Antimicrobial Activity of Mycelia of Oyster Mushroom Species (*Pleurotus spp.*) and their Liquid Filtrates (*In Vitro*)

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***Abstract*—*In vitro*, antimicrobial activity of four species of oyster mushrooms: *Pleurotus ostreatus* (grey and white strains), *Pleurotus cornucopiae* (bright yellow strain) and *Pleurotus salmoneostramineus* (pink stain) in form filtrates and mycelia were investigated against five standard strains of pathogenic bacteria and yeast. The filtrate of *P. salmoneostramineus* was best one compared with other filtrates against *Pseudomonas aeruginosa* ATCC 27853 and *Candida parapsilosis* ATCC 22019 respectively. *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* HIP10267 have low sensitive. Mycelia of *P. cornucopiae* inhibited colonies of the bacterium *Enterococcus faecalis* ATCC 29212 and yeast *Candida parapsilosis* ATCC 22019 at 5.21% and 29.19% respectively. *Escherichia coli* ATCC 25922 was sensitive for mycelia of *P. salmoneostramineus* only in significant (P< 0.05) inhibition value 6.67%.**

***Index Terms*—*pleurotus* spp. antimicrobial activity. culture filtrate. mycelia. interaction.**

I. Introduction

Oyster mushroom is an edible mushroom, can be cultivated on a wide variety of substrates containing lignin and cellulose. It has nutritional and medicinal properties [1]. *Pleurotus* spp. belongs to Basidiomycota, order Agaricales, and the family Tricholomataceae. The fungi is important source for some chemicals such enzymes and antibiotic [2]. Consequently, the antimicrobial activity of various polysaccharides from medicinal mushrooms is being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilizing the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics [3]. Medicinal mushrooms are able to synthesize a great amount of secondary metabolites that present antitumoral, antiviral and anti-inflammatory activities [4]. Mushrooms have been used as food supplement from times immemorial not only for their flavor, aroma and nutritive values but also for their medicinal properties as evident from ancient literature [5].

Currently, a large range of mushrooms species are grown in liquid medium. The obtained mycelium is used for various applications, such as obtaining dietary supplements, pharmaceutical applications, conversion of waste into biomass and production of enzymes [1]. "[6]" find the secondary metabolism of Pleurotus erngii was active against *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *C. glabrata* and *Epidermophyton* spp. Generally, many studies find different activity of extracts of mushrooms against different pathogenic bacteria [7, 8, 9]. *Pleurotus* spp. has changeable activity against Gram-negative pathogenic bacteria and strong inhibition against Gram-positive pathogenic bacteria: *Bacillus subtilis* and *Micrococcus luteus* [10]. However, this article showed antibacterial and anti-yeast activity of mycelial of four species of oyster mushrooms and their liquid culture filtrate *in vitro*.

II. Material and Methods

A. Strains

Four oyster mushrooms species: *Pleurotus ostreatus* (grey), *Pleurotus ostreatus* (white), *Pleurotus cornucopiae* var. *citrinopileatus* (bright yellow) and *Pleurotus salmoneostramineus* (pink) were obtained from Mushroom Box Company, Monmouth, UK, in form spawn and sub cultured it on Potato Dextrose Agar (PDA) medium at 25 C° for this experiment. Five standard strains of microbes, bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* HIP10267 and *Enterococcus faecalis* ATCC 29212 and yeast: *Candida parapsilosis* ATCC 22019 obtained from USA.

B. Media and Liquid Culture Filtrate Preparation

In this experiment, Potato dextrose broth (PDB) and potato dextrose agar (PDA) were prepared [11]. The oyster mushrooms were cultured in Potato Dextrose Broth (PDB) by using five centimeter disk of seven days old culture in flask (250 ml) contains 50 ml of PDB, incubated at 25±1 C° for 20 days with once shake every

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day. Culture filtrates collected by double filter paper Whatman No.1, edited pH to 7 (neutral) by drops of NaOH (1N). Culture filtrates of oyster mushrooms were diluted with fresh PDB medium separately to make 50% concentrations (v/v) of each filtrate, sterilized by Autoclave at 121 C° for 25 minutes and 1.5 psi, then poured in test tubes 10 ml to test activity of liquid culture filtrate. Fresh PDB used as control [12]. PDA poured in Petri dishes 85 mm and used to test the activity of Pleurotus spp. mycelia.

C. Bioactivity of Filtrate of Oyster Mushrooms Mycelium

Liquid filtrates 50% (v/v) were used to determine the inhibition against pathogenic bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* HIP10267, *Enterococcus faecalis* ATCC 29212 and *Candida parapsilosis* ATCC 22019 in broth. Test tubes filled 10 ml of filtrate 50%, inoculated by 100 microliter bacteria 24 h old culture and incubated at 35 C° for 24 h. The antibacterial activity of liquid filtrate of Pleurotus spp. was evaluated by determine the bacterial growth by reading OD at 600 nm by spectrophotometer after 12, 24 and 36 h [13]. The OD value liquid culture filtrate higher than the OD value of control (PDB) indicates no inhibition.

D. Bioactivity of Mycelia of Oyster Mushrooms

Interaction between mycelia of *Pleurotus* spp. and colonies of pathogenic bacteria and yeast was achieved (Fig. 1). Bioactivity of mycelia was tested to determine ability of growth of bacteria colonies on PDA by Weller's Method [11], [14]. Six millimeters disk of seven days old culture of *Pleurotus* spp. placed in one side of dish, incubated at 25±1 C° for 48 h and put out of incubation. In opposite side six millimeters disk of seven days old culture of bacteria placed away 3 cm in another side. Then incubated again at same conditions for five days, calculated zone inhibition for bacteria colonies and compared with colonies of bacteria alone. Percent inhibition of mycelial growth (PIMG) was determined by this formula:

PIMG = [(*diameter of bacteria colony alone - diameter of bacteria colony in dual culture*) / *diameter of bacteria colony alone*)]× *100*

E. Statistical Analysis

Experimental values are given as means. Statistical significance was determined by Completely Randomized Design (CRD) in two variance (two ways) analysis (ANOVA) by using GenStat Discovery Edition computer program version 7 DE3 (VSN International Ltd., UK). Differences at P< 0.05 were considered to be significant. The experiments used three replicates.

III. Results

A. Microbial Growth in Liquid Culture Filtrates of Pleurotus spp.

Properties of mycelia of *Pleurotus* spp. and its liquid filtrate appeared in Table I. Final pH of liquid culture filtrate was average 6.68 for all oyster mushroom species in this study. The best significant (P< 0.05) fresh biomass weight after 20 days was 8.09 g 50 ml^{-1} of broth for *Pleurotus ostreatus* (grey), followed by *P. salmoneostramineus* and *P. ostreatus* (white) 5.00 and 4.53 g 50 ml^{-1} respectively. Less fresh biomass weight was 3.99 g 50 ml^{-1} by *P. cornucopiae*. So the dry biomass weight of each oyster mushroom specie in same level.

B. Interaction Between Mycelia of Pleurotus spp. and Colonies of Pathogenic Bacteria

Table II and Fig. 2 showed that fungus *P. salmoneostramineus* more affect against pathogenic bacteria except colonies of *Staphylococcus aureus* HIP10267 which was resistance for all species of oyster mushrooms. Mycelia of *P. cornucopiae* inhibited the bacterium *Enterococcus faecalis* ATCC 29212 and yeast *Candida parapsilosis* ATCC 22019 at 5.21% and 29.19% respectively. *Escherichia coli* ATCC 25922 was sensitive for mycelia of *P. salmoneostramineus* only in significant (P< 0.05) inhibition value 6.67%.

TABLE I. Properties of Mycelia of *Pleurotus* spp. and its Liquid Culture filtrate

Pleurotus spp.	Properties of mycelia and its filtrate			
	First pH	Final pH	Fresh biomass (g 50 ml^{-1})	Dry biomass (g 50 ml^{-1})
P. ostreatus (grey)	7.00	6.63	8.09	0.190
P. ostreatus (white)	7.00	6.49	4.53	0.140
P. cornucopiae (bright yellow)	7.00	6.68	3.99	0.103
P. salmoneostramineus (pink)	7.00	6.92	5.00	0.160
Average of properties	7.00	6.68	5.41	0.148
LSD P< 0.05	0.000	0.027	0.665	0.0217

TABLE II. Inhibition Percentage of Mycelia of *Pleurotus* spp. against Pathogenic Bacteria in Solid Media After Five Days

Bacteria strains (B)	Mycelia of *Pleurotus* spp. (M)				Bacteria Average
	P. ostreatus (grey)	*P. ostreatus* (white)	*P. cornucopiae* (bright yellow)	*P. salmoneostramineus* (pink)	
Escherichia coli ATCC 25922	0.00	0.00	0.00	6.67	1.67
Pseudomonas aeruginosa ATCC 27853	7.23	6.02	0.00	23.29	9.14
Staphylococcus aureus HIP10267	0.00	0.00	0.00	0.00	0.00
Enterococcus faecalis ATCC 29212	6.25	6.25	5.21	6.25	5.99
Candida parapsilosis ATCC 22019	29.19	34.37	29.19	24.87	29.40
Oyster mushroom average (M)	8.53	9.33	6.88	12.22	9.24
LSD P< 0.05	B=0.909, M=0.813, B * M=1.817				

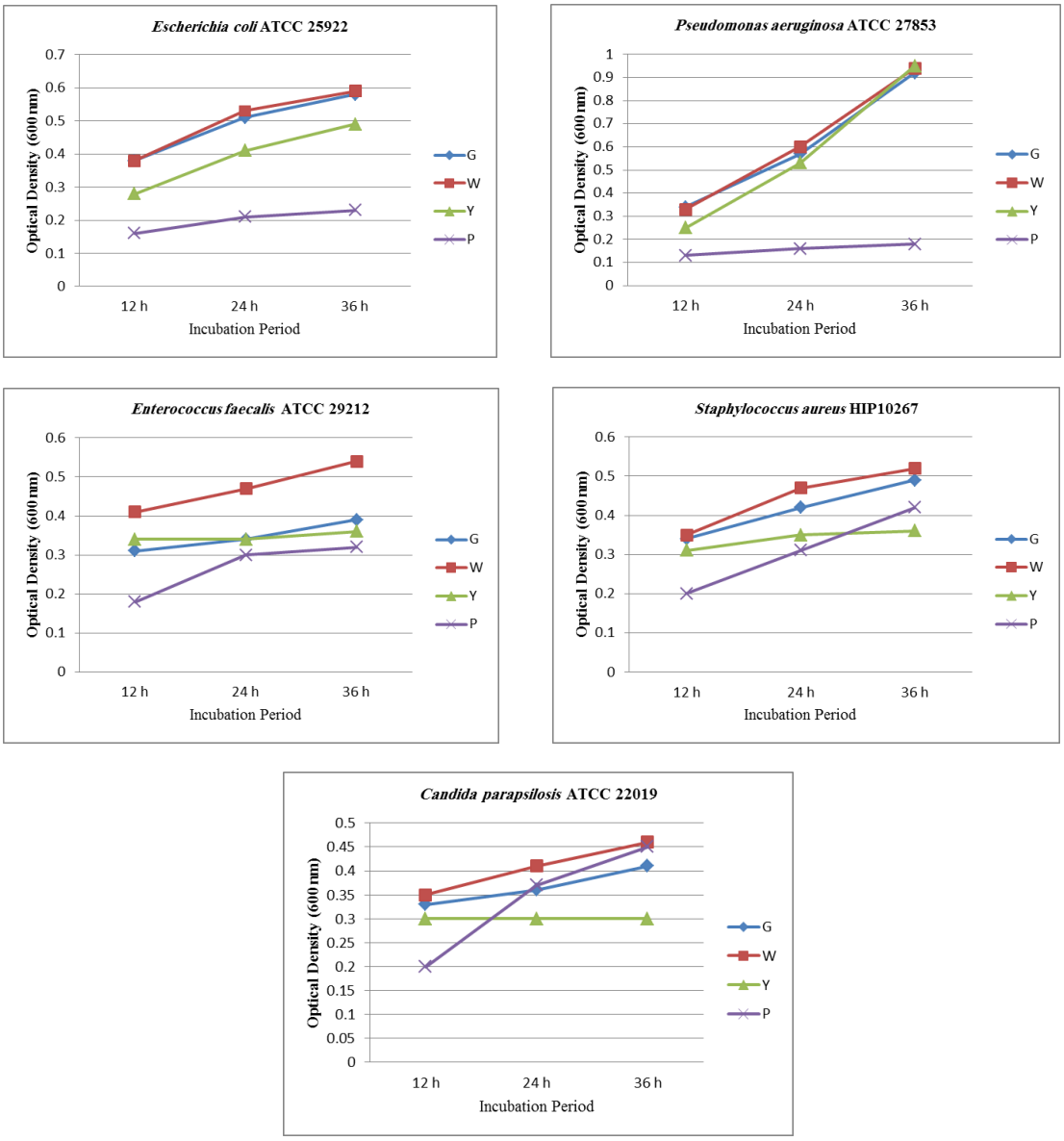
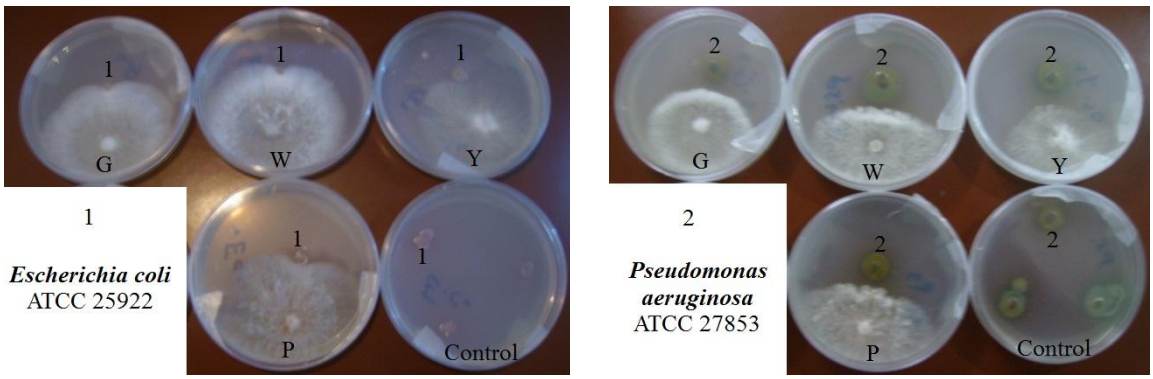


Figure 1. Growth of bacteria and yeast in liquid culture filtrate 50% (v/v) of *Pleurotus* spp. with PDB

G: liquid culture filtrate 50% (v/v) of *P. ostreatus* (grey) with PDB, **W**: liquid culture filtrate 50% (v/v) of *P. ostreatus* (white) with PDB, **Y**: liquid culture filtrate 50% (v/v) of *P. cornucopiae* with PDB, **P**: liquid culture filtrate 50% (v/v) of *P. salmoneostramineus* with PDB. The OD value liquid culture filtrate higher than the OD value of control indicates no inhibition.



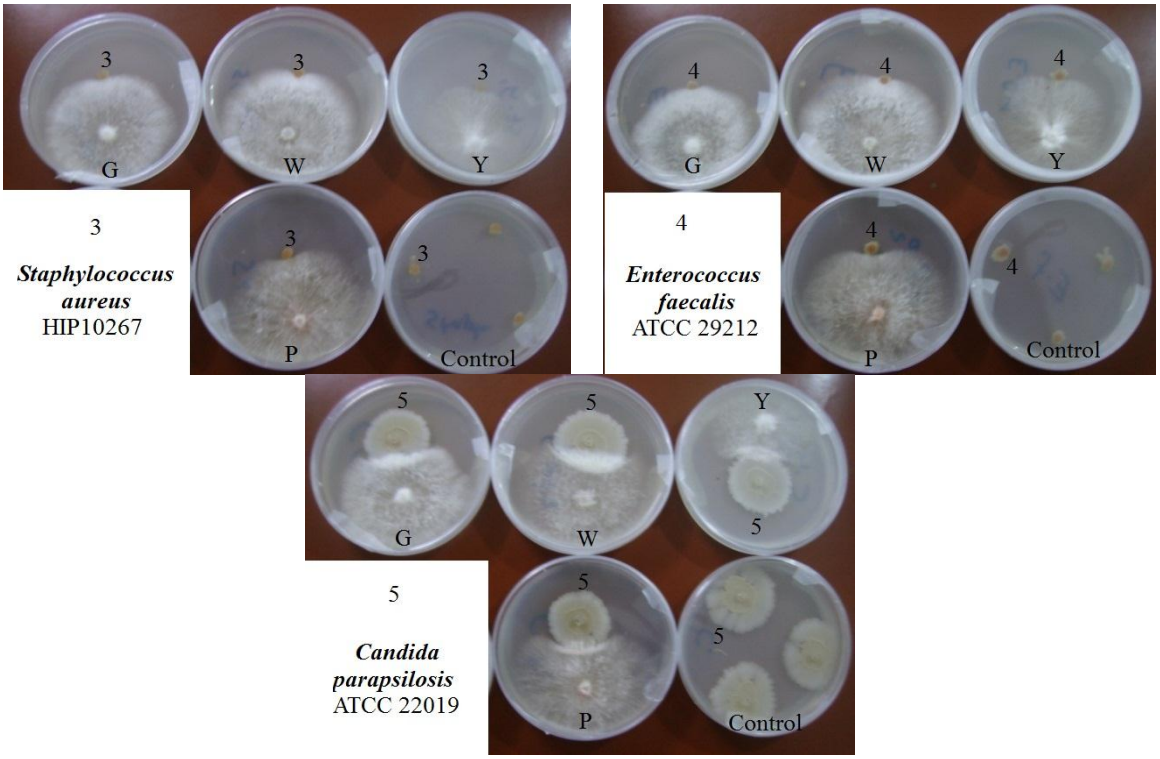


Figure 2. Interaction between mycelia of *Pleurotus* spp. and colonies of pathogenic bacteria after five days

G: *P. ostreatus* (grey), **W**: *P. ostreatus* (white), **Y**: *P. cornucopiae*, **P**: *P. salmoneostramineus*

IV. Discussion

Secondary metabolism in liquid medium was important against bacteria. Fig. 1 showed effect of *Pleurotus* spp. filtrate against growth of bacteria and yeast. The filtrate of *P. salmoneostramineus* was best one compared with other filtrates against *Pseudomonas aeruginosa* ATCC 27853 and *Candida parapsilosis* ATCC 22019 respectively. *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* HIP10267 have low sensitive due to the genetic characters of species of oyster mushroom which lead to different secondary metabolism. Metabolism affected on production bioactivity of this filtrate. Bioactivity of filtrate of *P. salmoneostramineus* may be belong to glycoprotein called Indolone was important for O_2 production in water [15]. The chemical composition of liquid filtrate of oyster mushroom is varying according to type of product of fungi [16].

The filtrate of pink strain *P. salmoneostramineus* has a higher antimicrobial activity as to comparison other filtrates and PDB (Fig. 1). And also, *P. cornucopiae* liquid filtrate was appeared low growth of Gram-positive bacteria *Staphylococcus aureus* HIP10267, *Enterococcus faecalis* ATCC 29212 and *Candida parapsilosis* ATCC 22019 (Fig. 1). None of the filtrates of *P. ostreatus* (grey and white strains) showed any activity against pathogenic bacteria and yeast.

Resistant bacteria were appeared in liquid filtrate because of loss some secondary metabolism bioactivity such proteins and polysaccharides by heat when sterilized [17]. Oyster mushrooms enable to produce metabolism products in 48 h to inhibit growth of pathogenic bacteria [18] due to produce polysaccharides, proteins, enzymes and triterpenoides of mycelia of *Pleurotus* spp. [19, 5]. The simple indolone was isolated from the edible mushroom *Pleurotus salmoneostramineus* as a glycoprotein conjugate by aqueous extraction. Indolone plays a role in the photochemical generation of oxygen from water, suggesting the possible involvement of indolone in photosynthesis [15] (Liu, 2004).

Results of this paper agree with results of "[20]" that referred to loss activity of *Agaricus bisporus* of spent mushrooms substrate against 19 bacteria and yeast after treated the filtrate broth by heat. And also, "[21]" searched three extracts of mushroom which decrease or loss of their effectiveness after being exposed to temperatures ranging between 60℃ and 100℃.

V. Conclusion

This is a comprehensive *In vitro* study reported the anti-bacterial and anti-yeast effects of *Pleurotus* spp. The liquid filtrate of *P. salmoneostramineus* was best one compared with other filtrates against *Pseudomonas aeruginosa* ATCC 27853 and *Candida parapsilosis* ATCC 22019 respectively. Mycelia of *P. cornucopiae* inhibited colonies of the bacterium *Enterococcus faecalis* ATCC 29212 and yeast *Candida parapsilosis* ATCC 22019 at 5.21% and 29.19% respectively. *Escherichia coli* ATCC 25922 was sensitive for mycelia of *P. salmoneostramineus* only inhibition value 6.67%, significantly ($P< 0.05$).

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