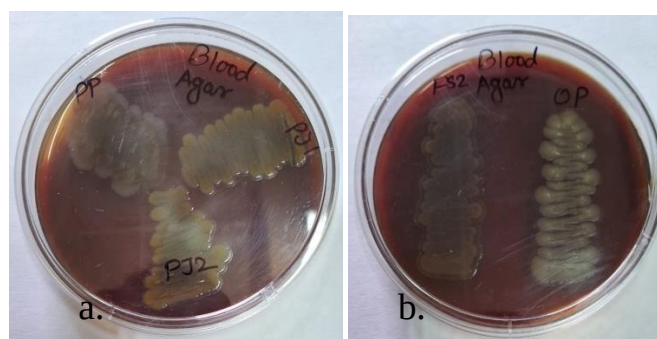


Figure 4.25.A. Blood agar test of endobacteria (a- PP, PJ1 and PJ2; b- OP and FS2)

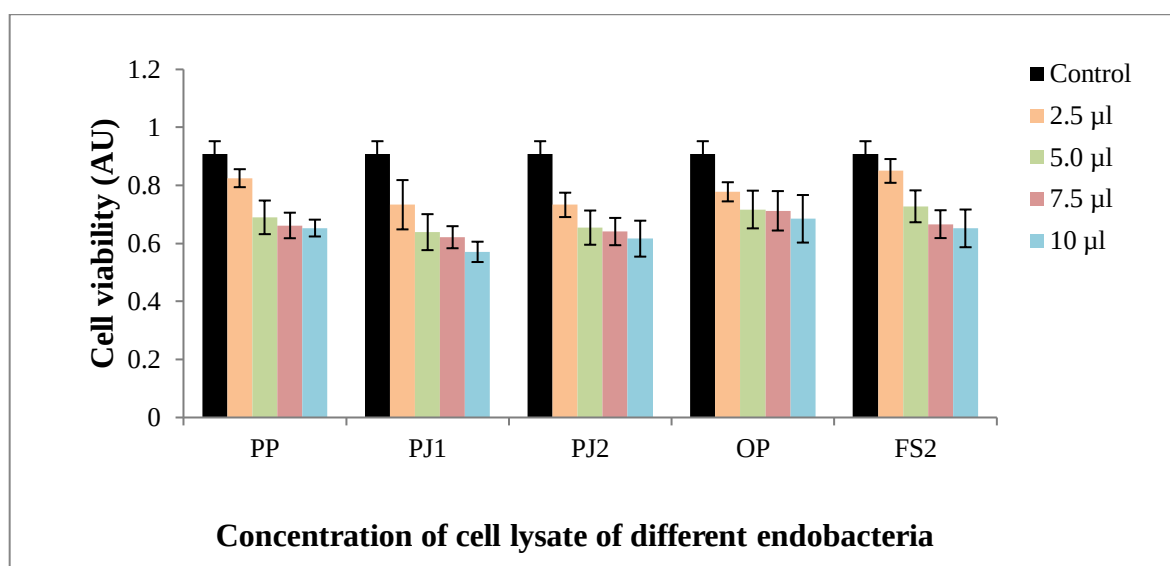


Fig 4.27.B. Cell cytotoxicity (MTT) assay of five endobacterial [PP, PJ1, PJ2, OP and FS2] cell lysate (2.5, 5, 7.5 and 10 µL) on Human Embryonic Kidney 293 (HEK 293) cell line

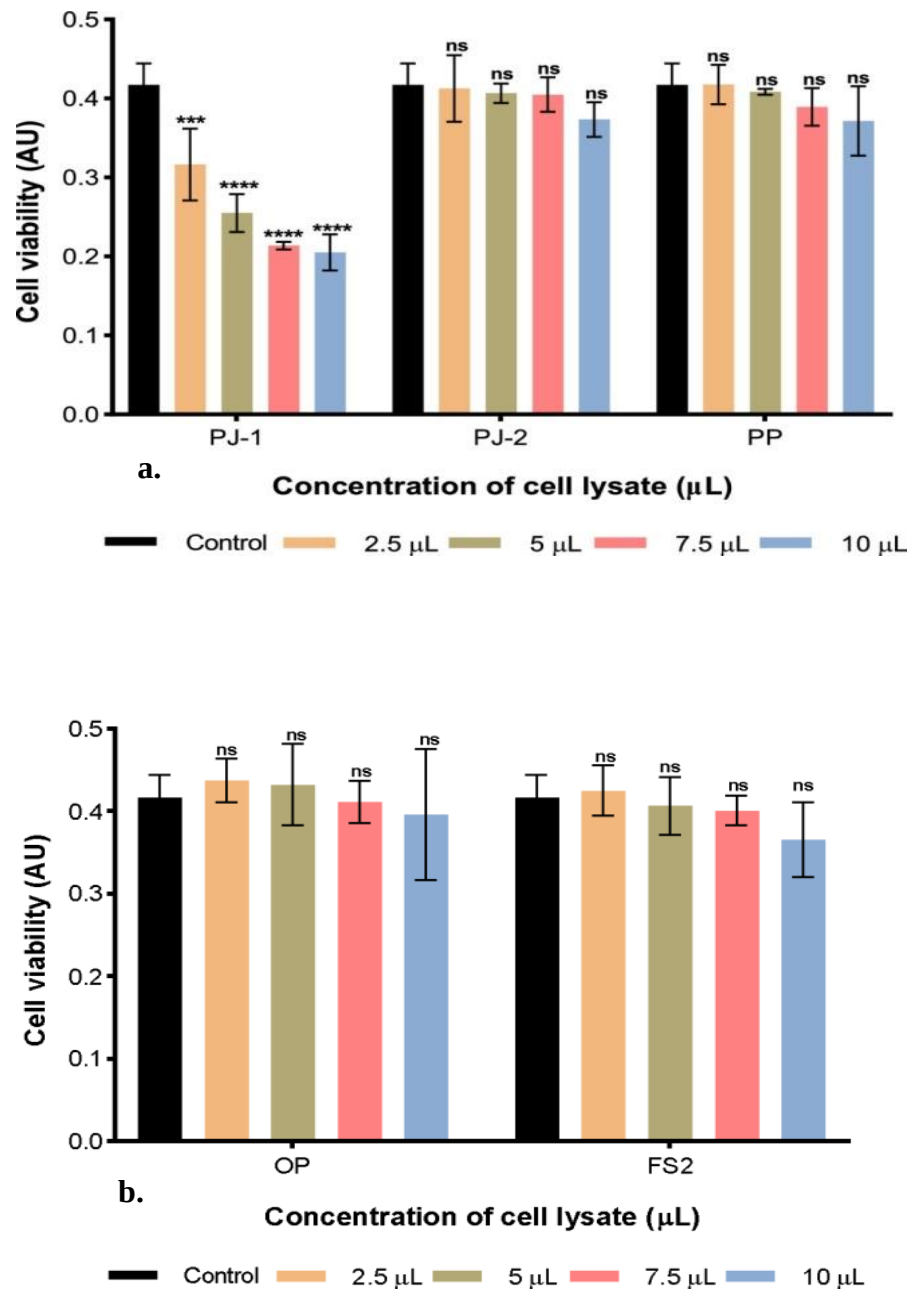


Figure 4.25.C. Cell cytotoxicity (MTT) assay of five endobacterial [a. PP, PJ1, PJ2; b. OP and FS2] cell lysate (2.5, 5, 7.5 and 10 μL) on Caco-2 human cancer cell line (a- PJ1, PJ2 & PP; b- OP & FS2). Mean values in each column do not differ significantly at $p < 0.05$. Comparison between control and treatment mean values was made by Sidak's multiple comparisons test. Where, "**" = $p < 0.0001$, "****" = $p < 0.001$, "*" = $p < 0.1$ and "ns" = data is non significant. Data were subjected to analysis of variance (ANOVA) test by using the GraphPad Prism 8.0.1 version software.**

4.3.6. Antibiotic sensitivity test:

In the present study twelve common antibiotic discs were taken for antibiotic sensitivity test. All the five bacteria showed sensitivity against all these twelve antibiotics. All the strains have identical response to each of the antibiotics though the extents of zone of inhibition vary from strain to strain (Table 4.13 and Figure 4.26).

Table 4.13. Antibiotic sensitivity test of endobacteria

ANTIBIOTIC SENSITIVITY					
Antibiotics	Diameter of inhibition zone (cm) of endobacteria				
	OP	PP	PJ1	PJ2	FS2
Amoxy/ Clav (Augmentin) AG (30 µg)	2	1.5	2.5	2.5	3.2
Tetracycline TE (30 µg)	2.2	2	2	2	2.2
Ciprofloxacin RC (5 µg)	2.5	2.5	3	3	3.2
Streptomycin S10/SM (10 µg)	2.3	2	2.2	2.5	2.5
Nitrofuranton FD (300 µg)	1.7	1.7	2	2	1.7
Imipenem IM (10 µg)	3	2.5	3.5	3.2	3
Meropenem MP (10 µg)	3.2	2.5	3.2	3.2	3
Co-trimoxazole BA (25 µg)	3.5	3	3	3	3.2
Vancomycin VA (30 µg)	2	2	2	2	2.2
Cefoperazone/Sulbactam CM (105 µg)	2.7	2	3.5	3.2	3.5
Chloramphenicol CH (30 µg)	3	2.5	2.5	2.5	2.5
Linezolid LZ (30 µg)	3	3.2	3	3	3.2

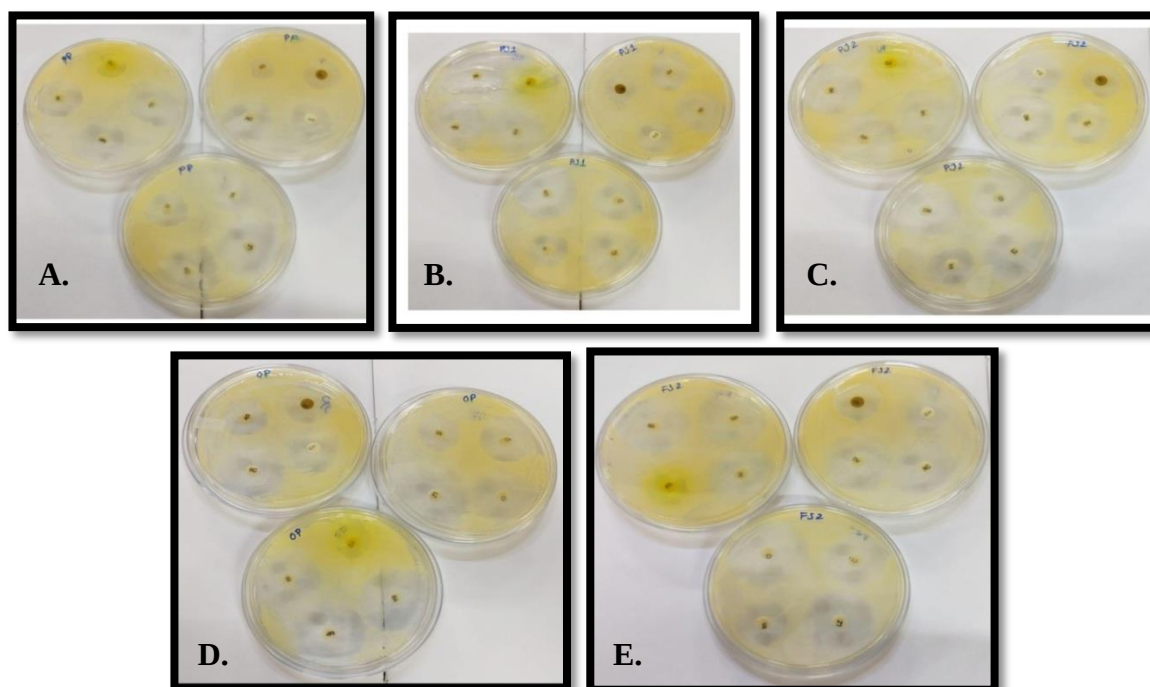


Figure 4.26. Antibiotic sensitivity test of endobacterial isolates (A- PP, B- PJ1, C- PJ2, D- OP, E- FS2)

Conclusion:

- A number of endobacteria were isolated from three tested *Pleurotus* species
- Within these endobacteria, five isolates PP, PJ1, PJ2, OP and FS2 were eventually selected for further studies on the basis of their *in vitro* growth promoting activities.
- PP was isolated from pileus and PJ1 and PJ2 were isolated from junction of the pileus and stipe of *P. pulmonarius*, OP was isolated from pileus of *P. ostreatus* whereas FS2 was isolated from stipe of *P. florida*.
- All of the five isolates produced good amount of IAA, Siderophore and ammonia which indicated their growth promoting capabilities.
- The biochemical and 16s rRNA sequencing studies indicated PJ1, PJ2 and FS2 as different strains of *Rossellomorea marisflavi* whereas PP and OP as two different species of *Priestia*.
- The 16S rRNA sequences of all these strains are submitted to GenBank and accession numbers are obtained as ON912004, ON851052, ON870959, ON912006 and ON872222 for PP, PJ1, PJ2, OP and FS2, respectively.
- PP, PJ1 and PJ2 were deposited in National Centre for Industrial Microorganisms, NCL, Pune, India and obtained the accession numbers as *Priestia* sp. NCIM 5828, *Rossellomorea marisflavi* NCIM 5829 and *Rossellomorea marisflavi* NCIM 5830, respectively whereas FS2 and OP were deposited to Microbial Type Culture Collections, IMTECH, Chandigarh, India as *Rossellomorea marisflavi* MTCC 13776 and *Priestia aryabhattai* MTCC 13777, respectively.

4.4. Determination of the role of endobacteria in growth, development, and nutraceutical compositions of *Pleurotus* spp.

4.4.1. Effect of bacterial supplementation on mycelial-growth promotion in plate culture:

In dual culture when *P. pulmonarius* supplemented with endobacteria PJ1 or PJ2 separately, 10 days were required to full cover the mycelia on the Petriplates (90 mm in diameter) whereas the control set (without endobacterial supplementation) required 12 days. When supplemented with endobacteria PP, 9 days were required to cover the mycelia on the plate. Mycelial growth rate of *P. pulmonarius* in the presence of PJ1 and PJ2 was 26.51 mm²/hr but in the presence of PP the growth rate was 29.46 mm²/hr whereas the growth rate of control set was 22 mm²/hr. When *P. ostreatus* supplemented with OP, 11 days was required to fully cover the mycelia whereas control set required 12 days. OP treated plate showed more denser mycelia than the control set. Mycelial growth rate *P. ostreatus* in presence OP was 24.10 mm²/hr whereas the growth rate of control set was 22 mm²/hr. When *P. florida* supplemented with FS2, 10 days was required to full cover the mycelia on the Petriplates whereas the control set required 12 days. The mycelial growth rate *P. florida* in presence FS2 was 26.51 mm²/hr whereas the growth rate of control set was 22 mm²/hr (Figure 4.27). The growth rate in terms of hyphal extension was maximum in PP (33.9%). OP showed minimum (9.23%) whereas PJ1, PJ2 and FS2 showed intermediate growth increase (20.5%) in solid agar plate culture.

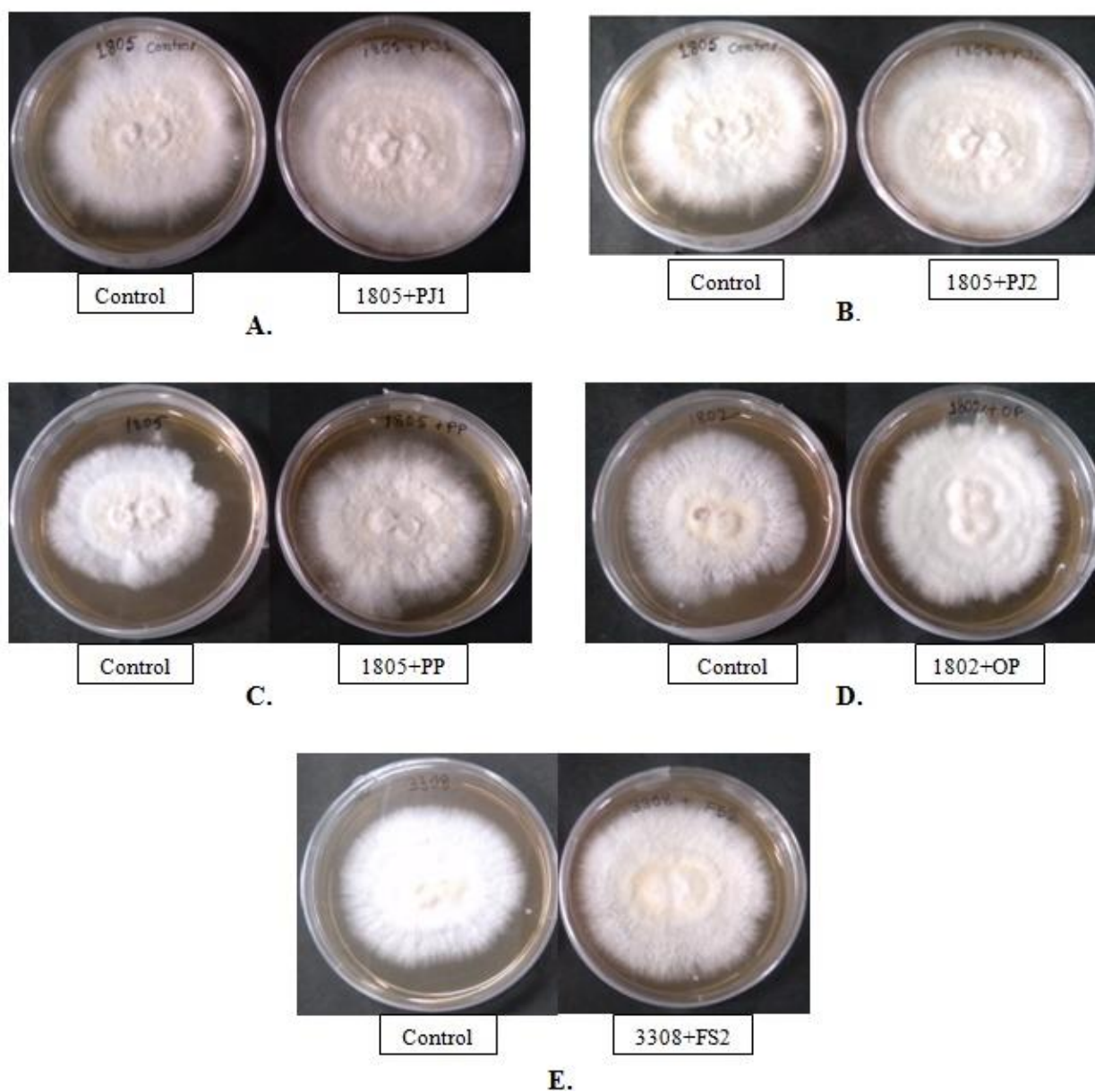


Figure 4.27. MTCC 1805 grown in presence of endobacteria PJ1, PJ2 & PP, respectively (A, B, C); MTCC 1802 (D) and ITCC 3308 (E) grown with endobacteria OP & FS2, respectively.

4.4.2. Study of growth of mycelial biomass in presence of endobacteria:

In submerged fermentation, supplementation of all the five bacterial isolates individually influenced the mycelial growth rate, as evidenced by increased mycelial dry weight. Highest biomass was found when MTCC 1805 supplemented with PP and PJ2, respectively. Lowest biomass was found in MTCC 1802 (Figure 4.28). The highest mycelial dry weight increase was found in FS2 supplemented *P. florida* (146%). The other bacteria increased the growth of respective hosts more or less similarly (86-90%).

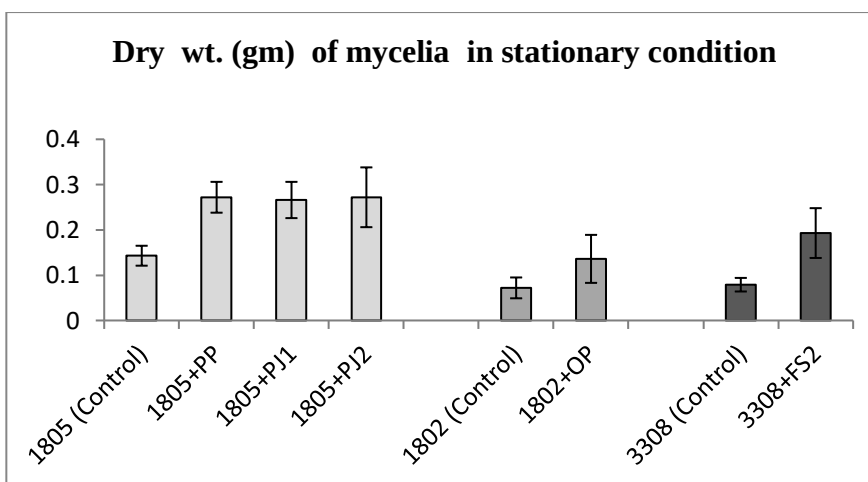


Figure 4.28. Bar graph showing the dry weight of mycelia of MTCC 1805, MTCC 1802 and ITCC 3308. Mushroom mycelia are grown in submerged culture in presence of respective endobacteria. The results were taken after 12 days of growth in stationary condition.

4.4.3. Laccase activity in presence of endobacteria:

All three mushroom strains produced laccase in PD media but none of the endobacteria produced laccase in PD, NB or LB media. When mushroom mycelia were supplemented with their respective endobacteria they produce more laccase than their control set up (without bacterial supplementations). *P. ostreatus* showed highest laccase activity (206

U/ml) on day 7 in OP supplemented set up whereas the control set showed 21 U/ml laccase activity (Figure 4.29.B). *P. pulmonarius* showed highest activity on day 9 after bacterial supplementation with their respective endobacteria. In *P. pulmonarius* maximum laccase was produced when supplemented with PJ2 (190.26 U/ml) followed by PJ1 (145.69 U/ml) and PP (135.9 U/ml) in contrast to control set (77.98 U/ml) (Figure 4.29.A). *P. florida* showed 190.44 U/ml laccase activity under supplementation of endobacteria FS2 whereas the control set produced 57.27 U/ml enzyme on day 8 (Figure 4.29.C). Figure 4.29.D showed the Zymogram of laccase using guaiacol as substrates.

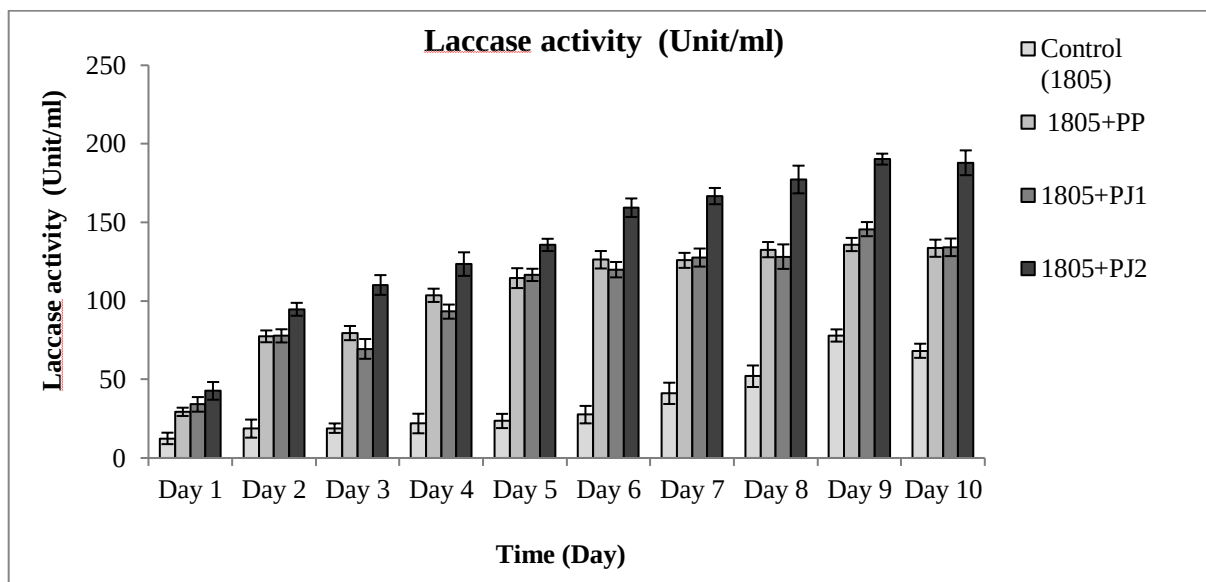


Figure 4.29.A. Laccase activity of *P. pulmonarius* in supplementation of endobacteria (PP, PJ1 and PJ2)

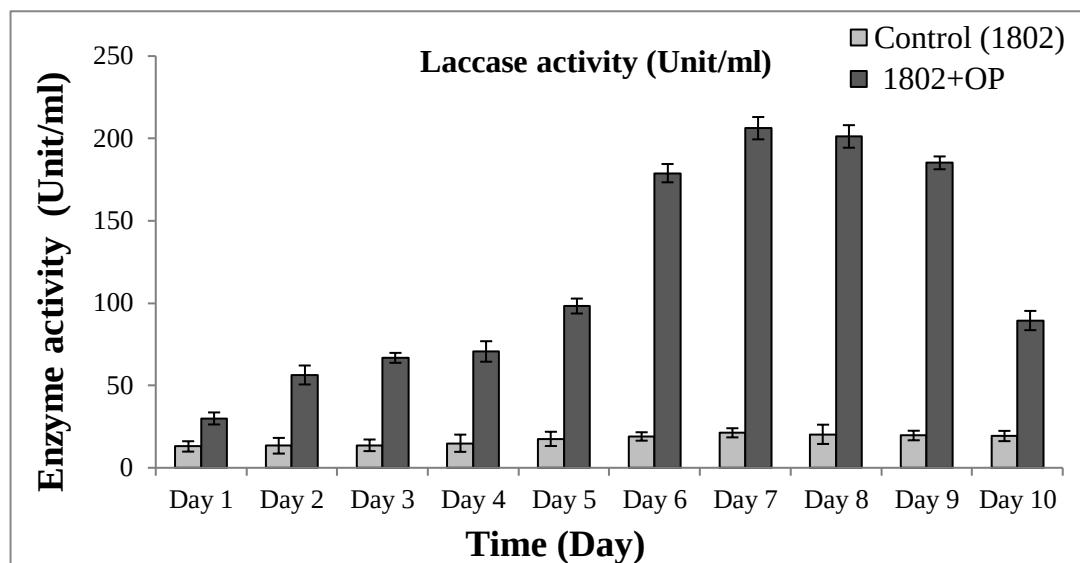


Figure 4.29.B. Laccase activity of *P. ostreatus* in supplementation of endobacteria (OP)

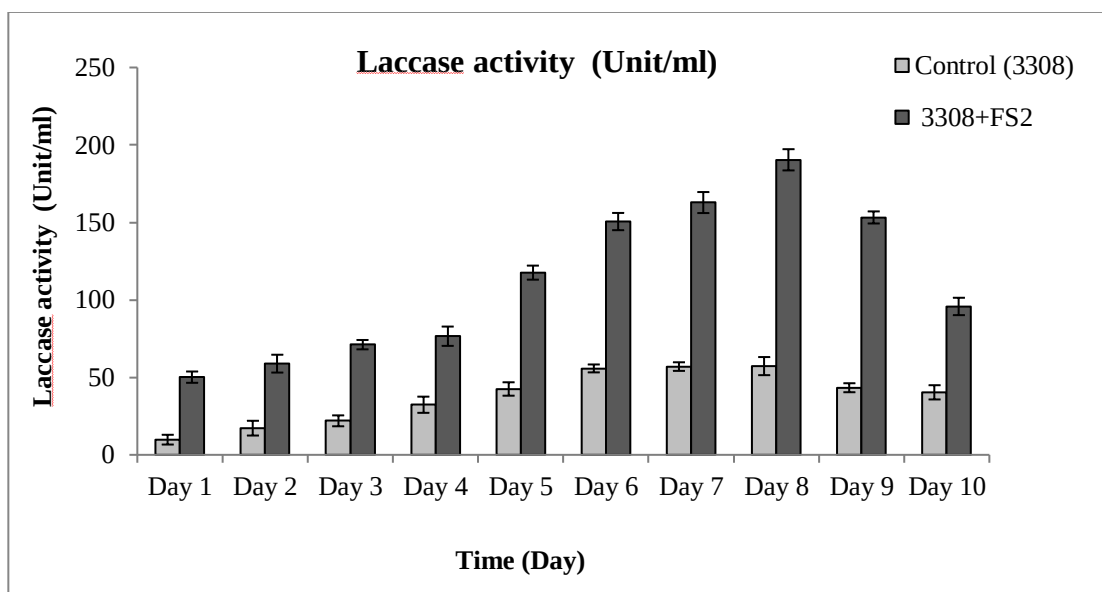


Figure 4.29.C. Laccase activity of *P. florida* in presence of endobacteria (FS2)

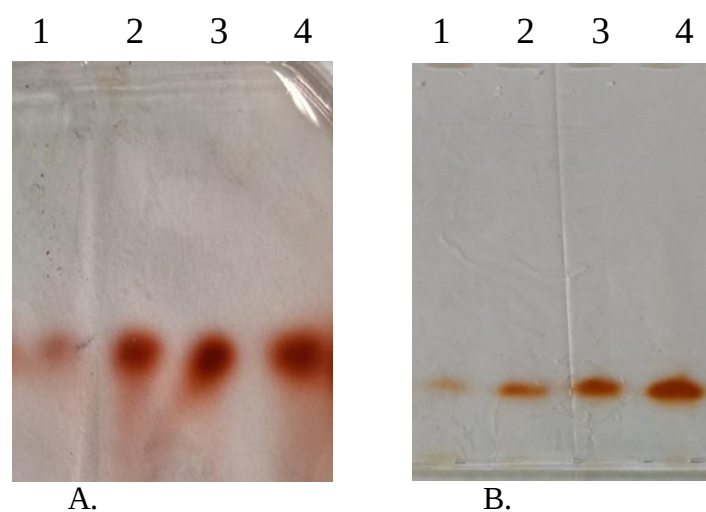


Figure 4.29.D. Zymogram of laccase. A. lane 1- 1805, lane 2- 1805+PJ1, lane 3- 1805+PJ2, lane 4- 1805+PP; B. lane 1- 3308, lane 2- 3308+FS2, lane 3- 1802, lane 4- 1802+OP

4.4.4. Biological efficiency of *Pleurotus* spp in presence of endobacteria at different concentration:

MTCC 1805, MTCC 1802 and ITCC 3308 strains were supplemented with their respective endobacteria at different concentrations. The concentrations per 100 gm dry substrates (Rice straw) were 0.5×10^8 , 0.8×10^8 , 1×10^8 , 3×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 & 1×10^9 CFU. Maximum BE was observed at 8×10^8 concentration for endobacteria PP, PJ1 & PJ2 and 1×10^8 concentration for endobacteria OP and FS2. In *P. pulmonarius* maximum BE (68%) was recorded in PJ2 supplemented sets followed by PJ1 (57.66%) and PP (56%) whereas control set showed BE of about 42.3% (Figure 4.30). OP supplemented *P. ostreatus* showed 62% of biological efficiency whereas control set showed 53.45% BE (Figure 4.31). FS2 supplemented *P. florida* showed 73.5 % BE whereas control set showed only 60.5 % BE (Figure 4.32). So, subsequent studies were done using 8×10^8 cells per 100 gm substrates (dry) for PP, PJ1 & PJ2 and 1×10^8 cells per 100 gm substrates (dry) for OP and FS2

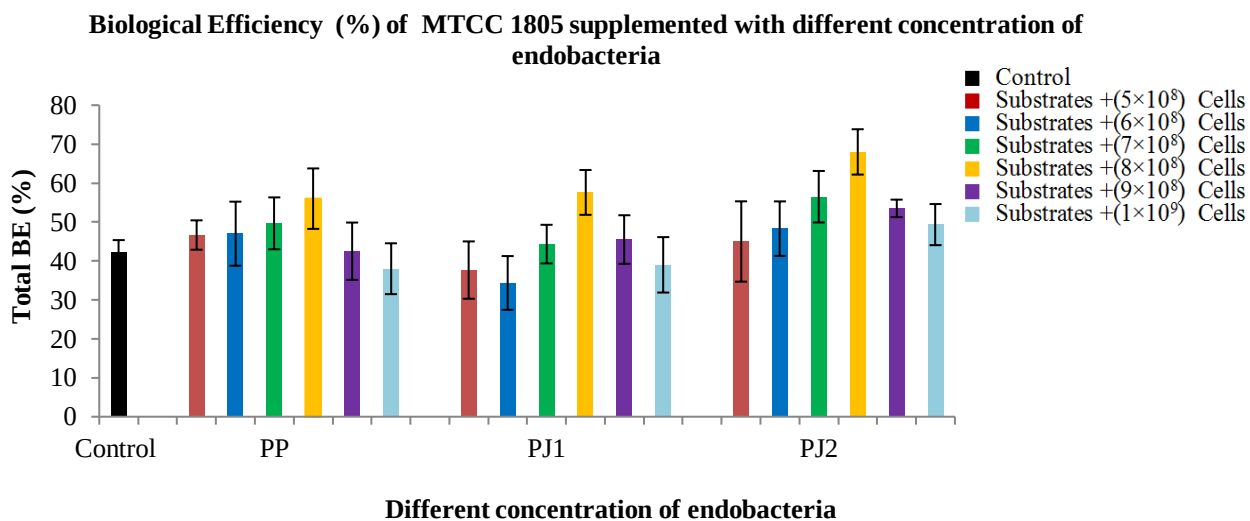


Figure 4.30 BE of *P. pulmonarius* (MTCC 1805) supplemented with different concentration of endobacteria (PP, PJ1 and PJ2)

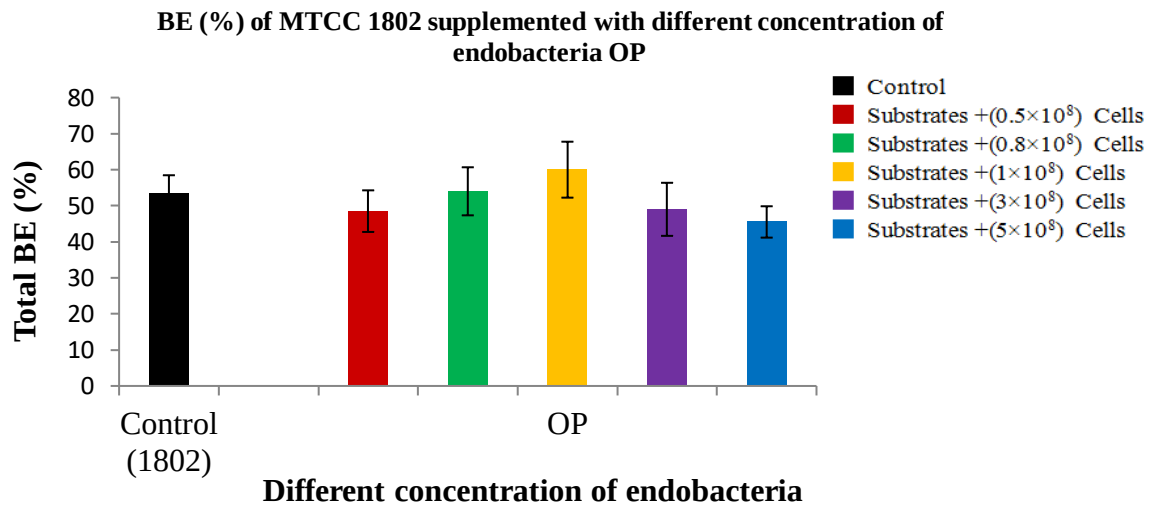


Figure 4.31. BE of *P. ostreatus* supplemented with different concentration of endobacteria (OP)

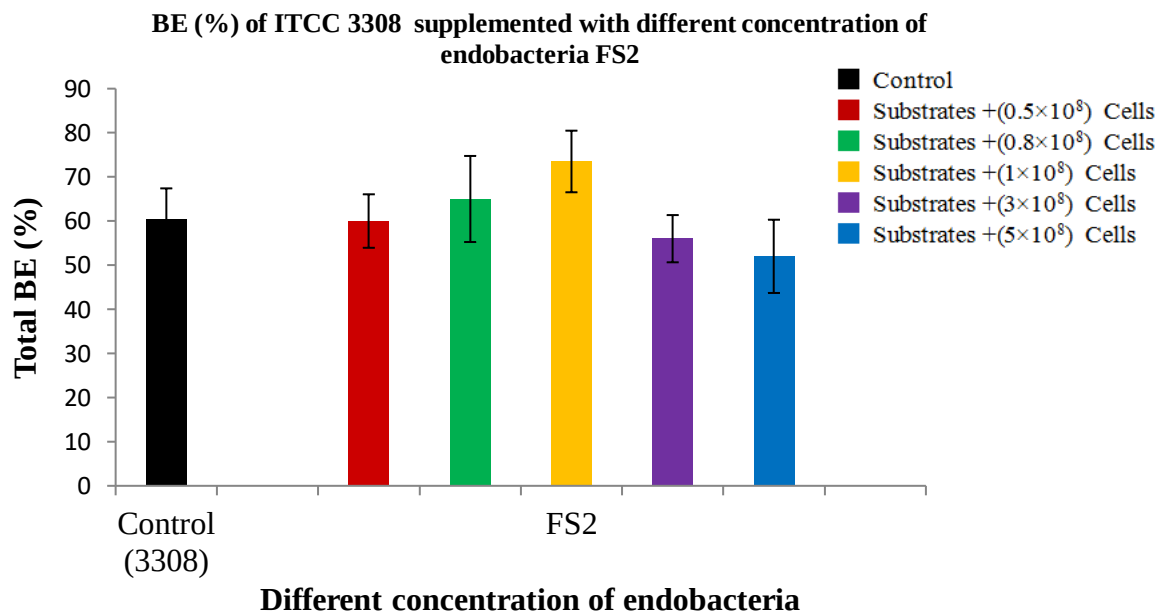


Figure 4.32. BE of *P. florida* supplemented with different concentration of endobacteria (FS2)

4.4.5. Study of fruiting body production in presence of endobacteria:

In all the bacterial supplementations fruiting bodies production were enhanced in all the tested oyster mushroom species irrespective of their incorporation with spawn, substrate or both spawn and substrate (Figure 4.33.A, 4.33.B, 4.33.C).

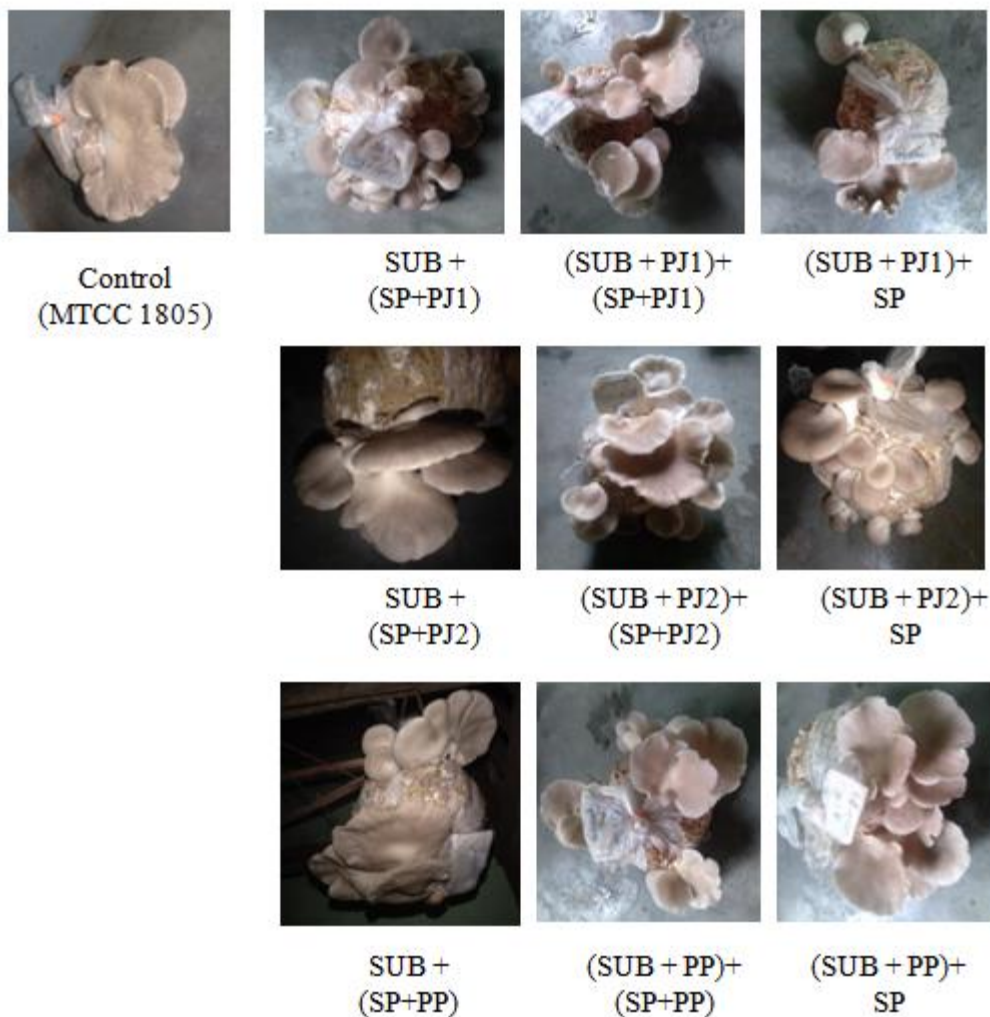


Figure 4.33.A. Fruiting body production of *P. pulmonarius* in supplementation of endobacteria (PJ1, PJ2 and PP). SUB=Substrates, SP= Spawn



Figure 4.33.B. Fruiting body production of *P. ostreatus* in supplementation of endobacteria (OP). SUB=Substrates, SP= Spawn

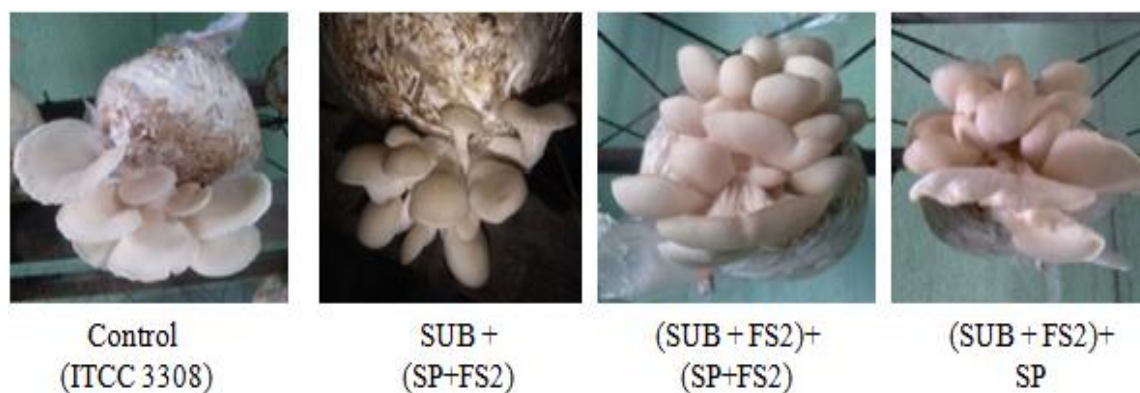


Figure 4.33.C. Fruiting body production of *P. florida* in supplementation of endobacteria (FS2). SUB=Substrates, SP= Spawn

4.4.6. Biological efficiency (BE) of *P. pulmonarius*, *P. ostreatus* and *P. florida* growing in presence of different supplementations:

All the endobacterial isolates enhanced biological efficiencies of the tested three species of oyster mushrooms under all the supplementations. When endobacteria PP was mixed with the spawn 47% BE was increased, when PJ2 was mixed with rice-straw substrates 64% BE was increased, in comparison to control set (Figure 4.34 & Table 4.14). In case of endobacteria OP 24% BE was increased when mixed with both substrate and spawn and in case of endobacteria FS2 26% BE was increased when mixed with only substrate in comparison to control set (Figure 4.35, 4.36 & Table 4.14). In Figures 4.34, 4.35, and 4.36, the p-value for the t-test result is displayed at the top of each bar. According to the results of the analysis, the treatment groups outcomes (PJ2 + SUB), [(PJ1 + SP) + (PJ1 + SUB)], [OP + SP) + (OP + SUB)], (FS2 + SUB), and [(FS2+ SP) + (FS2 + SUB)] differ significantly from those of the corresponding control groups (p-values 0.0412, 0.0465, 0.0187, 0.0143, and 0.0136, respectively). Time required for fruiting in all combinations are given in table 4.14.

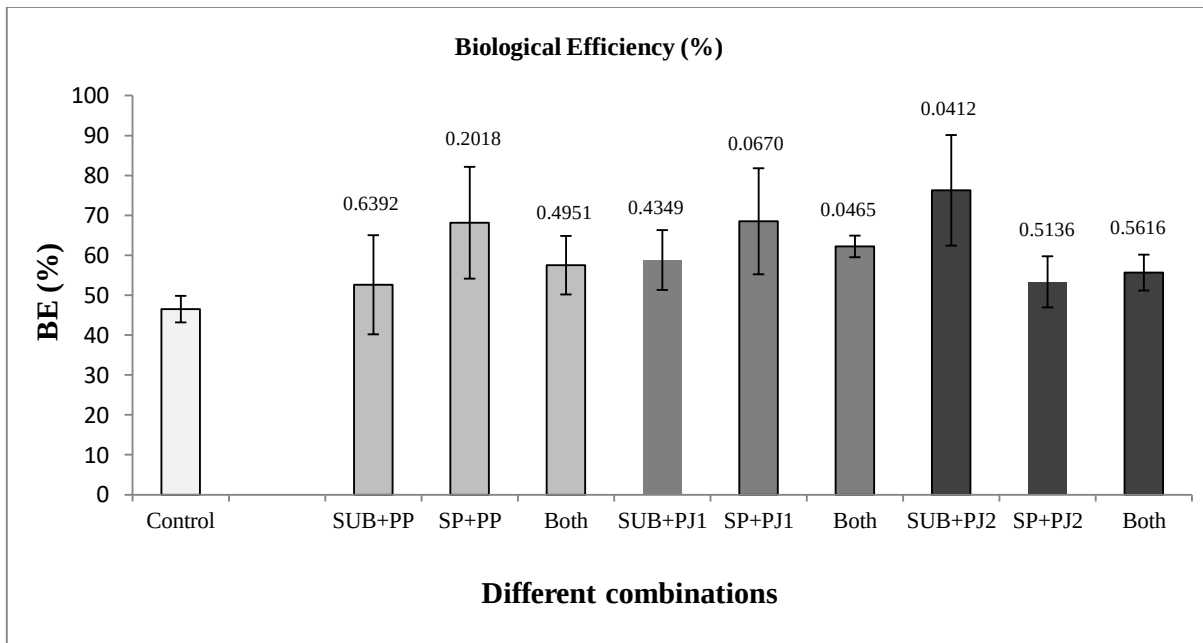


Figure 4.34. The bar graph showing the Biological efficiency of *P. pulmonarius* growing on different combinations using 10% spawn. p-values are given at the top of the error bar. 8×10^8 endobacterial cells were used for supplementation. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate. In the bar diagram, the p-value for the t-test result is displayed at the top of each bar.

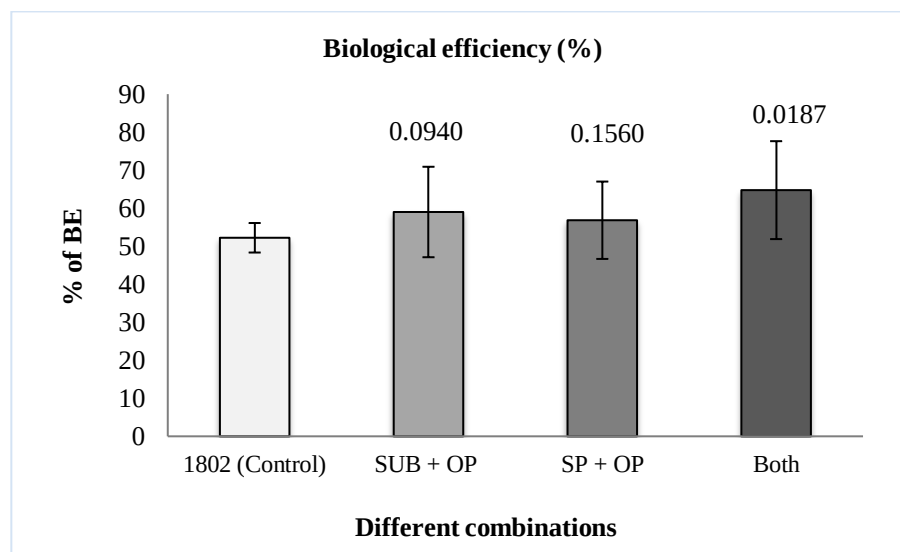


Figure 4.35. The bar graph showing the BE of *P. ostreatus* growing on different combinations. p-values are given at the top of the error bar. 1×10^8 endobacterial cells were used for supplementation. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate. In the bar diagram, the p-value for the t-test result is displayed at the top of each bar.

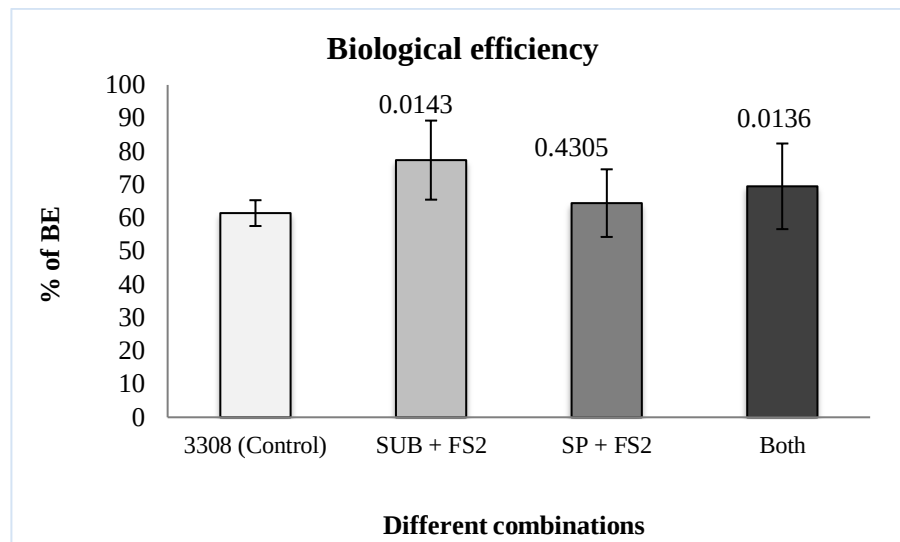


Figure 4.36. The bar graph showing the BE of *P. florida* growing on different combinations. using 10% spawn. p-values are given at the top of the error bar. 1×10^8 endobacterial cells were used for supplementation.

SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate. In the bar diagram, the p-value for the t-test result is displayed at the top of each bar.

Table 4.14. Comparison of increased BE and fruiting time in presence of different endobacterial- supplementation combinations

Host mushrooms	Treatment	Increase in total BE (%)	Average fruiting period (Days)
<i>Pleurotus pulmonarius</i> (MTCC 1805)	Control		23.7±1.50
	Spawn+PP	46.51	22.3±2.17
	Substrate+PP	13.11	23.4±4.79
	(Spawn+ PP)+(Substrate+PP)	23.65	24.7±2.41
	Spawn+PJ1	47.31	22.24±2.60
	Substrate+PJ1	26.45	24.1±2.35
	(Spawn+ PJ1)+(Substrate+PJ1)	33.76	23.2±2.15

Host mushrooms	Treatment	Increase in total BE (%)	Average fruiting period (Days)
<i>Pleurotus pulmonarius</i> (MTCC 1805)	Spawn+PJ2	14.68	24.7±3.30
	Substrate+PJ2	63.97	23.5±2.71
	(Spawn+ PJ2)+ (Substrate+PJ2)	19.68	22.1±1.80
<i>Pleurotus ostreatus</i>	Control		27.3±2.50
	Spawn+OP	8.82	26.5±1.62
	Substrate+OP	12.98	24.6±3.21
	(Spawn+ OP)+ (Substrate+OP)	23.99	25.1±2.15
<i>Pleurotus florida</i>	Control		25.8±2.40
	Spawn+ FS2	4.88	24.9±3.29
	Substrate+FS2	25.94	24.3±2.45
	(Spawn+ FS2)+ (Substrate+FS2)	13.11	25.2±1.95

4.4.7. Analysis of nutraceuticals in sporophores of oyster mushrooms developed in supplementations of endobacteria:

Supplementation of all the endobacterial isolates enhanced the nutraceutical contents than their respective control set. In *P. pulmonarius* highest protein content was recorded when both substrates and spawn was supplemented with all the three endobacteria individually. Among all the three endobacteria- supplemented set highest result has been found in PJ2 (66 µg/mg dry weight) followed by PJ1 (47.4 µg/mg dry weight) and lowest in PP (47 µg/mg dry weight). Control set showed 15.06 µg/mg dry weight (Figure 4.37). In *P. florida* and *P. ostreatus*, highest protein contents were found when FS2 (29 µg/mg dry weight) or OP (33 µg/mg dry weight) was supplemented with only substrates (Figure 4.38 & 4.39). Carbohydrate content was highest in *P. pulmonarius* when PP was supplemented in both spawn and substrate (260 µg/mg dry weight) followed by PJ2 supplemented with both spawn and substrate (131.6 µg/mg dry weight) whereas the control set showed only 51.3 µg/mg dry weight of carbohydrate (Figure 4.40). In 1802 and 3308, highest carbohydrate contents were found when OP and FS2 were supplemented with substrates and the values are 117 µg/mg dry weight and 98 µg/mg dry weight respectively (Figure 4.41 and 4.42).

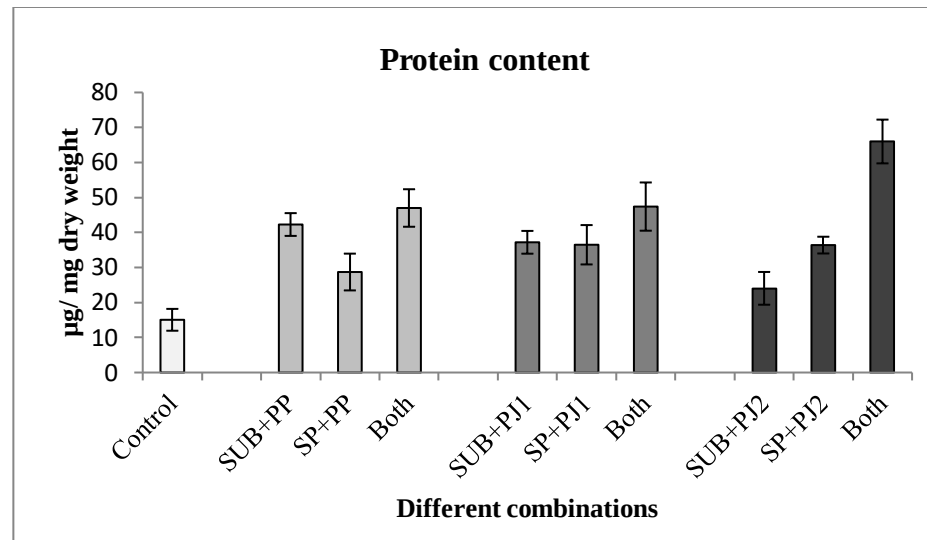


Figure 4.37. Graphical illustration showing the Protein contents of *P. pulmonarius* supplemented with isolates PP, PJ1 and PJ2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

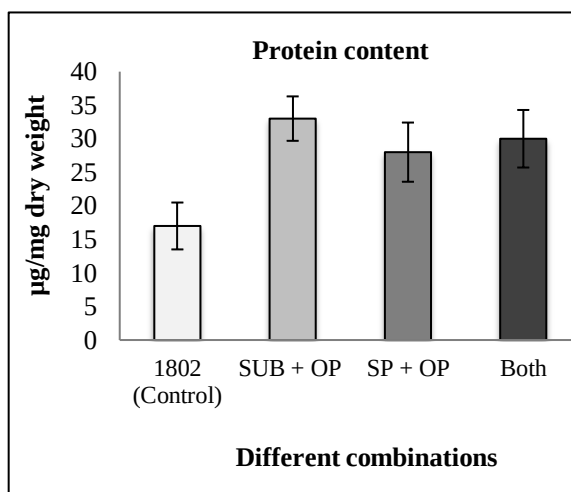


Figure 4.38. Graphical illustration showing the Protein content of *P. ostreatus* supplemented with isolate OP in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

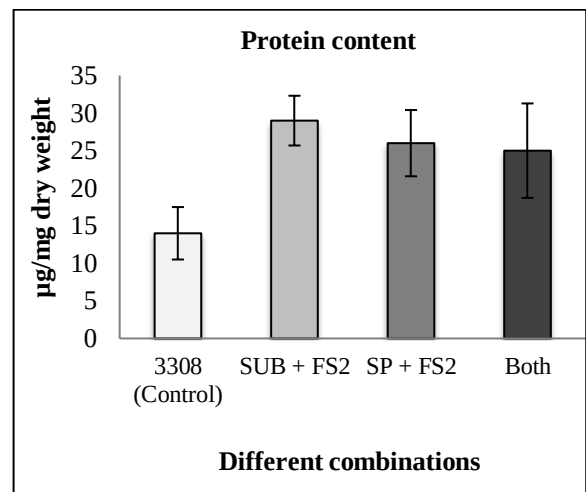


Figure 4.39. Graphical illustration showing the Protein content of *P. florida* supplemented with isolate FS2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

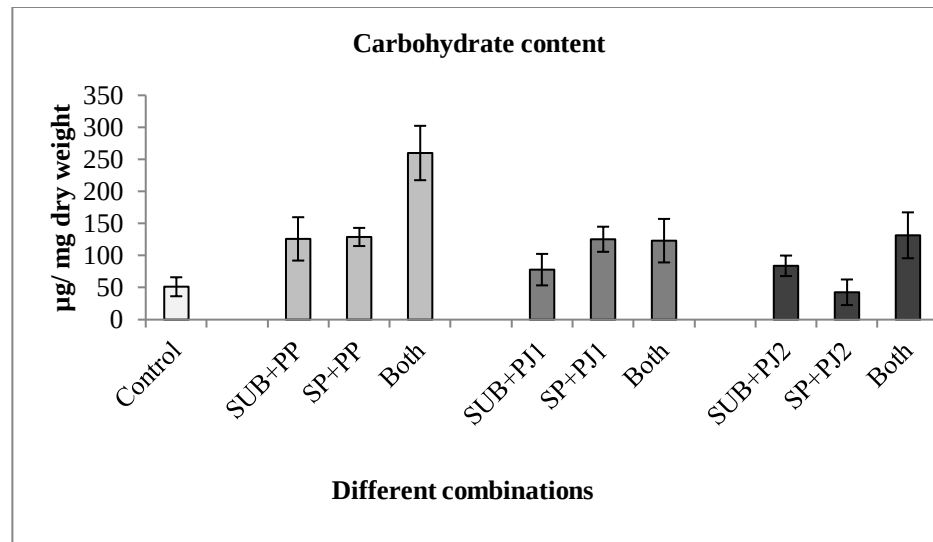


Figure 4.40. Graphical illustration showing the Carbohydrate content of *P. pulmonarius* supplemented with isolates PJ1, PJ2 and PP in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

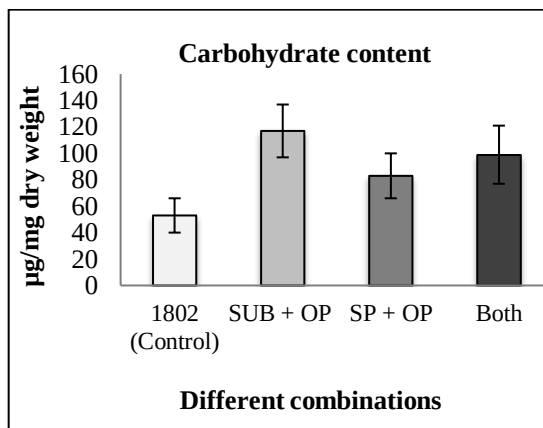


Figure 4.41. Graphical illustration showing the Carbohydrate content of *P. ostreatus* supplemented with isolate OP in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

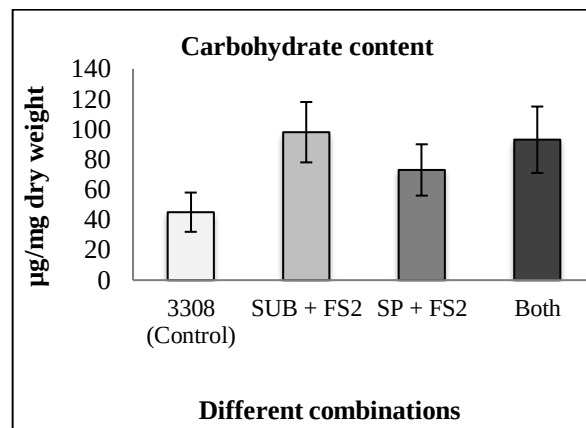


Figure 4.42. Graphical illustration showing the Carbohydrate content of *P. florida* supplemented with isolate FS2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

Polyphenol contents were increased in all the supplementations when bacteria were supplemented with substrates (Figure 4.43; 4.44 and 4.45).

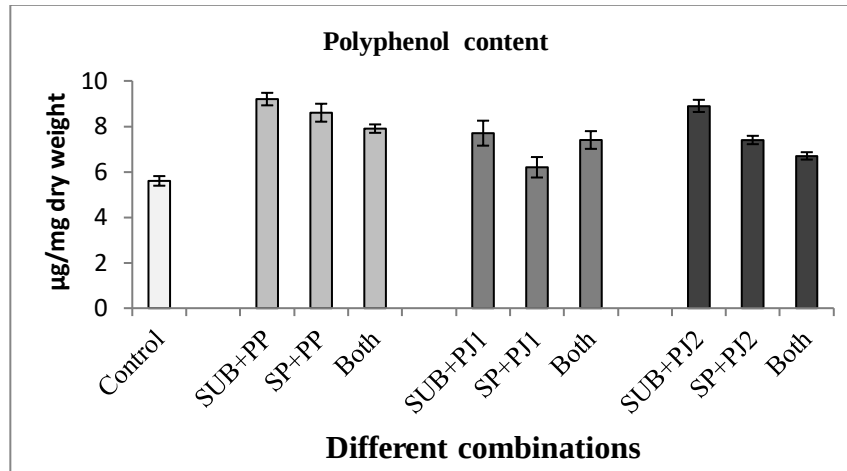


Figure 4.43. Graphical illustration showing the Polyphenol content of *P. pulmonarius* supplemented with isolates PP, PJ1 and PJ2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

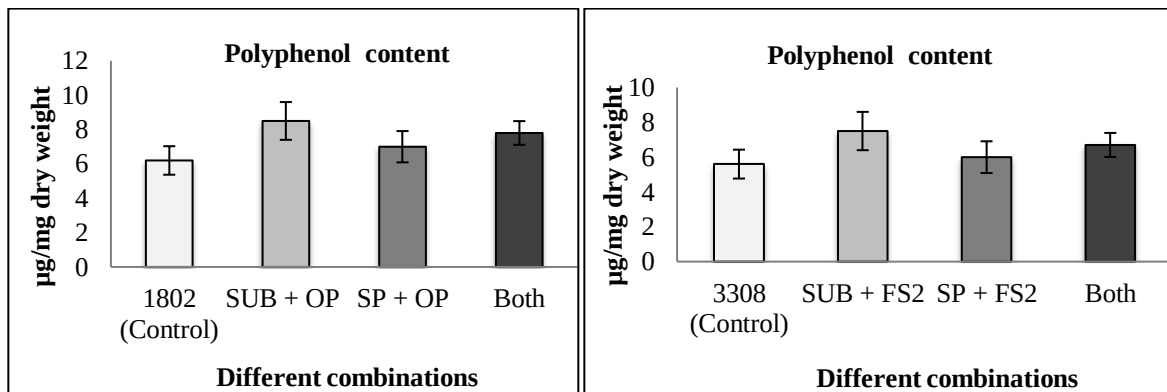


Figure 4.44. Graphical illustration showing the polyphenol content of *P. ostreatus* supplemented with isolate OP in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

Figure 4.45. Graphical illustration showing the Polyphenol content of *P. florida* supplemented with isolate FS2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

Flavonoid contents were also increased when endobacteria were supplemented during mushroom cultivation. Highest flavonoid contents were found when the respective endobacteria were supplemented with the substrates (Figure 4.46; 4.47 and 4.48).

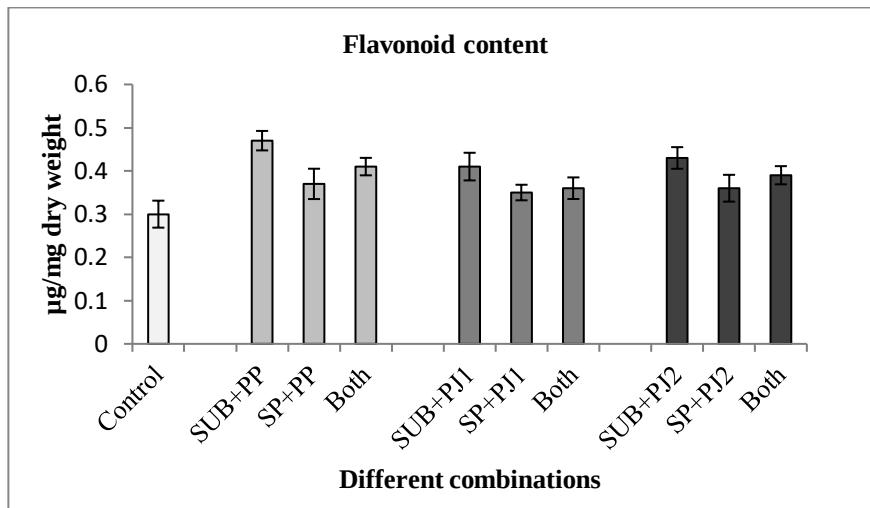


Figure 4.46. Graphical illustration showing the Flavonoid content of *P. pulmonarius* supplemented with isolates PP, PJ1 and PJ2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

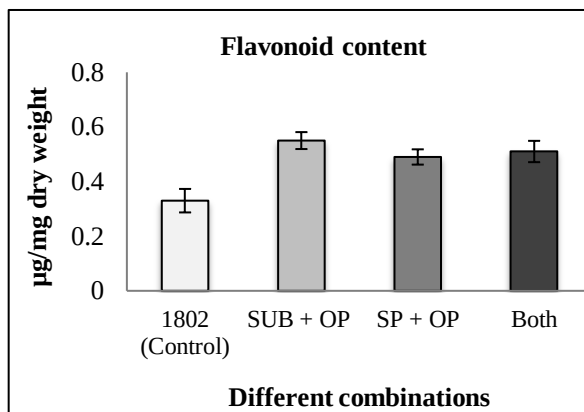


Figure 4.47. Graphical illustration showing the Flavonoid content of *P. ostreatus* supplemented with isolate OP in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

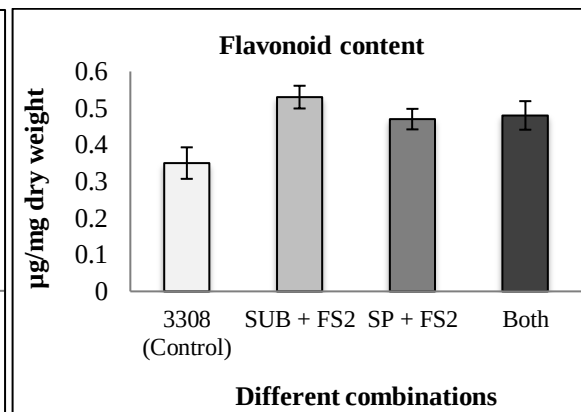


Figure 4.48. Graphical illustration showing the Flavonoid content of *P. florida* supplemented with isolate FS2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

Antioxidant activities were improved in the fruiting bodies derived from endobacteria-supplemented beds. Highest antioxidant activities were found when bacteria were supplemented with the substrates in all the tested sets (Figure 4.49, 4.50 and 4.51).

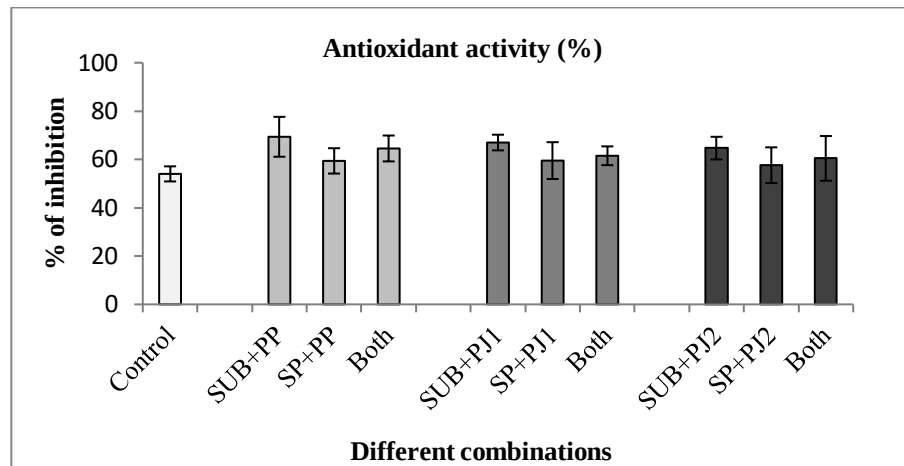


Figure 4.49. Graphical illustration showing the Antioxidant activity (DPPH reduction method) of *P. pulmonarius* supplemented with isolates PP, PJ1 and PJ2 in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

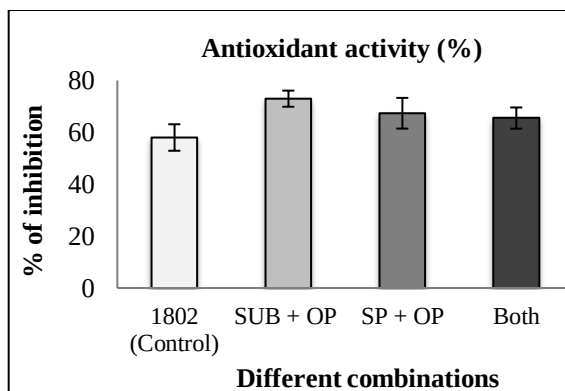


Figure 4.50. Graphical illustration showing the Antioxidant activity (DPPH reduction method) of *P. ostreatus* supplemented with isolate OP in different combinations. SUB=Substrates, SP= Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

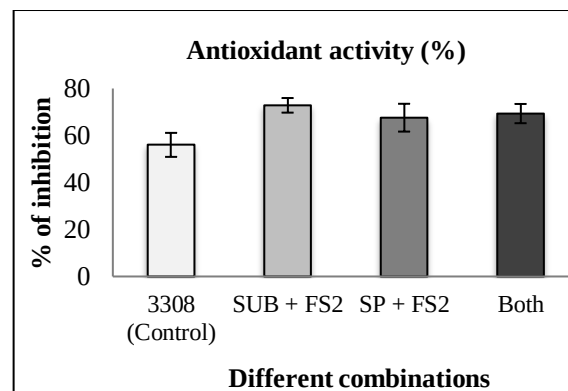


Figure 4.51. Graphical illustration showing the Antioxidant activity (DPPH reduction method) of *P. florida* supplemented with isolate FS2 in different combinations. SUB=Substrates, SP=Spawn, Both=Supplementation of endobacteria with both spawn and substrate.

Conclusion:

- Different concentrations of endobacteria were supplemented with substrate to evaluate the BE of different *Pleurotus* spp. Highest biological efficiency was recorded at the concentration of 8×10^8 cells of PP, PJ1 & PJ2 in *P. pulmonarius* and 1×10^8 cells of OP & FS2 for *P. ostreatus* and *P. florida* respectively.
- Mycelial growth of all the tested mushrooms was increased under all endobacterial supplementations in both solid agar media & liquid submerged conditions.
- Endobacteria did not produce laccase but they induced the laccase production of their respective host mushroom.
- All the bacterial isolates significantly increased the BE and nutraceutical compositions of tested oyster mushrooms in supplementations.

4.5. Studies on bioremediation potentialities and antimicrobial activities of *Pleurotus* spp. and their endobacteria

4.5.1. Bioremediation potentialities of three *Pleurotus* spp.

4.5.1.1 Pesticide tolerance and degradation:

All the three tested oyster mushrooms tolerated insecticide Imidacloprid and fungicide Carbendazim at the concentration of 0.01 % and 0.05 %, respectively (Figure 4.52). Mushroom mycelia were placed on Petriplate containing PDA media supplemented with two different concentrations of respective pesticides and incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Time required to fully cover the Petriplate are tabulated (Table 4.15). None of the mushroom strain degraded any of the pesticides as they didn't grow in pesticide supplemented MS media. Though all the five endobacteria were grown in pesticide (0.01% and 0.05 %) supplemented Nutrient Broth media (Figure 4.53 A, B, C, D, E) but they were unable to degrade the pesticides. Only endobacteria PJ1 and P2 show poor growth in Imidacloprid supplemented media. The growth of all the five endobacteria in Carbendazim supplemented media was very poor.

Table 4.15. Time (Days) required to fully cover the Petriplate (90 mm) containing different pesticides at different concentration

Mushroom strains	Time (Days) required to fully cover the Petriplate (90 mm)				
	Control	Imidacloprid		Carbendazim	
		0.01%	0.05%	0.01%	0.05%
MTCC 1805	12	12	13	26	40
MTCC 1802	13	18	20	24	32
ITCC 3308	12	12	20	26	38

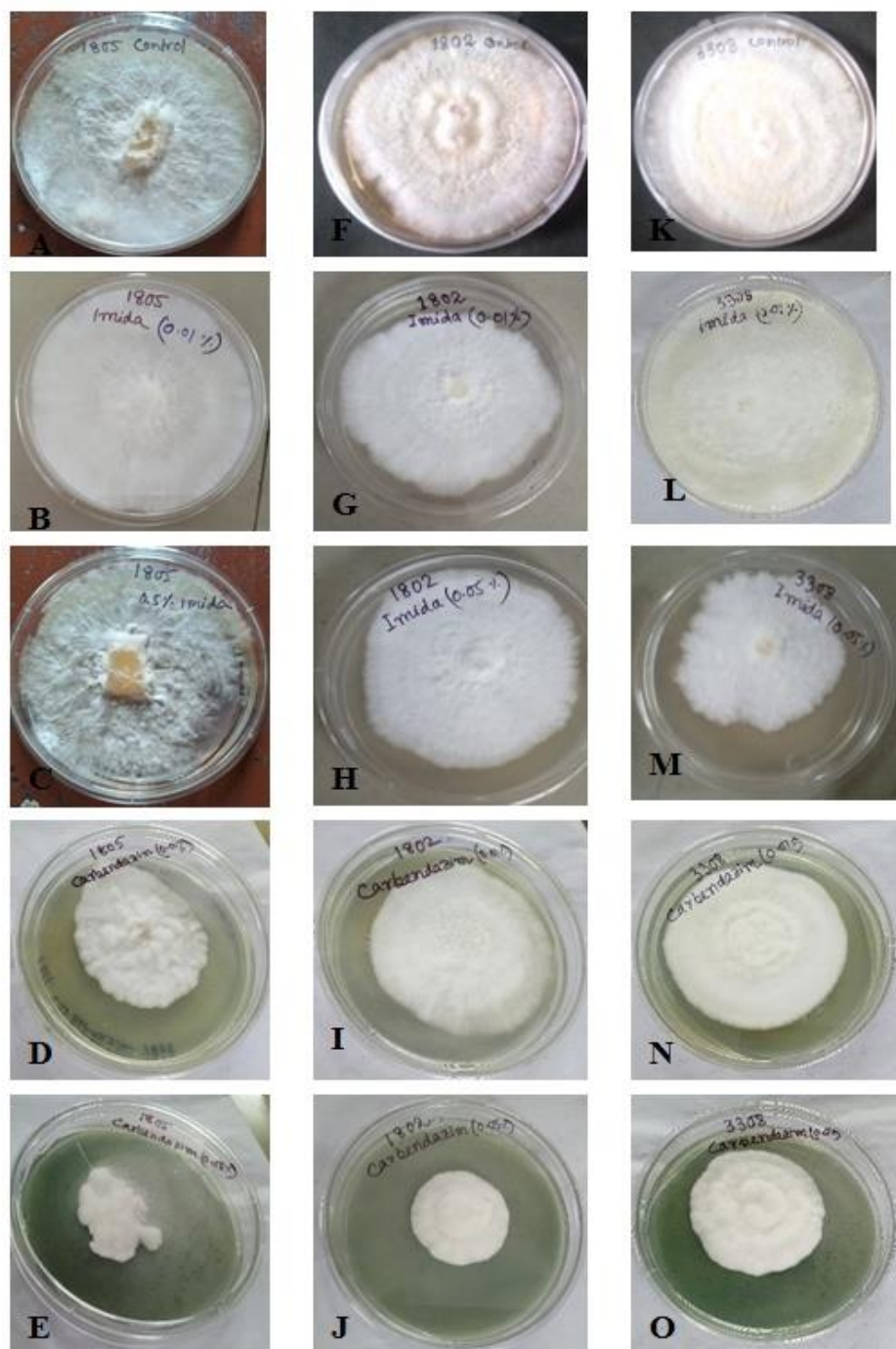


Figure 4.52. Pesticide tolerance by *Pleurotus* spp. A- E- MTCC 1805 (Control, 0.01% Imidacloprid, 0.05% Imidacloprid, 0.01% Carbendazim and 0.05% Carbendazim, respectively; F-J- MTCC 1802 (Control, 0.01% Imidacloprid, 0.05% Imidacloprid, 0.01% Carbendazim and 0.05% Carbendazim respectively; K-O- ITCC 3308 (Control, 0.01% Imidacloprid, 0.05% Imidacloprid, 0.01% Carbendazim and 0.05% Carbendazim respectively.

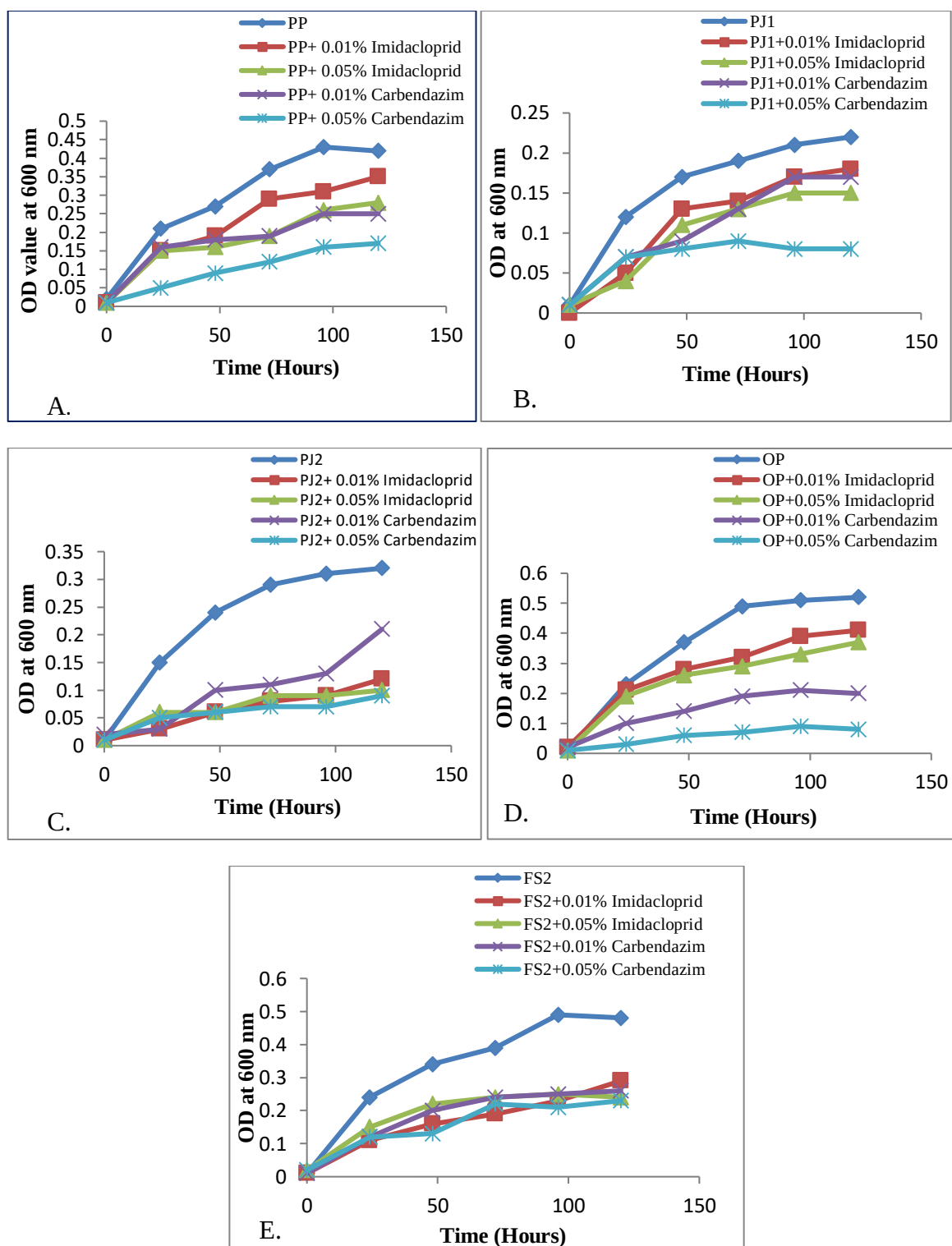


Figure 4.53. Graphical representation showing the Pesticide tolerance (Imidacloprid and Carbendazim) by endobacteria (A. PP, B. PJ1, C. PJ2, D. OP, E. FS2)

4.5.1.2 Dye decolorization:

All the three oyster mushrooms were able to decolorize four different industrial dyes namely Congo red, Bromocresol green, Malachite green and Acridine orange. Qualitative as well as quantitative assay were done. Qualitative assay was done using agar plate supplemented with respective dye and incubated for 10 days (Figure 4.53). Quantitative assay was done in submerged fermentation with shaking condition and Incubated for 7 days (Figure 4.54). The percentage of decolorization are given in the Table 4.16.

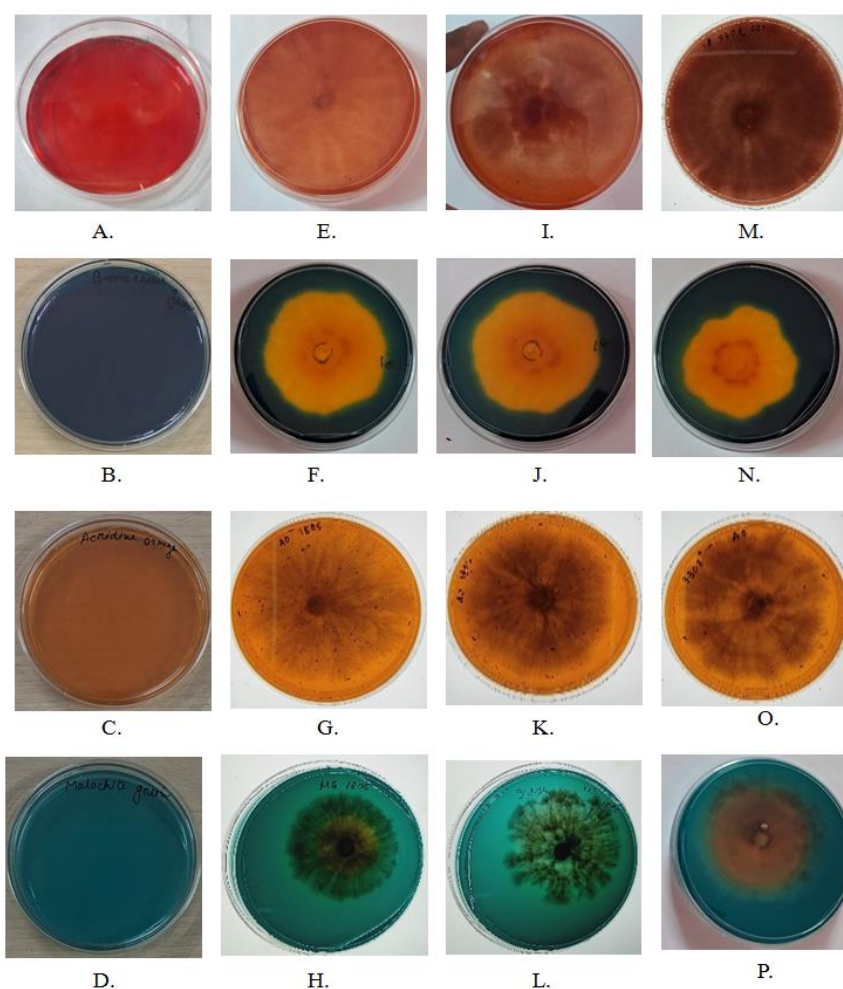


Figure 4.54. Dye decolorization by *Pleurotus* spp. A- D. Control (A-CR, B-BCG, C-AO, D-MG); E-H. MTCC 1805 (E-CR, F-BCG, G-AO, H-MG); I-L. MTCC 1802 (I-CR, J-BCG, K-AO, L-MG); M-P. ITCC 3308 (M-CR, N-BCG, O-AO, P-MG). Names of the dyes CR- Congo red, BCG- Bromocresol green, MG- Malachite green, AO- Acridine orange

Table 4.16. Percentage of decolorization[#] of dye

Mushroom strains	% of decolorization of dye			
	Congo red	Bromocresol green	Malachite green	Acridine orange
MTCC 1805	89.9	97.3	93.9	67.5
MTCC 1802	87.4	94.3	85.3	72.4
ITCC 3308	87.8	95.7	88.2	78.6

After 7 days of incubation in shaking condition.



Figure 4.55. Decolorization of dyes by mushroom strains. *P. pulmonarius* (A- CR, B- BCG, C- MG, D- AO); *P. ostreatus* (E- CR, F- BCG, G- MG, H-AO) and *P. florida* (I- CR, J- BCG, K- MG, L- AO). Names of the dyes CR- Congo red, BCG- Bromocresol green, MG- Malachite green, AO- Acridine orange

4.5.2. Antimicrobial activity of three *Pleurotus* spp.

All the three tested mushrooms extracts showed antibacterial potentialities against both Gram positive as well as Gram negative bacteria. Maximum inhibition zone was found by ITCC 3308 extracts (2.1 cm) against *Bacillus safensis*. Mushroom extracts didn't show any antifungal activity (Table 4.17& Figure 4.54) in present experimental conditions. Endobacteria didn't show antimicrobial activities (Table 4.18).

Table 4.17. Antimicrobial activity of different *Pleurotus* spp.

Mushroom strains	Antibacterial activity			Antifungal activity		
	Test organisms	Diameter of Inhibition zone (cm)		Test organisms	Diameter of Inhibition zone (cm)	
		Mushroom extract#	Amoxicillin (20µg/well)		Mushroom extract #	Fluconazole (20µg/well)
MTCC 1805	<i>Bacillus cereus</i>	1.5	1.5	<i>Alternaria</i> sp	Nil	1.5
	<i>B. safensis</i>	1.4	1.3	<i>Rhizoctonia solanii</i>	Nil	1.4
	<i>Escherichia. coli</i>	-	1.4	<i>Fusarium oxysporum</i>	Nil	1.7
	<i>Pseudomonas donghuensis</i>	1.4	1.6			
	<i>Klebsiella pneumoniae</i>	1.3	1.3			

Mushroom strains	Antibacterial activity			Antifungal activity		
	Test organisms	Diameter of Inhibition zone (cm)		Test organisms	Diameter of Inhibition zone (cm)	
		Mushroom extract#	Amoxicillin (20µg/well)		Mushroom extract #	Fluconazole (20µg/well)
MTCC 1802	<i>Bacillus cereus</i>	1.9	1.5	<i>Alternaria</i> sp	Nil	1.5
	<i>B. safensis</i>	1.7	1.3	<i>Rhizoctonia solanii</i>	Nil	1.4
	<i>Escherichia coli</i>	1.1	1.4	<i>Fusarium oxysporum</i>	Nil	1.7
	<i>Pseudomonas donghuensis</i>	-	1.6			
	<i>Klebsiella pneumoniae</i>	1.4	1.3			
MTCC 3308	<i>Bacillus cereus</i>	2.0	1.5	<i>Alternaria</i> sp	Nil	1.5
	<i>B. safensis</i>	2.1	1.3	<i>Rhizoctonia solanii</i>	Nil	1.4
	<i>Escherichia coli</i>	1.3	1.4	<i>Fusarium oxysporum</i>	Nil	1.7
	<i>Pseudomonas donghuensis</i>	-	1.6			
	<i>Klebsiella pneumoniae</i>	1.7	1.3			

In each well 25 µl of crude mushroom extracts were given

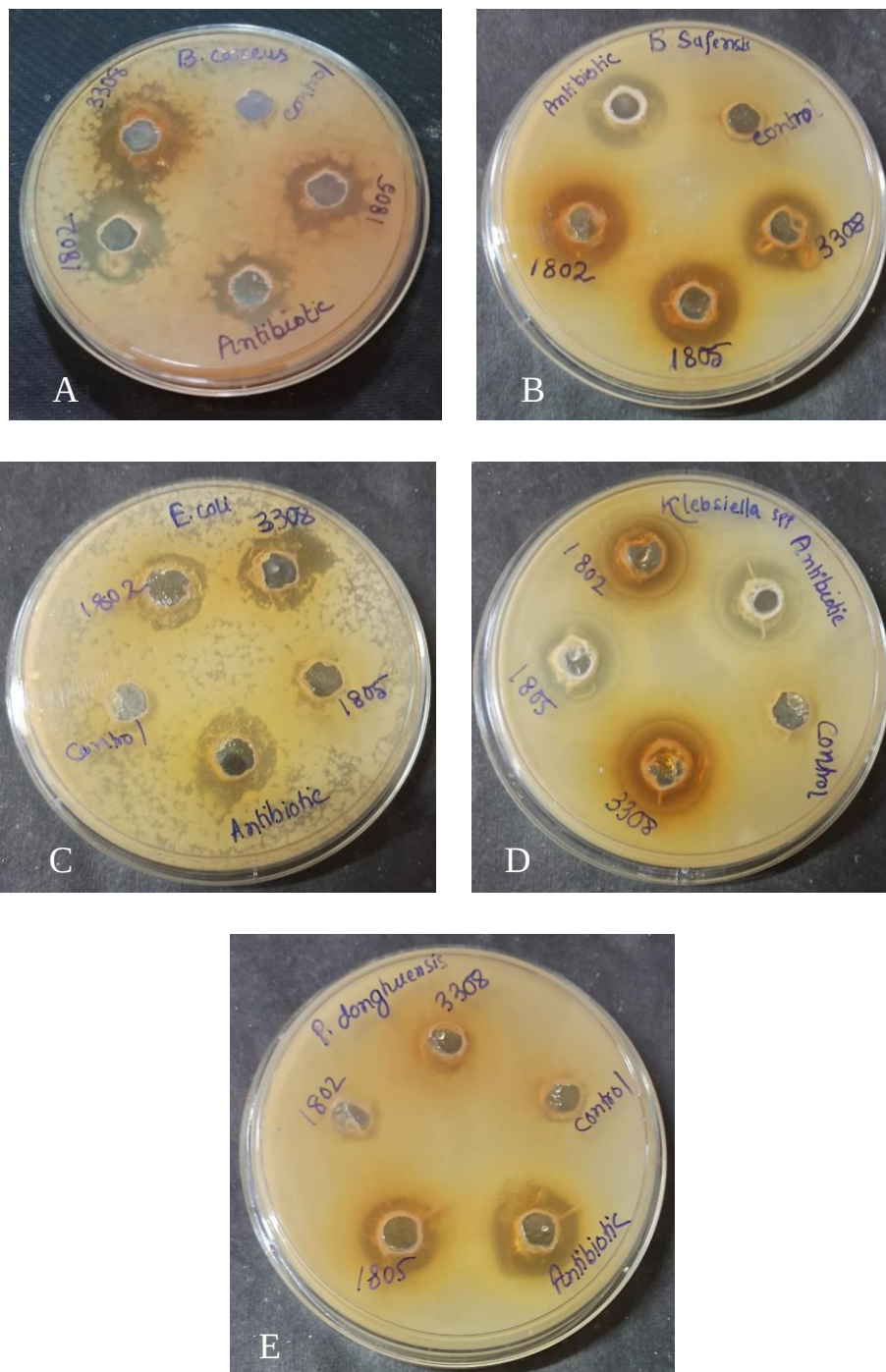


Figure 4.56. Antibacterial activity of mushroom extracts against five test organisms. A- *B. cereus*, B- *B. safensis*, C- *E. coli*, D- *Klebsiella sp.* and E- *P. donghuensis*.

Table 4.18. Antimicrobial activity of five different endobacteria

Endo-bacterial strains	Antibacterial activity			Antifungal activity		
	Test organisms	Diameter of Inhibition zone (cm)		Test organisms	Diameter of Inhibition zone (cm)	
		Endo-bacterial Culture filtrate	Amoxicillin (20µg/well)		Endo-bacterial culture filtrate	Fluconazole (20µg/well)
PP,PJ1, PJ2, OP and FS2	<i>B. cereus</i>	Nil	1.5	<i>Alternaria</i> sp	Nil	1.5
	<i>B. safensis</i>	Nil	1.3	<i>R. solanii</i>	Nil	1.4
	<i>E. coli</i>	Nil	1.4	<i>F. oxysporum</i>	Nil	1.7
	<i>P. donghuensis</i>	Nil	1.6			
	<i>K. pneumoniae</i>	Nil	1.3			

Conclusion:

- All the tested *Pleurotus* spp. tolerated 0.01% and 0.05% pesticides (Imidacloprid and Carbendazim) but didn't degrade any of these pesticides.
- All the tested *Pleurotus* spp. efficiently decolorized four industrial dyes (Congo red, Bromocresol green, Malachite green and Acridine orange). All the five endobacteria also tolerated pesticides but none of the endobacteria degraded any of the pesticides or decolorized the tested dyes.
- Extract of all the three mushroom strains showed antibacterial activities against some Gram positive and Gram negative bacteria but didn't show antifungal activity. On the other hand, none of the endobacteria showed any antimicrobial activity.

CHAPTER V

DISCUSSION

Mushroom cultivation is one of the oldest biotechnologies that does not require land, utilizes low-cost substrates, and demands minimal water input (Mistry and Parmar, 2024). It is considered the second most widely adopted biotechnological process, ranking just after yeast fermentation (Pathak et al. 2009). According to Sanchez (2010), oyster mushrooms rank second in global consumption, following the button mushroom *Agaricus bisporus*.

In addition to their rich nutrient content, the popularity of oyster mushrooms (*Pleurotus* spp.) can be attributed to their affordability, the abundance of substrates, and the ease of cultivation (Das and Mukherjee, 2007). There is, however, some controversy regarding the number of species in the genus *Pleurotus*. While Buchanan (1993) reported approximately twenty species, Kong (2004) identified about seventy. Most species are native to tropical and subtropical rainforests, with several being commercially cultivated (Bonatti et al. 2004).

Oyster mushrooms are widely known as a delicious functional food, rich in minerals, proteins and vitamins, while being low in fat. They can be cultivated on a broad range of lignocellulosic substrates like wood, various agro-wastes and even weed plants (Gregori et al. 2007; Das and Mukherjee, 2007).

In the present study, three species were investigated: *Pleurotus pulmonarius* (MTCC 1805), *Pleurotus ostreatus* (MTCC 1802), and *Pleurotus florida* (ITCC 3308). All these oyster mushrooms were successfully cultivated at $30 \pm 2^{\circ}\text{C}$ in the laboratory using sugarcane bagasse and rice straw as substrates (Figure 4.1).

Morphometric characteristics of the fruiting bodies were analyzed. Although rice straw resulted in mushrooms with greater average length and width, a higher number of fruiting bodies were observed on sugarcane bagasse (Figure 4.2).

Dubey et al. (2019) demonstrated that rice straw yielded mushrooms with longer stipes and larger pileus diameters compared to sugarcane bagasse. Similarly, Radwan (2005) found that rice straw produced the largest cap diameters in mushroom fruiting bodies. All three species exhibited higher biological efficiency (BE) when cultivated on rice straw compared to sugarcane bagasse (Figure 4.3). These findings are consistent with reports by Dubey et al. (2019) and Gorai and Sharma (2018).

The highest BE (63%) was recorded for *P. florida*, followed by *P. ostreatus* (54.5%) on rice straw, across both the first and second flushes. On sugarcane bagasse, *P. florida* again showed the highest BE (49%), followed by *P. ostreatus* (41%) (Figure 4.3). Gorai and Sharma (2018) reported that the BE of *Pleurotus* species ranges from 67.6% to 41.6% on sugarcane bagasse. According to Zhang et al. (2002), *P. sajor-caju* yields on rice straw were 10% greater than those on wheat straw.

Moisture content is closely related to dry matter in all living organisms. Among the rice straw-derived mushrooms, *P. ostreatus* exhibited the highest moisture content (89.2%), followed by *P. florida* (88.5%). For sugarcane bagasse-derived mushrooms, maximum moisture was observed in *P. ostreatus* (88.6%), and the lowest in *P. pulmonarius* (84.5%) (Table 4.1).

The traits of the basidium and basidiospores are important from a taxonomical standpoint since *Pleurotus* is a member of the subdivision Basidiomycotina (Figure 4.4). These morphological features, relevant to taxonomic studies, are listed in Table 4.2.

Oyster mushrooms are considered one of the best groups of edible fungi due to their rich nutritional profile (Owaid, 2019). Various researchers have reported that the proximate composition of mushrooms varies depending on the substrate used (Patil et al. 2010; Khan et al. 2013). Rice straw-derived mushrooms generally showed slightly higher protein content compared to those grown on sugarcane bagasse. Wang et al. (2001) emphasized that the substrate significantly influences the protein content of mushrooms, as both the quantity and the nature of the substrate's protein impact mushroom protein synthesis.

In this study, the highest protein content was found in *P. ostreatus* (18 µg/mg dry weight), followed by *P. pulmonarius*, with the lowest in *P. florida* (Figure 4.5). Chang et al. (1981) reported that the crude protein content in mushroom sporophores ranges from 26.6% to 34.1%. Similarly, Alam et al. (2007) found that protein content ranging from 28.0% to 31.8% (w/w) in *P. florida* and *P. sajor-caju* cultivated on various substrates. The carbohydrate content was highest in *P.ostreatus* in sugarcane bagasse (Figure. 4.6) whereas the ash content was highest in Rice straw –grown *P. florida* (Figure 4.7). Fiber, another important constituent in mushroom was found more or less similar in both rice straw and sugarcane bagasse (Figure 4.8).

Because of their hydroxyl groups, phenolic compounds were found as powerful antioxidants by Duh et al. (1999) and as important agents in scavenging free radicals by Hatano et al. (1989). The high phenolic content of mushrooms is the main factor responsible for their antioxidant qualities (Chirinang and Intarapichet, 2009; Khatun et al. 2015). In our present study, polyphenol concentrations were also assessed. Among the tested species, *P. ostreatus* cultivated on rice straw exhibited the highest polyphenol content (6.11 µg/mg), while *P. pulmonarius* showed the lowest (Figure 4.9).

Several studies have demonstrated that diets rich in phenolic compounds may reduce the risk of cardiovascular diseases by preventing the formation of atherosclerotic plaques (Elmastas et al. 2007). Because of its abundance of nutraceuticals, the members of *Pleurotus* are regarded as a medicinal mushroom (Gregori et al. 2007; Paul et al. 2017).

Among these nutraceuticals, flavonoids-a significant group-were detected in all tested *Pleurotus* species. According to Ferguson (2001) and Rice-Evans et al. (1996), flavonoids lowers the risk of developing tumor, menopausal symptoms, coronary heart disease, and a number of disorders linked to oxidative damage to nucleic acids, proteins and membranes. All three species in this study exhibited appreciable levels of flavonoids. *P. florida* showed the highest flavonoid content (0.38 µg/mg) when grown on both rice straw and sugarcane bagasse, while *P. pulmonarius* exhibited the lowest (0.22 µg/mg) (Figure 4.10).

Antioxidants present in mushrooms play a protective role against oxidative damage from free radicals, which cause ageing and the onset of illnesses including diabetes and cancer (Gregori et al. 2007; Lee et al. 2007; Paul et al. 2017). One common method to evaluate antioxidant potential is through DPPH radical scavenging assays. All three mushroom strains-MTCC 1805, MTCC 1802, and ITCC 3308 demonstrated comparable antioxidant capacities (Figure 4.11). Effiong et al. (2024) demonstrated that the biochemical contents of Oyster mushroom *P. ostreatus* varied depending upon the nature of solvent for extraction. The methanol (100%) extract of *P. ostreatus* showed 87.67% DPPH. scavenging activity. The lower antioxidant activity may be due to the use of concentration of solvent (70% methanol) during sporophore extraction.

All living organisms, including plants, animals, and humans, host diverse range of microbial communities throughout their lifespan (Proctor, 2011). These microbes, along

with environmental interactions, contribute to ecological balance and sustainability. Similar to plants and animals, fungi including wild and cultivated mushrooms harbor diverse microbial populations during their life cycles (Deveau et al. 2018), with bacteria being especially prevalent. Though there are a substantial amount of work on endobacteria of animals and plants but fungi are neglected in this context though the presence of bacteria in fungal system is noteworthy (Bonfante and Desir`o, 2017).

Variations in the microbial community can significantly influence mushroom yield, quality, and fruiting behavior. Recent studies have shown that mushrooms harbor not only surface-associated bacteria but also internal, endobacterial communities. These microbiomes are thought to play a crucial role in the growth and development of mushrooms, as demonstrated in species such as *Tricholoma* and *Rhizopogon* (Oh et al. 2018; Pramoj et al. 2019).

The mushroom microbiome comprises beneficial, harmful, symbiotic, and opportunistic organisms that can influence not only the host mushroom's life cycle but also nearby agricultural crops and even human health (Carrasco and Preston, 2020). Hamidizadeh et al. (2024) identified wild mushrooms as reservoirs of bacteria capable of causing plant diseases. These bacteria inhabit in both the external surfaces and internal tissues of mushrooms. Although some can be cultured on conventional media, many are not amenable to traditional microbiological methods. Traditional culture-based methods have limitations, as many bacteria are non-culturable on artificial media. Therefore, metagenomic analysis using high-throughput sequencing provides a more comprehensive understanding of microbial diversity.

Metagenomics has revolutionized microbial biotechnology by enabling the direct extraction and analysis of genetic material from environmental samples without the need for culturing (Madhavan et al. 2017). Unlike genomics, which targets specific organisms, metagenomics allows for a comprehensive analysis of all genomes within a given sample. By generating hundreds of millions of sequence reads, it is possible to reconstruct nearly complete genomes of dominant prokaryotic species (Wrighton et al. 2012; Baeza et al. 2017).

In the present study, a metagenomic analysis of the sporophores of three *Pleurotus* species was conducted to investigate the diversity and functional roles of both endogenous and exogenous bacterial populations. The microbial communities associated with these oyster mushrooms were analyzed, and their relative abundance and taxonomic distribution were determined at various hierarchical levels (Figures 4.12.A- 4.12.F).

The findings revealed that the majority of identified microbes belonged to the phylum Pseudomonadota (formerly *Proteobacteria*), followed by Actinomycetota, Bacillota, and Bacteroidota. Proteobacteria constituted over 80% of the total microbiome, making them the most dominant phylum. Specifically, more than 80% of sequences from sample MTCC 1802 and MTCC 1805 were identified as *Proteobacteria*, predominantly from the class Gammaproteobacteria. Previous studies have similarly reported that fresh produce, including button mushrooms, harbor high proportions of *Proteobacteria*, sometimes exceeding 90% (Qiu et al., 2019; Chang et al., 2022).

Species richness was higher in *P. ostreatus* and *P. pulmonarius* compared to *P. florida*, as indicated by higher Chao1 index values (Figure 4.13.A). The Simpson Diversity Index, which ranges from 0 to 1 (with values closer to 1 indicating greater diversity), further

supported these observations. In *P. pulmonarius*, a Simpson index of 0.393 suggested dominance by a single species (*Pseudomonas*), while in *P. florida*, a value of 0.821 indicated more even distribution among species (Figure 4.13.B).

All three *Pleurotus* species contained a rich diversity of beneficial bacteria, including *Pseudomonas*, *Stenotrophomonas*, *Priestia*, *Rhizobium*, *Burkholderia*, *Rossellomorea* etc. While bacterial associations with mushrooms such as *Pleurotus* have been reported previously (Yara et al. 1999, 2006; Bianciotto et al. 2000; Febriansyah et al. 2018), this study may represent the first comprehensive report on the total microbiome of the fruiting bodies of three mature, fresh *Pleurotus* species.

According to Cho et al. (2003), certain *Pseudomonas* species found on the mycelial surface of *P. ostreatus* can stimulate vegetative development and primordia initiation. Similarly, composting substrates of *P. eryngii* (Chen et al. 2022a) and *P. floridanus* (Wei et al. 2024) were found to contain numerous bacteria that promote mushroom growth. On the other hand, Lim et al. (2008) identified eight bacterial isolates from diseased *P. eryngii* mycelia that inhibited fruiting body formation. Adamski and Pieter (2018) also reported bacterial isolates from eight *P. ostreatus* strains, although their specific roles were not clarified.

Most previous metagenomic studies have focused on the compost used for oyster mushroom cultivation, showing that microbial communities are influenced by the compost's physicochemical parameters such as temperature, pH, C/N ratio, and organic matter content (Kong et al. 2020). Ban et al. (2022) observed that when oyster mushrooms were grown in bottles or on shelves, Pseudomonadaceae and Enterobacteriaceae were more abundant in mushrooms from supermarkets and outdoor markets, respectively. They attributed this variation to the higher temperatures in open-air markets, which may favor

the growth of spoilage bacteria like *Pseudomonas spp.*, known to degrade mushroom quality and cause brown blotch disease (Venturini et al. 2011). According to Frey-Klett et al. (2011) the hyphosphere of fungi offers microhabitats for colonization of different bacterial communities.

The presence of a diverse group of bacteria within the fungal system may dictate and modulate the life pattern of the interacting organisms. The fungal-bacterial interactions (FBI) ranging from antagonistic to mutualistic, affect various aspects of fungal and bacterial biology and ecology, including growth, reproduction, motility, nutrient acquisition, stress tolerance, and pathogenicity etc. These outcomes are shaped by the nature of their physical associations (such as biofilms, free-living cells, or intracellular localization), molecular communication between the partners (direct or indirect), and the surrounding environmental conditions and/or host responses (Deveau et al. 2018). Despite their genetic and functional diversity, bacterial communities associated with fungi represent a distinct and novel type of microbiota, referred to as the fungal microbiota. According to Desirò et al. (2014), the most intimate form of interaction occurs when bacteria reside within fungal cells as endobacteria.

Thus we aimed to isolate endobacteria with growth-promoting traits from the internal tissues of sporophores of these *Pleurotus* species. After random screening and evaluation of their growth-promoting ability, five endobacterial isolates were selected: PP, PJ1 and PJ2 from *P. pulmonarius*, OP from *P. ostreatus*, and FS2 from *P. florida* (Table 4.4-4.6). PP was isolated from the pileus part whereas PJ1 and PJ2 from the junction of stipe and pileus of *P. pulmonarius*; OP from the pileus part of *P. ostreatus* and FS2 from the stipe part of *P. florida* indicating the universal presence of endobacteria throughout the

sporophore of *Pleurotus* species. Figure 4.17 confirmed the mutual relation of the host and endobacteria as the mushroom mycelia and respective endobacteria were grown side by side without hampering each other in co-culture. These bacteria were characterized morphologically and biochemically (Figure 4.18- 4.21 and Table 4.7 & 4.8). Based on 16S rRNA sequencing, PJ1, PJ2, and FS2 were identified as *Rossellomorea marisflavi*, PP as *Priestia paraflexa*, and OP as *P. aryabhatai* (Table 4.9). The endobacterial isolates grouped primarily within the genera *Rossellomorea* and *Priestia*. PJ1, PJ2, and FS2 are clustering closely with *Rossellomorea marisflavi*, PP as *Priestia paraflexa*, and OP as *P. aryabhatai* (Table 4.9 Figure 4.22), indicating high sequence resemblance and potential taxonomic relationship. Some other members of the family *Bacillaceae* formed well-supported clades, including species from *Bacillus*, *Evansella*, *Alteribacter*, and *Robertmurraya*. The phylogenetic tree was rooted with *Pseudomonas aeruginosa* (NR_026078.1) as the out group to provide orientation. The result suggests that these endobacteria represent phylogenetically diverse members of the *Bacillaceae* family, with close relationships to known environmental and endophytic strains. The clustering pattern and high bootstrap support additionally specify potential ecological or functional associations with the fungal host. The 16s sequences are deposited in NCBI and all the cultures are deposited in culture collections. PP, PJ1 and PJ2 are deposited in National Centre for Industrial Microorganism, Pune whereas OP and FS2 are deposited in Microbial Type Culture Collections, IMTECH, Chandigarh, India (Table 4.10).

Interestingly, despite *Pseudomonas* being the most dominant genus in the metagenomic analysis of all three mushrooms, none of the selected beneficial growth promoting isolates belonged to this genus.

Similar to pathogens, lytic enzymes are the arsenal of endogenous microorganisms for their transmission and nutrient accumulation. The endobacteria PP and OP produced cellulase, chitinase and pectinase (Fig 4.23 and Table 4.11). The other organisms produced very less lytic enzymes. Here most of the endogenous bacteria are not harmful to the host mushroom so the amount of lytic enzymes is very less. In addition to fungal cellulase, bacteria also produced cellulase for efficient degradation of cellulose into glucose. Pectinase though not directly affect the fungal host, but it could assist degradation of plant materials, indirectly increasing nutrition of the fungal hosts. The chitinase produced by endobacteria possibly involved in fungal cell wall remodeling or turnover. As chitin is a key structural component of fungal cell walls, so, endogenous regulation of its degradation is essential during hyphal extension, branching, or sporulation (Araldi-Brondolo et al 2017). The endobacteria *Paraburkholderia* secrete chitinase through Type II system, which is necessary for the bacteria to enter the hyphae of host *Rhizopus* sp. (Moebius et al. 2014).

All the endobacteria produced siderophore, ammonia and IAA which are indirectly or directly required for growth promotion (Figure 4.24 and Table 4.12). They produced IAA in tryptophan-supplemented media (Figure 4.24.C). All five isolates enhanced the growth of mycelia in both solid agar cultures and submerged fermentation when co-cultured with their respective host mycelia. IAA, a key phytohormone, plays a crucial role in plant and fungal growth by promoting cell elongation and acting as a signaling molecule in plant–microbe interactions (Matsuda et al. 2018; Zhao, 2010). The endobacteria in our investigation generated significantly higher amount of IAA (33.75–45 mg/l) than *Bacillus aryabhattai*, which was isolated from *P. ostreatus* and produced only 3.17 µg/ml

(Febriansyah et al., 2018). Prior studies also noted that exogenous IAA promotes growth in *P. sajor-caju* (Mukhopadhyay et al. 2005).

As we are dealing with some bacteria and finding their various roles in growth and development of some edible mushroom, so, it is very much essential to know their level of toxicity. The blood agar culture (Figure 4.25A) followed by MTT assays (Figure 4.25B & 4.25 C) with both human embryonic kidney 293 (HEK 293) cell line and Caco 2 human cancer cell lines showed they are not cytotoxic. The PJ1 endobacteria did not inhibit the growth of HEK 293 cell line but showed the inhibition of cancer cells (Caco 2 cell lines) which indicate their probable role in anticancer activity. But thorough research is needed to establish the therapeutic activity of PJ1. Mushrooms including *Pleurotus* spp. are reported to show anticancer activity by various researchers (Akanni et al. 2010). The endobacteria are very much sensitive to a number of common antibiotics (Table 4.13 and Figure 4.26) which again indicate their harmless nature which is very much expected from the endogenous population of an edible oyster mushroom.

To confirm their growth-promoting potential, the isolates were exogenously applied at different stages of mushroom growth. The endobacteria enhanced vegetative growth of mushrooms in solid media by increasing the mycelial extension (Figure 4.27). The growth rate in terms of hyphal extension was maximum in PP (33.9%) supplemented media, OP showed minimum (9.23%) whereas PJ1, PJ2 and FS2 supplemented media showed intermediate growth increase (20.5%) of their respective host mycelia in solid agar plate culture. In submerged culture, also the supplementation of endobacteria increased the mycelial biomass of different *Pleurotus* species as evidenced from the mycelial dry weight (Figure 4.28). The highest mycelial dry weight increase was found in FS2 supplemented *P.*

florida (146%). The other bacteria increase the growth of respective hosts more or less similarly (86-90%). MGPB generally increase the vegetative growth of different mushroom strains (Suarez et al. 2019). Growth promotion can be done by direct application of the bacteria or the application of cell free broth of the bacteria. The cell-free fermentation broths of *Bacillus cereus*, increased the growth of *P. eryngii* (Chen et al. 2022a) and *Glutamicibacter arilaitensis* isolated from soil (Kumari & Narain, 2021) increased the growth of mushroom *P. florida*. While high concentrations of *Azotobacter* broth reduced growth, low concentrations increased *P. eous* mycelial development by 18.28% (Eyini et al. 2005). In our study, all five endobacterial isolates significantly increased mycelial dry weight, confirming their positive impact on vegetative growth of the mushroom.

Laccase, a lignin degrading enzyme is very common in WRF including *Pleurotus* species. (Das et al. 1997). Although none of the endobacterial isolates produced laccase individually, increased laccase activities were observed when they were co-cultured with the tested mushroom species. The maximum laccase activities were observed between 7-9 days after endobacterial supplementation (Figure 4.29.A-4.29.C). The OP-containing culture of *P. ostreatus* exhibited the highest laccase activity (Figure 4.29.B). *Pleurotus* laccase activity has previously been recognized in mycelial growth and sporophore development (Das et al. 1997, 2011; Sun et al. 2011; Kues & Liu, 2000; Hultberg et al. 2020). Das et al (2011) found a growth-regulating laccase in different *Pleurotus* species including *P. ostreatus*, *P. pulmonarius* and *P. florida*. The present observation support the notion that bacterial isolates stimulate the laccase production of respective host mushroom, thereby enhancing their growth. Chen et al. (2022a) proposed that bacterial extracellular

metabolites such as cyclopeptides increase nitrogen availability, reduce the Carbon-to-Nitrogen ratio, and induce the release of enzymes like amylase and laccase.

After confirmation of the role of endobacteria on vegetative growth and mycelial extension of their host mushroom we have tried to see their role in fruiting body development. The concentration of supplemented endobacteria is one of the most important criteria. The optimum concentration for *P. pulmonarius* was 8×10^8 CFU/ml whereas the optimum bacterial concentration for *P. ostreatus* and *P. florida* supplementations was determined to be 1×10^8 CFU/ml (Figures 4.30–4.32).

Time of application of endobacteria is another important parameter. So we mixed the above concentrations of endobacteria during preparation of spawn, preparation of substrates and in both conditions and evaluated the fruiting efficiency separately. Despite limited data on endobacteria's role in fruiting body induction, our results showed that all the endobacterial isolates promoted fruiting regardless of application method (Figures 4.33.A–4.33.C). Notably, PJ2 enhanced biological efficiency (BE) of *P. pulmonarius* by 64.84% when applied to the substrate (Figure 4.34), OP enhanced the BE of *P. ostreatus* by 24% when mixed with both spawn and substrates (Figure 4.35), whereas FS2 increased the BE of *P. florida* by 26% when mixed with substrates (Figure 4.36). These BE increase are also statistically significant though there are increase of BE in various combinations but the changes are not significant (Figures 4.34–4.36). *Glutamicibacter arilaitensis* MRC119 increased BE of *P. florida* by 58% (Kumari & Naraian, 2021), while Suarez et al. (2019) observed no BE improvement when bacteria were added to spawn but reported reduced spawn running time of *P.ostreatus* supplemented with *Micromonospora lupini*. In our study, spawn running time remained more or less unchanged (Table 4.14).

In the present investigation, it was also found that endobacteria not only boost the mycelial growth or fruiting body production, nonetheless, bacterial supplementation also increased the nutritional content improving protein, carbohydrate, polyphenol, flavonoid and antioxidant levels of fruiting bodies (Figures 4.37-4.51). This is the first report of modulation of nutrient contents in Oyster mushroom after bacterial supplementation.

Several mushroom strains showed the bioremediation potentialities against different pollutants (Sadiq et al. 2015). In present investigation *Pleurotus* species were exposed against some pesticides and industrial dyes. The isolated endobacteria were also cultured individually as well as in presence of host mushrooms and exposed against pesticides and industrial dyes to observe their involvement in bioremediation process.

Pleurotus spp. and the isolated endobacteria were grown on media supplemented with Imidacloprid and Carbendazim (Figures 4.52 & 4.53). Though Lang et al. (1996), Ponting (2001) reported the involvement of *Pleurotus* spp. in bioremediation of a wide range of organic pollutants, various pesticides, PAHs, and polychlorinated aromatic compounds but in present investigation none of the tested oyster mushroom species or endobacteria degraded either pesticide (as they were unable to grow in pesticide-supplemented minimal media) in present experimental condition but tolerated both Imidacloprid and Carbendazim pesticides at low concentrations. Roy and Das (2017) showed that two soil bacteria *Bacillus safensis* and *Bacillus cereus* can grow in pesticide -supplemented minimal media and can use the pesticide as their carbon source after degradation.

Here all the mushroom species could decolorize industrial dyes including Congo red, Bromocresol green, Malachite green, and Acridine orange (Figures 4.54 & 4.55; Table 4.16). However, the endobacteria alone did not decolorize any of these dyes. Previous

studies demonstrated that *P. ostreatus* could decolorize Synazol red by up to 94% over 30 days (Hashmi and Saleem, 2013), and other reports have confirmed their capability to decolorize Congo red and other dyes using free and immobilized laccase enzymes (Jamil et al. 2018; Dewi et al. 2019). Laccase production was elevated in all experimental sets supplemented with bacteria, supporting the conclusion that endobacteria stimulate host laccase activity.

Therefore, these endobacteria not only enhanced vegetative growth and fruiting body formation but also improved enzymatic and nutritional properties of their host mushrooms. Their role in dye decolorization likely stems from their ability to induce laccase production in the host, even though they did not produce the enzyme themselves.

In our present investigation, fruiting body extracts of the tested oyster mushroom species exhibited inhibitory effect against certain Gram-positive as well as Gram-negative bacteria (Table 4.17 and Figure 4.56). However, no antifungal activity was observed. According to earlier research, *P. ostreatus* crude extracts have antimicrobial potentiality against a variety of bacteria, including both Gram-positive and Gram-negative ones, as well as fungus like *Aspergillus niger* (Gerasimenya et al. 2002). *P. eryngii* extracts have been demonstrated to suppress the development of *Bacillus* species (Nagi and Ng, 2006), whilst *P. sajor-caju* extracts have been demonstrated to decrease the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nagi and Ng, 2004).

Oyetayo and Ariyo (2013) reported that ethanolic extracts of *P. ostreatus* fruiting bodies demonstrated antimicrobial potentiality against *E. coli*, *S. aureus*, *B. subtilis*, and *Candida albicans*. Furthermore, *P. eryngii* has been shown to possess antifungal qualities that prevent the growth of *Fusarium oxysporum* and *Mycosphaerella arachidicola* (Wang and

Ng, 2004). Das et al. (2012) found that both aqueous and 70% ethanolic extracts of fresh mycelia and sporophores of *P. floridanus* exhibited antibacterial properties. Interestingly, while the mycelial extract of *P. florida* did not inhibit bacterial growth, the basidiocarps of *P. florida* were capable of suppressing the growth of certain bacteria. None of the isolated endobacterial strains showed antibacterial or antifungal activities in present experimental conditions.

Mushroom growth-promoting bacteria (MGPB) have previously been isolated predominantly from ecological niches such as compost, soil, casing materials, and mushroom surfaces. Some of these environmental isolates pose potential health risks to humans. In contrast, the bacterial isolates used in our study are endogenous to edible mushrooms and are considered safe for use. These isolates hold significant potential for the development of bacterial bio-inoculants intended for commercial mushroom cultivation. Such bio-inoculants could contribute to the sustainable production of mushroom fruiting bodies. Given their endogenous origin, these bacteria are unlikely to disrupt the native endobacterial communities already present within the mushrooms.

While this study demonstrates the effectiveness of endobacterial inoculants in promoting sporophore formation, successful commercial application will require optimization of various factors, including bacterial concentration, timing of inoculation, pH, temperature, and other ecological conditions. Beyond their role in enhancing sporophore development, these endobacterial isolates also show promise for future applications in the production of probiotics and other valuable secondary metabolites.

A diverse population of bacteria is found on the surface and within the internal tissues of oyster mushroom fruiting bodies. The growth, development, and nutritional properties of

these edible mushrooms result from complex interactions between the associated bacteria, mushroom species, and environmental factors. Manipulating mushroom growth-promoting bacteria (MGPB), particularly those of endogenous origin, can enhance nutrient solubilization and presents a promising strategy for improving production efficiency and promoting environmental sustainability in mushroom cultivation. Future research should also investigate the molecular mechanisms underlying these interactions to develop beneficial microbial consortia tailored not only for large-scale commercial mushroom production but also for the optimal synthesis of bioactive compounds with pharmaceutical importance.

KEY FINDINGS

- The three *Pleurotus* spp. i.e *Pleurotus florida* (ITCC 3308), *Pleurotus ostreatus* (MTCC 1802) and *Pleurotus pulmonarius* (MTCC 1805), were successfully cultivated on both rice straw and sugarcane bagasse. Rice straw showed better BE than Sugarcane bagasse
- The fruiting bodies were found to be rich in protein, carbohydrate, flavonoid, polyphenol and antioxidant activities.
- The Metagenomic analysis of sporophores of oyster mushrooms showed a great diversity of compositions of microbiota, the largest of which is phyla Pseudomonodota (proteobacteria) followed by Bacillota, Bacteroidota, Actinomycetota etc.
- Isolation of some beneficial bacteria with growth promoting activities from the endogenous tissue of the sporophore of these mushrooms was carried out.
- Five growth promoting endobacteria were finally screened from the three *Pleurotus* species namely PP, PJ1, PJ2, OP & FS2 and studied extensively.
- PP was isolated from pileus and PJ1 and PJ2 were isolated from junction of the pileus and stipe part of *P. pulmonarius*, OP was isolated from pileus part of *P. ostreatus* and FS2 was isolated from stipe part of *P. florida*.
- All of the five isolates produced good amount of IAA, Siderophore and ammonia which indicated their growth promoting capabilities.
- In co-culture the endobacteria increased the radial mycelial growth and increased the biomass of mushroom mycelia.
- Though the endobacteria did not produce laccase but they induced the laccase production of their respective hosts. The induction of laccase might one of the possible reasons for increased mycelial growth.

- PP was identified as *Priestia paraflexa* and deposited in NCIM, Pune having accession no. NCIM 5828, PJ1 was identified as *Rossellomorea marisflavi* having accession numbers NCIM 5829 and PJ2 was identified as *Rossellomorea marisflavi* having accession number NCIM 5830.
- OP was identified as *Priestia aryabhattai* and FS2 was identified as *Rossellomorea marisflavi* and deposited in MTCC, IMTECH, Chandigarh.
- Different concentrations of endobacteria were inoculated to evaluate the BE of different *Pleurotus* spp. Highest biological efficiency was recorded when 8×10^8 endobacterial cells (PP, PJ1 & PJ2) were added with MTCC 1805 and 1×10^8 endobacterial cells (OP & FS2) were added with MTCC 1802 and ITCC 3308, respectively. So further studies were performed with these concentrations of inoculum.
- The endobacterial isolates not only induced the BE but also enhanced the nutraceutical compositions (Protein, Carbohydrate, Flavonoid, Polyphenol, Antioxidant activities etc.) of the host mushrooms when supplemented externally.
- All the three *Pleurotus* spp showed bioremediation potentialities like decolorization of dyes (Congo red, Bromocresol green, Malachite green & Acridine orange) but none of the endobacteria showed this property.
- All the mushroom strains tolerated both Imidacloprid and Carbendazim pesticides which may be due to the presence of endobacteria as the isolated endobacteria also showed this property.
- Supplementation of endobacteria may open a new horizon for sustainable agricultural practices of Oyster mushroom.

SIGNIFICANCE OF THE STUDY:

The present research holds significant implications for multiple domains, including agriculture, biotechnology, and environmental science. Here are the key points highlighting the significance of the work:

Agricultural Impact:

1. **Enhanced Biological Efficiency (BE):** The study exhibits that rice straw is superior substrate compared to sugarcane bagasse for cultivating *Pleurotus* spp., leading to better yields. This observation can guide farmers in choosing the most effective substrates for mushroom cultivation, optimizing their production and profitability.
2. **Growth-Promoting Endobacteria:** The isolation and identification of growth-promoting endobacteria that augment the mycelial growth rate of *Pleurotus* spp. indicate a potential method for increasing mushroom yield. This can lead to more competent mushroom farming practices.

Nutritional and Health Benefits:

3. **Enriched Nutritional Profile:** Due to the presence of ample carbohydrates, proteins, polyphenols, flavonoids, and antioxidants, these highly nutritious edible

mushrooms are beneficial for human health. This highlights their assessment as a functional food with potential health benefits.

4. **Nutraceutical Enhancement:** The supplementation with endobacteria not only improves yield but also enhances the nutraceutical compositions of the mushrooms, adding value to the crop and making it more appealing to health-conscious consumers.

Microbiological and Biotechnological Insights:

5. **Microbiota Diversity:** The metagenomic analysis reveals a diverse microbiota composition within the sporophores, primarily comprising Pseudomonadota (proteobacteria), Bacillota, Bacteroidota, and Actinomycetota. This diversity is very much essential to the general well-being and development of the mushrooms.
6. **Identification of Beneficial Bacteria:** The identification and characterization of beneficial bacteria like *Priestia paraflexa*, *Rosellomorea marisflavi*, and *Priestia aryabhattai* provide valuable insights into their roles in mushroom growth promotion and laccase induction. These findings can lead to further biotechnological applications in mushroom cultivation and other agricultural practices.

Environmental Significance:

7. **Bioremediation Potential:** The *Pleurotus* species exhibit significant bioremediation capabilities, such as decolorizing dyes like Congo red, Bromocresol green, Malachite green and Acridine orange. This positions them as potential agents for environmental cleanup and pollution control.
8. **Pesticide Tolerance:** Pesticide-tolerant character may allow mushrooms to be grown on agricultural byproducts or low-quality biomass that may contain pesticide residues, helping to recycle waste more efficiently.

Future Research and Applications:

- 1. Sustainable Agriculture:** The successful use of endobacteria to improve mushroom growth and yield as well as to biocontrol harmful pathogens, paves the way for developing sustainable agricultural practices which can reduce the need of chemical fertilizers and pesticides thus promote eco-friendly agricultural practices.
- 2. Further Studies:** The work sets a foundation for future research on optimizing endobacteria concentrations and exploring their effects on other mushroom species. In addition to agriculture these endobacteria may be used in production of different secondary metabolites and probiotics. The deposition of beneficial bacteria indicates a growing repository of useful microbial strains for agricultural/industrial biotechnology.

Overall, these results offer novel insights into the applications of microbe-microbe interactions in fungal biology and are potentially useful for formulation of valuable endobacteria-based bioinoculants in the mushroom industry for improving mushroom production, enhancing nutritional value, promoting sustainable agricultural practices, and contributing in environmental remediation efforts.

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PUBLICATIONS

1. **Paul C**, Roy T, Roy M, Rajeev AC, Pan A, Maitra M, Das N (2025) Genome wide analysis of *Priestia aryabhattai*_OP, an endobacterium, modulating growth, development and biochemical compositions of sporophores in Edible Oyster Mushroom *Pleurotus ostreatus* (MTCC 1802) **World Journal of Microbiology and Biotechnology** 41:194. <https://doi.org/10.1007/s11274-025-04438-z>
2. **Paul C**, Pal N, Maitra M, Das N (2024) Laccase-assisted bioremediation of pesticides: Scope and challenges. **Mini Reviews in Organic Chemistry** 21(6): 633-654. <https://doi.org/10.2174/1570193X20666221117161033>
3. **Paul C**, Maitra M, Das N (2023) Study of growth improving and sporophore-inducing endobacteria isolated from *Pleurotus pulmonarius*. **World Journal of Microbiology and Biotechnology** 39:349. <https://doi.org/10.1007/s11274-023-03776-0>
4. **Paul C**, Maitra M, Das N (2021) An Overview of Endophytic Bacteria in the Production of Bioactive Compounds of Nutraceutical Importance. In Hand book of agriculture and plant sciences.(Ed. Sinha D). **ABS Books Publisher and Exporter**. Delhi .Pp.118-145. ISBN: 978-93-91002-25-1.
5. **Paul C**, Maitra M, Das N (2020) Fungal lignocellulolytic enzymes: Physiological roles and biotechnological applications .In Research advances in the fungal world- Culture, isolation, identification, classification, characterization, properties and kinetics. Chapter 16 PK Chaurasia SL Bharati (editors) **Nova Science Publishers, Inc.**, New York. Pp. 321-371. ISBN: 978-1-53617-197-6
6. **Paul C**, Maitra M, Das N (2019) Comparative study of three *Pleurotus spp.* cultivated on two different substrates with special reference to their biochemical and antibacterial activities. **Mushroom Research** 28 (1): 39-46.



Genome wide analysis of *Priestia aryabhattai*_OP, an endobacterium, modulating growth, development and biochemical compositions of sporophores in edible oyster mushroom *Pleurotus ostreatus* (MTCC 1802)

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Abstract

The increasing global interest in the consuming and producing of edible oyster mushrooms (*Pleurotus* spp.) is driven by their well-documented nutritional and health benefits. The metagenomic analysis of fruiting body revealed a distinct microbial composition in *P. ostreatus*, predominantly comprising *Pseudomonadota* (~82%) and *Bacillota* (~10%). An endobacterium *Priestia aryabhattai*_OP, associated with internal tissue of *P. ostreatus* (MTCC 1802), was isolated and characterized through biochemical and microscopic analyses as well as 16 S rRNA and whole genome sequencing. Co-cultivation of *P. ostreatus* with this bacterium significantly enhanced the in vitro production of laccase, a key growth-promoting enzyme. Additionally, the endobacterium improved the biological efficiency (BE) of the mushroom, enriched its nutraceutical profile, and facilitated the biosynthesis of beneficial compounds, including IAA, siderophores, and antimicrobials like lassopeptides, phosphonates, non-ribosomal iron-binding siderophores (NI- siderophore), carotenoids, paenimodins, synechobactins, and surfactins. The present findings offer novel insights into microbe-microbe interactions and their pivotal roles in fungal biology, with significant implications for sustainable mushroom production as well as nutrient enrichment and biotechnological advancements.

Keywords Functional food · Laccase · Mushroom growth promoting Bacteria · Microbiome · Nutraceuticals · Whole genome sequencing

Introduction

Microorganisms play pivotal roles in global biogeochemical cycles. They also responsible in maintaining the health of plants and animals, and supporting various biotechnological

processes (Kleiner et al. 2017). They can be found in the surface and internal tissues of most of the living systems and offer a crucial role in the growth and development of their host organisms (Proctor 2011; Carrasco and Preston 2020). The microbiome, which comprises the collective

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REVIEW ARTICLE

Laccase-assisted Bioremediation of Pesticides: Scope and Challenges

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Abstract: Laccase (Benzenediol: oxygen oxidoreductase; E.C.1.10.3.2), a multicopper oxidase that is a known lignin-degrading enzyme, can catalyze an ample array of substrates, from phenolic, non-phenolic compounds, aromatic amines, diamines, heterocyclic compounds to organic/inorganic metal compounds *etc.*, bestowed they have not too high redox potentials. Despite many laccase-producing organisms like bacteria, insects, plants, and animals, white rot filamentous fungi are the best producers of this enzyme. In the presence of laccase, pesticides (fungicides, herbicides, insecticides, *etc.*) of various chemical compositions (organophosphates, organochlorines, carbamates, pyrethrin & pyrethroids *etc.*) are oxidized into the water with collateral reduction of four electrons of molecular oxygen with various efficiencies. Bioremediation efficiency can be increased in the presence of various natural or synthetic mediators, viz. ABTS, violuric acid, 1-hydroxy benzotriazole, vanillin, syringaldehyde, PEG, *etc.* Immobilized laccase on various supporting materials increased the enzyme's stability, reliability, and reusability for continuous application, particularly for industrial processes. The present review discusses the structure, catalytic cycle, general mechanism of oxidation, and various scopes and challenges of pesticide degradation by this multifaceted biocatalyst which could lead to a green sustainable environment.

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1. INTRODUCTION

Pesticides are either single or a mixture of substances frequently utilized in present agricultural practices to efficiently produce various foodstuffs for destroying, controlling or preventing any pest attack, including vectors of human diseases, animal diseases, and invasive animal or plant species. To feed more than 7.90 billion population with a limited area of agricultural land, the use of pesticides is of the utmost necessity. However, the indiscriminate use of pesticides compromises the existence of the present biome and our successors. Approximately 2 million tons of pesticides (herbicides, insecticides, fungicides, nematicides, *etc.*) are produced and consumed [1]. More than 95% of sprayed herbicides and 98% of sprayed insecticides end up in places where their intended targets are absent, including nontarget species in the water, soil, and air [2]. Pesticides have a high risk of polluting the environment, including rivers, marine waters, streams, ground water, *etc.*, because of their wide-spread and extensive usage [3], and several pesticides are bioaccumulated and biomagnified through our food chain [4–6]. Moreover, pesticide exposure is linked to several det-

ritional health impacts, including respiratory issues, skin damage, congenital disabilities, mental disorders, depression, miscarriages, cognitive defects, hematopoietic cancer, and even death [7–9]. Sharma *et al.* [10] reported the occurrence of 385 million cases of unpremeditated pesticide poisoning globally per year as per the database of WHO.

For the sake of ecological sustainability and survival, removing these hazardous substances from soil, wastewater, and our ecosystem is imperative. Physical or chemical approaches (flocculation, photocatalytic degradation, ozonation, coagulation, and Fenton oxidation) to degrade organic pollutants are expensive, time-consuming, primarily inefficient, and can result in the production of more recalcitrant compounds [11, 12]. More recently, researchers have investigated that microorganisms play a significant role in degrading different organic contaminants and pesticides. Bioremediation involves microorganisms, *i.e.*, fungi, archaea, and bacteria (either naturally occurring or introduced) in the degradation of pollutants [13]. It is well known that many bacterial and fungal organisms degrade organic contaminants metabolically and concurrently [14]. Microbial communities, mainly filamentous fungi and bacteria, can interact chemically or physically with various pesticides, leading to degradation or structural alterations and transformation of pesticide molecules. The soil's most significant pesticide breakdown pathway is microbial metabolism [15]. Therefore, effective bioremediation strategies should be implemented to elimi-

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Study of growth-improving and sporophore-inducing endobacteria isolated from *Pleurotus pulmonarius*

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Abstract

Several *Pleurotus* species (oyster mushrooms) are commercially cultivated in India owing to the favorable tropical agro-climatic conditions. However, there are only a few studies on the microbiome of mushrooms, especially oyster mushrooms. The aim of this study was to assess the effect of endobacteria on mycelial growth, spawning, sporophore development, and proximate composition of *P. pulmonarius*. We isolated several bacterial strains from the sporophores of *P. pulmonarius* and assessed the in vitro production of indole acetic acid, ammonia, and siderophores. The selected bacteria were individually supplemented with spawn, substrate, or both for sporophore production. Three of 130 isolates were selected as mycelial growth-promoting bacteria in both solid and submerged fermentation. These bacterial isolates were identified through Gram staining, biochemical characterization, and 16S rRNA sequencing. Isolate PP showed 99.24% similarity with *Priestia paraflexa*, whereas isolates PJ1 and PJ2 showed 99.78% and 99.65% similarities, respectively, with *Rossellomorea marisflavi*. The bacterial supplementation with spawn, substrate, or both, increased the biological efficiency (BE) and nutrient content of the mushrooms. The bacterial supplementation with substrate augmented BE by 64.84%, 13.73%, and 27.13% using PJ2, PP, and PJ1, respectively; under similar conditions of spawn supplementation, BE was increased by 15.24%, 47.30%, 48.10%, respectively. Overall, the supplementation of endobacteria to improve oyster mushroom cultivation may open a new avenue for sustainable agricultural practices in the mushroom industry.

Keywords Endobacteria · Indole acetic acid · Mushroom growth-promoting bacteria · *Priestia paraflexa* · *Pleurotus pulmonarius* · *Rossellomorea marisflavi*

Introduction

All living organisms, including humans and plants, are associated with different microorganisms throughout their lifecycles (Proctor 2011). The continuous interaction among higher organisms, environment, and microbes accounts for the ecological balance and sustainability. The microbes, collectively termed microbiomes, may be beneficially,

harmfully, symbiotically, or opportunistically associated with the hosts and play a pivotal role in the growth and development of the hosts (Carrasco and Preston 2020; Proctor 2011).

The interactions between hosts and endogenous organisms have been described as balanced host–pathogen antagonism for secondary metabolite production of the associates, with endophytes typically causing no harm to their host plants (Schulz et al. 1999). These interactions range from mutualism to commensalism to parasitism. The complex host–endophyte relationships represent a unique ecological niche (Ancheeva et al. 2020; Aslani et al. 2018; Carrasco and Preston 2020). Endophytes play a major role in improving the uptake of nutrients, controlling pathogen infection, enhancing or inhibiting host development, and producing bioactive compounds (Aslani et al. 2018; Chen et al. 2022a, b). Similar to plants, mushrooms contain numerous endobacteria as a part of their microbiome (Rossouw and Korsten 2017).

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An Overview of Endophytic Bacteria in the Production of Bioactive Compounds of Nutraceutical Importance

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Introduction

Nutraceuticals is amalgamation of the words “nutrition” and “pharmaceuticals”, is any substance considered as a food or its part which have health and therapeutic benefits and also provide protection against chronic disease, proposed by scientist Stephen de Felice (Biesalski, 2001). About 2500 years ago

Chapter 16

**FUNGAL LIGNOCELLULOLYTIC ENZYMES:
PHYSIOLOGICAL ROLES AND
BIOTECHNOLOGICAL APPLICATIONS**

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ABSTRACT

Lignocellulose, a complex of cellulose and hemicelluloses, encrusted with lignin is one of the major components of biomass. Their abundant presence in agricultural and forest residues very often is considered as wastes and is destroyed by burning which creates environmental pollution. The structural integrity of lignocellulosic materials is generally resistant to most of the microorganisms except some white rot fungi which produce different lignocellulolytic enzymes mainly to collect the resources for their growth and development. The lignocellulolytic enzymes comprised of a number of enzymes which includes cellulases, hemicellulases, peroxidases and laccases. In addition to mycelial growth, they may help in different physiological processes in producer organisms viz. fruiting body development, sporulation, melanin formation, pathogenesis etc. Based on lignocellulosic degradation, a number of biotechnological applications of these enzymes are suggested including fermentation, food and fodder production, biobleaching, biopolishing, bioremediation etc. The application of various lignocellulolytic enzymes in agriculture, alcohol and brewing industry, paper and pulp industry, textile industry as well as in different medical practices are also discussed in this chapter.

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Comparative study of three *Pleurotus* spp. cultivated on two different substrates with special reference to their biochemical and antibacterial activities

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ABSTRACT

Cultivation of oyster mushroom (*Pleurotus* spp) is a very simple low cost technology. Three *Pleurotus* spp. (*P. ostreatus*, *P. florida* and *P. pulmonarius*) were successfully cultivated in $30 \pm 2^\circ\text{C}$ on two different substrates viz. rice straw and sugarcane bagasse. The biological efficiency (BE) was better in rice straw than sugarcane bagasse when used as substrate. The highest BE was found in *P. florida* followed by *P. ostreatus*. All the biochemical studies were done using 70% methanolic extract of fresh mushroom fruiting bodies except protein and carbohydrate determination. When cultivated on sugarcane bagasse, *P. pulmonarius* showed the highest carbohydrate content (103.46 g/mg dry wt.) whereas *P. ostreatus* showed the highest protein content (12.16 g/mg dry wt.). *P. ostreatus* showed the highest polyphenol content (6.11 g/mg dry wt.) and *P. pulmonarius* (1.66 g/mg dry wt) showed the lowest when grown in rice straw. Flavonoid contents were highest in *P. florida* when grown in sugarcane bagasse. Antioxidant activities of the mushroom extract in terms of DPPH oxidation was estimated in all the species and there was no notable differences on the effect of substrates. *P. ostreatus* showed the highest DPPH oxidation whereas *P. pulmonarius* showed the lowest. Methanolic extract of all the fruiting bodies showed the antibacterial activities. There was no remarkable differences of antimicrobial activities among the mushrooms derived from two different substrates. Rice straw grown *P. pulmonarius* showed the maximum growth inhibition of bacteria.

Keywords: Antioxidant activity, biological efficiency, oyster mushroom, *Pleurotus* spp.

Mushrooms are plethora of fungi mainly from the division basidiomycotina of the kingdom mycota. From time immemorial they are regarded as delicious food. According to Kurtzman (1976) mushroom protein are intermediary between that of animals and vegetables whereas Purkayastha and Nayak (1981) considered them as of superior qualities due to the presence of all the essential amino acids. Oyster mushrooms (*Pleurotus* spp.) are grown in the wild in tropical, sub tropical and temperate regions as well as commercially cultivated throughout the world (Li, 2017). According to Sanchez (2010) *Pleurotus* spp. positioned second in the world in respect to their consumption which is just after *Agaricus* spp. They are most popular edible

mushrooms in India due to their effortless cultivation procedures within an ample range of temperatures ($15 - 30^\circ\text{C}$) on different varieties of substrates like agro forest residues, weeds and wastes (Das *et al.*, 2007; Gregory *et al.*, 2007; Obodai *et al.*, 2003). Oyster mushrooms have been regarded as healthy foods, low in calories, rich in protein, fibre, chitin, vitamins and minerals (Das *et al.*, 2015; Jayakumar *et al.*, 2009; Akindahunsi and Oyetayo, 2006; Manzi *et al.*, 2004). They not only regarded as nutritious food but also produce a number of enzymes and secondary metabolites which are considered as nutraceuticals (Das and Mukherjee, 2007; Gregory *et al.*, 2007). These nutraceuticals can be used for prevention and

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SEMINAR AND PRESENTATIONS

1. **Paul C**, Maitra M, Das N (2025) Exploring the Role of Endobacterium *Rossellomorea marisflavi* FS2: A Sustainable Approach to Boosting *Pleurotus florida* Cultivation and Environmental Remediation. Poster Presentation in one day International conference on **Frontiers In Biological Scinces (chapter IV)**. 14th February. **St. Xavier's College** (Autonomous), Kolkata. (Secured 3rd position).
2. **Paul C**, Maitra M, Das N (2023) Role of Endobacteria in Growth Promotion and Sporophore Development in *Pleurotus ostreatus* and *Pleurotus florida*. Oral Presentation at **International conference BIOSPECTRUM on Biotechnology and Biological Sciences** in (16th -18th November, 2023). **University of Engineering and Management**, Kolkata in collaboration with **Universiti Sains Malaysia** School of Health sciences. Kolkata
3. **Paul C**, Mondal A, Maitra M, Das N (2023) Comparative Studies of Biochemical Compositions and Antioxidant Activities of Mycelia and Fruiting Bodies of *Pleurotus pulmonarius* Grown With or Without Bacterial Supplementation. Poster Presentation in **International seminar on Recent Development Trends in Biological Research**. 15th July. **Acharya Prafulla Chandra College**. Kolkata.
4. **Paul C**, Maitra M, Das N (2022) Isolation and Characterization of Some Growth Promoting Bacteria from *Pleurotus pulmonarius*. Poster Presentation in **International conference on Plants and Microbes: Health & Food**. 28th -29th December. Department of Botany, **University of Calcutta**. Kolkata.
5. **Paul C**, Maitra M, Das N (2019) Evaluation of five *Pleurotus spp.* for Their Biological Efficiency, Biochemicals and Antibacterial Activities. Poster Presentation in **National seminar on Application of Statistics in Natural Sciences**. 16th – 17th December. **St. Xavier's College** (Autonomous), Kolkata.
6. **Paul C**, Maitra M, Das N (2019) Comparative Study of Three *Pleurotus spp.* Cultivated on Two Different Substrates with Special Reference to Their Biochemical and Antibacterial Activities. Poster Presentation in 10^{6th} **Indian Science Congress**. 3rd -7th January. **Lovely Professional University**, Phagwara, Jalandhar

APPENDICES

MEDIA COMPOSITIONS

CAS Agar Medium: **Solution 1-** 50 ml of aqueous solution of 0.06gm CAS, **Solution 2-** 10 ml of 1mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (in 10 mM HCl), **Solution 3-** 40 ml of aqueous solution of 0.73 gm HDTMA, mix all three solutions. **Solution 4-** 3.0 gm of Casamino Acid in 30 ml water and filter sterilized, **Solution 5-** 2gm glucose, 2.0 gm mannitol, 439 mg MgSO_4 , 11 mg CaCl_2 , 1.17 mg MnSO_4 , 1.4 mg H_3BO_3 , 0.04 mg CuSO_4 , 1.2mg ZnSO_4 , 1.0 mg Na_2MoO_4 dissolved in 70 ml of water, **Solution 6-** 3.0 gm KH_2PO_4 , 8.3 gm NaCl and 10 gm NH_4Cl in 750 ml of water, **Solution 7-** 32.24gm PIPES dissolved in to 750 ml of salt solution (solution 6), NaOH solution was added to raise the pH-6.8. 20 gm Agar powder was mixed with solution 7. Finally 1000 ml volume was adjusted and autoclaved separately and mixed well before plating.

Glucose Broth Medium: Glucose-5.0 gm, Yeast extract-0.5gm, Beef extract-1.5 gm, Peptone- 5 gm, MgSO_4 - 0.2 gm, K_2HPO_4 -1.0 gm, NaCl-5.0 gm, $(\text{NH}_4)\text{H}_2\text{PO}_4$ -1.0 gm, Methyl Red- 0.08gm, dd H_2O -1 lit., pH -7.

Lactose Broth Medium: Lactose-5.0 gm, Yeast extract-0.5gm, Beef extract-1.5gm, Peptone- 5.0 gm, MgSO_4 - 0.2 gm, K_2HPO_4 -1.0 gm, NaCl-5.0 gm, $(\text{NH}_4)\text{H}_2\text{PO}_4$ -1.0 gm, Methyl Red- 0.08gm, dd H_2O -1 lit., pH -7.

Lysine Decarboxylase Broth Medium: Peptone- 5.0 gm, Yeast extract-3.0 gm, Dextrose- 1.0 gm, L- Lysine- 5.0 gm, Bromocresol Purple- 0.02gm, dd H_2O -1 lit., pH- 6.8.

Mannitol Broth Medium: Mannitol-5.0 gm, Beef extract-1.0 gm, Peptone-10 gm, NaCl-5.0 gm, Bromocresol Purple-0.015gm, ddH₂O-1 lit., pH -6.8.

MRVP Broth Medium: Peptone- 7.0 gm, Dextrose- 5.0gm, Potassium Phosphate-5.0 gm, ddH₂O-1 lit., pH -6.9.

Minimal Salt Medium: NaCl- 1.0 gm, (NH₄)₂SO₄- 1.0 gm, MgSO₄- 0.05 gm, K₂HPO₄- 0.5gm, ddH₂O-1 lit., pH 7.0.

NBRIP Medium: Glucose- 10 gm, Tri-calcium Phosphate (TCP)- 5.0gm, MgCl₂- 5.0gm, MgSO₄- 0.25gm, KCl- 0.20gm, (Na₄)₂SO₄- 0.10 gm, Bromophenol Blue- 0.025gm, Agar- 20gm, ddH₂O-1 lit., pH 7.0.

Nitrate Broth Medium: KNO₃ -1.0 gm, Peptone-5.0 gm, Beef extract-3.0 gm, ddH₂O-1 lit., pH 7.0.

Nutrient Agar Medium: Peptone- 5gm, NaCl- 5gm, Beef extract- 1.5gm, Yeast extract- 1.5 gm, Agar powder- 20gm, ddH₂O-1 lit., pH- 7.0.

Nutrient Broth Medium :Peptone- 5gm, NaCl- 5gm, Beef extract- 1.5gm, Yeast extract- 1.5gm, ddH₂O-1 lit., pH- 7.0.

Nutrient Gelatin Medium: Peptone-5gm, Beef extract-3 gm, NaCl- 5 gm, Gelatin- 15 gm, ddH₂O-1 lit., pH- 7.0.

Peptone Water: Peptone- 10 gm, NaCl- 5 gm, ddH₂O-1 lit., pH -7.2.

Potato Dextrose Agar Medium: Potato extract- 200 gm, Dextrose- 20 gm, Agar powder- 20 gm, ddH₂O-1 lit., pH- 7.0.

Potato Dextrose Broth Medium: Potato extract- 200 gm, Dextrose- 20 gm, ddH₂O-1 lit., pH- 7.0.

Simmons Citrate Agar Medium: Sodium Citrate-2.0 gm, MgSO₄ -0.2 gm, (NH₄)H₂PO₄ - 1.0 gm, K₂HPO₄ -1.0 gm, NaCl-5.0 gm, Bromothymol Blue-0.08 gm, Agar-20 gm, ddH₂O- 1 lit., pH -7.0.

Starch Agar Medium: Starch-20 gm, Beef extract-3.0 gm, Peptone-5gm, Agar-20 gm, ddH₂O-1 lit., pH-7.0.

Sucrose Broth Medium: Sucrose-5.0gm, Yeast extract-0.5gm, Beef extract-1.5gm, Peptone- 5.0 gm, MgSO₄ -0.2 gm, K₂HPO₄ -1.0 gm, NaCl-5gm, (NH₄)H₂PO₄ -0.1 gm Methyl Red-0.08gm, ddH₂O-1 lit., pH -7.0.

Tryptone Broth Medium: Tryptone- 10 gm, ddH₂O-1 lit., pH -7.0.

Urea broth Medium: Urea- 20 gm, Yeast extract 0.1 gm, K_2HPO_4 -9.5 gm, KH_2PO_4 - 9.0 gm, Phenol Red- 0.01 gm, ddH₂O-1 lit., pH -7.0.

REAGENTS

Barritt's reagent: VPI (5% 1-naphthol dissolved in absolute alcohol) & VPII reagent (40% KOH solution).

DNS reagent: 3,5-dinitrosalicylic acid 1 gm in 50 ml ddH₂O, Potassium sodium tartrate tetrahydrate-30 gm, 2(N) sodium hydroxide solution- 20 ml, final volume 100 ml.

Kovac's reagent: p-dimethylaminobenzaldehyde-15 gm, Isoamyl alcohol-150 gm, HCl-75 ml.

Nitrate test reagent: Zinc Chloride-20 gm, Starch-4.0 gm, Potassium iodide-2.0 gm, ddH₂O-1 lit, diluted H₂SO₄-(1:3)

Nessler's reagent: 2 gm of potassium iodide was added to 5 ml of distilled water. Then 3 gm of mercury (II) iodide was added to this solution and made to 20 ml by adding water. Finally, 40 g potassium hydroxide (30 %) was added to it.