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Antimicrobial Activities of Basidiocarp, Submerged Mycelium and Exopolysaccharide of Some Native Basidiomycetes Strains

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Abstract

In this study, extracts from basidiocarps, submerged grown mycelia and crude exopolysaccharide precipitates of *Clavariadelphus truncatus* (Quél.) Donk (*Gomphaceae*), *Coprinus comatus* (O.F. Müll.) Pers. (*Agaricaceae*), *Ganoderma carnosum* Pat. (*Ganodermataceae*), *Cerrena unicolor* (Bull.) Murrill, *Laetiporus sulphureus* (Bull.) Murrill, *Lentinus strigosus* (Schwein.) Fr., *Lenzites betulina* (L.) Fr. and *Polyporus arcularius* (Batsch) Fr. (*Polyporaceae*) were tested for antimicrobial activity. The activity was evaluated by hole-plate diffusion and disk diffusion tests using bacteria and yeasts. Vancomycine and fluconazole were used as positive controls for bacteria and yeasts, respectively. The crude exopolysaccharides of *Ganoderma carnosum, Polyporus arcularius, Lenzites betulina, Cerrena unicolor*; and basidiocarps of *Ganoderma carnosum, Laetiporus sulphureus, Coprinus comatus, Lenzites betulina* and *Clavariadelphus truncatus* have relatively high antimicrobial activity. Mycelial extracts from *Cerrena unicolor* showed the highest antimicrobial activity against the test cultures.

Key words: Antimicrobial activity, Basidiocarp, Exopolysaccharide, Macrofungi, Submerged Mycelia

INTRODUCTION

It has been known that macrofungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity [1]. Dioscorides, first century Greek physician, knew that *Laricifomes* (*Fomitopsis*) *officinalis* (Vill.) Kotl. & Pouzar (*Fomitopsidaceae*) can be used for treatment of "consumption", a disease now known as tuberculosis [2]. The famous 5300 year-old Otzi, or Ice Man, had *Piptoporus betulinus* (Bull.) P. Karst. (*Fomitopsidaceae*) and *Fomes fomentarius* (L.) J.J. Kickx (*Polyporaceae*) with him when his body was discovered [2].

It is believed that mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Antimicrobial compounds could be isolated from many mushroom species and some proved to be of benefit for humans [3]. As an antifungal and antibacterial compound, Sparassol was isolated in the early 1920s from *Sparassis crispa*. Since then, several antifungal and antibacterial compounds have been isolated from different macrofungi species. In early studies performed by Anchel, Hervey and Wilkins in 1941, diverse antibiotic activity was detected in basidiocarp or mycelial culture extracts of more than 2000 fungal species [4]. It was succeeded by the isolation and identification of pleuromutilin [5]. This compound has served for the development of the first commercial antibiotic of Basidiomycete origin. Then, other compounds with antimicrobial activity were isolated and characterized from macrofungi such as mucidin [6], aegeritin [7], enokipodin C and D [8], hericenols [9] and so forth. But antibiotic compounds from microscopic fungi are dominant on market [3].

In recent *in vitro* studies, screening for the antimicrobial activity of basidiomycete strains, some studies were done both of in basidiocarp and in submerged culture. Antimicrobial activities of basidiomycete strains from different countries were screened in submerged culture [4, 10, 11]. Suay et al. (2000) reported diverse antibiotic activity in mycelial cultures of 204 macrofungi species. Forty-five percent of 317 isolate inhibited growth of a wide variety of microorganisms [10]. Similarly, Rosa et al. (2003) detected 14 mushroom isolates with significant activity against one or more of the target microorganisms [4]. Zjawiony (2004) observed that 75% of polypore fungi that have been tested show strong antimicrobial activity [12].

Antibacterial activities of mushroom exopolysaccharides such as lentinan (from *Lentinus edodes*), schizophyllan (from *Schizophyllum commune*) and PSK ("Polysaccharide Kureha" from *Trametes versicolor*) have also been reported [2, 13-16]. As a matter of fact, macrofungi produce a large number of metabolites that show antibacterial and antifungal activity. Thus, these fungi may be a source of new and useful bioactive compounds. In this context, the antimicrobial activity of basidiocarps, submerged mycelia and exopolysaccharides of nine newly isolated mushroom strains are reported here.

MATERIALS and METHODS

Fungal organisms

Clavariadelphus truncatus (Quél.) Donk (*Gomphaceae*), *Coprinus comatus* (O.F. Müll.) Pers. (*Agaricaceae*), *Ganoderma carnosum* Pat. (*Ganodermataceae*), *Cerrena unicolor* (Bull.) Murrill, *Laetiporus sulphureus* (Bull.) Murrill, *Lentinus strigosus* (Schwein.) Fr. *Lenzites betulina* (L.) Fr. and *Polyporus arcularius* (Batsch) Fr. (*Polyporaceae*) were used in

screening for the antimicrobial activity. All macrofungi were collected during field trips in Eskişehir, Sakarya and Bartın - Turkey. The morphological and ecological characteristics of the collected macrofungi were recorded and photographed in their natural habitats. Dried specimens were numbered and placed in locked bags. The specimens were identified by their macroscopic and microscopic features [17-21].

Basidiocarp extract preparation (BC)

The basidiocarps were cut into small pieces, air-dried and ground to fine powder. The powdered material (20 g) was extracted with 100 ml of n-heptane, diethyl ether, chloroform and dichloromethane with stirring 150 rpm for 1 day. The extracts were filtered and the solvents were completely evaporated to dryness at 40°C using a rotary vacuum evaporator.

Submerged mycelium preparation (SM)

Mycelial cultures of the fungi were obtained from the living tissues of the basidiocarps in potato dextrose agar (PDA; Merck) medium. All isolates were kept on PDA slants at 4°C. The mycelia were grown at 25°C in submerged liquid cultures in Potato Malt Peptone Medium (PMP; Malt extract 10 g/L, peptone 1 g/L, Potato dextrose broth 24 g/L). One hundred milliliters of the medium in 250 mL Erlenmeyer flasks was inoculated by five mm diameter mycelial agar plugs taken from the PDA plates. The inoculated flasks were shaken at 150 rpm at 25°C for 7 days. The culture fluid was separated from pellets by filtration. Mycelial extracts were prepared like basidiocarp extracts.

Exopolysaccharide preparation

Fresh potato dextrose agar (PDA) cultures of strains were used as pre-inocula. Five mm diameter agar disks of these cultures transferred into the seed medium. The liquid seed cultures were prepared in a 250 ml flask containing 50 ml of potato malt peptone (PMP) medium at 25ºC, 150 rev/min, for 4 days. Then, the flasks were homogenized and inoculated to 4% (v/v) PMP medium of the seed culture. The flask cultures were incubated at 25ºC, 150 rev/min, for 7 days, filtered to separate fungal biomass. To precipitate exopolysaccharide (EPS), the resulting culture filtrates were mixed with four

Table 1. Antimicrobial activities of basidiocarps of basidiomycetes strains

Fungus Species and Code	Solvent[a]	Inhibition zone[b, c]							
		A	B	C	D	E	F	G	H
Cerrena unicolor (D 30)	Hp	-	+	-	-	-	-	-	+
	De	-	+	-	+	+	-	+	+
	Kl	-	-	-	-	-	-	+	+
	Dm	-	+	-	-	-	-	-	+
	Ea	-	-	-	-	-	-	++	+
Clavariadelphus truncatus (T 192)	Hp	-	+	-	-	-	-	+	+
	De	-	+	-	+	-	-	+	+
	Kl	+	+	++	++	-	-	++	+
	Dm	-	+	-	+	-	-	++	+
Coprinus comatus (M 118)	Hp	-	-	-	-	-	+++	-	-
	De	-	+	-	++	+	-	-	++
	Kl	-	-	-	++	-	+	-	++
	Dm	-	-	-	-	-	-	-	++
	Ea	-	-	-	-	+	-	-	-
Ganoderma carnosum (M 88)	Hp	+	+	-	-	-	-	+	+
	De	-	+	-	-	-	-	+	+
	Kl	-	+	-	-	-	-	+	+
	Dm	-	+	-	-	-	-	+	+
	Ea	-	+	-	+	-	-	+	+
Laetiporus sulphureus (M 107)	Hp	-	+	-	+	+	-	-	+
	De	-	+	-	+	+	+	+	+
	Kl	-	+	+	-	+	+	+	+
	Dm	-	+	-	-	+	+	+	+
	Ea	-	+	-	-	+	-	-	+
Lentinus strigosus (D 26)	Hp	-	-	-	++	+	+	-	-
	De	-	-	-	++	-	-	-	+
	Kl	-	-	-	-	-	+	-	++
	Dm	-	-	-	-	-	-	-	++
	Ea	-	-	-	-	+	-	-	-
Lenzites betulina (S 2)	Hp	-	+	-	+	-	-	-	+
	De	-	+	-	+	-	+	+	+
	Kl	-	-	+	+	-	+	+	+
	Dm	-	+	+	+	-	+	+	+
	Ea	-	-	-	-	-	-	-	+
Polyporus arcularius (T 438)	Hp	-	+	-	-	-	-	-	-
	De	-	+	-	-	-	-	-	-
	Kl	-	+	-	-	-	-	-	-
	Dm	-	+	-	-	-	-	-	-
	Ea	-	+	-	-	-	-	-	-
Standard Antibiotics[d] (30 µg/disk)		++++	++	++	++	++	-	++	++

[a] Hp: Heptane, De: Diethylether, Kl: Chloroform, Dm: Dichloromethane, Ea: Ethyl acetate

[b] A: *Bacillus subtilis* (NRRL B-3711) B: *Staphylococcus aureus* (ATCC 25923) C: *Micrococcus luteus* (NRRL B-1018) D: *Enterococcus faecium* (NRRL B-2354) E: *Eschericha coli* (ATTC 25992) F: *Proteus vulgaris* (NRRL B-123) G: Candida albicans (NRRL Y-12983) H: Candida glabrata (isolate obtained from Eskişehir Osmangazi University, Faculty of Medicine)

[c] +: <10 mm, ++: 10-15 mm, +++: 15-20 mm, ++++: >20 mm, -: Without activity, NT: Not tested

[d] Vancomycine for bacteria and Fluconazole for fungi

volumes of ethanol, stirred vigorously and left overnight at 4ºC. The precipitated EPS was recovered by centrifugation at 10,000 rpm for 10 min and the supernatant was discarded. The precipitate of crude EPS was lyophilized [22].

All extracts and mycelial cultures were stored at 4°C until use for assaying their activities.

Determination of antimicrobial activity

In vitro antimicrobial susceptibility studies were performed using the following strains; *Bacillus subtilis* (NRRL B-3711), *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (NRRL B-1018), *Enterococcus faecium* (NRRL B-2354), *Eschericha coli* (ATTC 25992), *Proteus vulgaris* (NRRL B-123), *Candida albicans* (NRRL Y-12983), *Candida glabrata* (isolate obtained from Eskişehir Osmangazi University, Faculty of Medicine). Antimicrobial activities of all extracts and fractions were screened by the disk diffusion and agar well diffusion methods.

Disk diffusion method

Test microorganisms were activated in Mueller Hinton Broth (37°C, 150 rpm, 24 h). The Mc Farland (No: 0.5) standard is used to adjust the turbidity to prepare inoculum from overnight grown bacteria and yeast cultures. Then, 100 µL of inocula were uniformly spread on the surfaces of Mueller-Hinton agar (Oxoid) for bacteria and Saburoud dextrose agar (Merck) plates for yeasts. 30 µg of crude macrofungus extract dissolved in 15 µl of 20% DMSO were applied to a 6 mm diameter paper disk (Whatman No: 1). Paper disks were placed onto the MHA plates inoculated with test microorganisms. The inoculated plates were incubated overnight at 37°C. Antimicrobial activity was assayed by measuring the inhibition zones around the disks. [23].

Agar well method

The plates were inoculated with the test organisms by the above-mentioned method. 15 µl of EPS solutions (2 mg/ml) were added to each well (6 mm diameter holes cut in the agar gel). The plates were incubated at 37°C for 24 h for bacteria, and at 30°C for 48 h for yeasts. Antimicrobial activity was determined by measuring the radius of the clear inhibition zone around each well [24].

Standard antimicrobial agents, vancomycine and fluconazole (30 µg/disk) were used as positive controls for bacteria and yeasts, respectively. Disks injected with 20 µL of 20% DMSO were observed as negative control. Each experiment was replicated three times and the results were expressed as average values.

RESULTS and DISCUSSION

In this study, antimicrobial activity of basidiocarp, submerged mycelial growth and exopolysaccharides of several basidiomycete strains against test microorganisms was compared with the results of positive control antibiotics of vancomycine and fluconazole. Among the 94 extracts studied, 86 (91%) show antimicrobial activity against at least one of the test microorganisms (Table 1-3). But, the extracts show weak antimicrobial activities, in general.

Antimicrobial activity of basidiocarp, submerged mycelial growth and exopolysaccharides of basidiomycete strains

Table 2. Antimicrobial activities of submerged mycelium of basidiomycetes strains[a]

Fungus Species and Code	Solvent	Inhibition zone							
		A	B	C	D	E	F	G	H
Cerrena unicolor (D 30)	Hp	-	+	+	+++	+	-	-	+
	De	-	+	+	+	+	-	-	+
	Kl	-	++	++	+++	+	-	-	+
	Dm	-	++	++	+++	+	-	-	++
	Ea	-	+	+	+++	+	-	-	+
Clavariadelphus truncatus (T 192)	De	-	+	+	+	+	-	+	-
	Dm	-	-	-	-	-	-	+	-
	Ea	-	+	-	-	-	-	+	-
Coprinus comatus (M 118)	Hp	-	-	-	-	-	-	-	+
	De	-	-	-	-	-	-	-	++
	Kl	-	-	-	-	-	-	-	+
	Dm	-	-	-	-	-	-	-	+
	Ea	-	-	-	-	-	-	-	+
Ganoderma carnosum (D 21)	Hp	+	-	-	-	-	+	+	+
	De	-	-	-	-	-	-	-	+
	Kl	+	-	-	-	-	-	-	+
	Dm	-	-	+	-	-	-	-	+
	Ea	-	-	-	-	+	-	-	+
Ganoderma carnosum (M 88)	Hp	-	-	-	-	-	-	-	++
	De	-	-	-	-	-	-	-	+
	Kl	-	-	-	-	-	-	-	+
	Dm	-	-	-	-	-	-	-	+
	Ea	-	-	-	-	-	-	-	+
Laetiporus sulphureus (M 107)	Hp	-	-	-	-	-	-	+	+
	De	-	-	-	-	-	-	-	+
	Kl	-	-	-	-	-	-	-	+
	Dm	-	-	-	-	-	-	-	+
	Ea	-	-	-	-	-	-	-	+
Lentinus strigosus (D 26)	Hp	-	-	-	-	-	-	-	++
	De	-	-	-	+	-	-	-	+
	Kl	-	-	-	+	-	-	-	+
	Dm	-	-	-	+	-	-	-	+
	Ea	-	-	-	+	-	-	-	+
Lenzites betulina (S 2)	Hp	-	-	-	+	-	-	-	-
	De	-	-	-	+	-	-	-	-
	Kl	-	-	-	-	-	-	+	++
	Dm	-	-	-	-	-	-	+	+
	Ea	-	-	-	-	-	-	+	-
Polyporus arcularius (T 438)	De	-	-	-	-	-	-	-	+
	Kl	-	-	-	-	-	-	-	+
	Dm	-	-	-	-	-	-	-	+
	Ea	-	-	-	-	-	-	+	+

[a] All abbreviations used are the same as those of Table 1

Table 3. Antimicrobial activities of exopolysaccharides of basidiomycetes strains[a]

Fungus Species and Code	Inhibition zone							
	A	B	C	D	E	F	G	H
Cerrena unicolor (D 30)	-	-	-	++	+	-	-	-
Clavariadelphus truncatus (T 192)	-	-	-	-	-	-	-	-
Coprinus comatus (M 118)	-	-	-	-	-	-	-	-
Ganoderma carnosum (D 21)	-	-	+++	++	-	-	+++	-
Ganoderma carnosum (M 88)	+	-	-	++	+	-	-	-
Laetiporus sulphureus (M 107)	-	-	-	-	-	-	-	-
Lentinus strigosus (D 26)	-	-	-	-	-	-	-	-
Lenzites betulina (S 2)	++	-	++	-	+	-	-	-
Polyporus arcularius (T 438)	-	++	++	-	-	-	-	-

[a] All abbreviations used are the same as those of Table 1

against test microorganisms were given in Table 1, 2 and 3, respectively. The antimicrobial activity of mushroom samples varied according to the solvent, strain and life form. Among all mushroom strains tested, *Cerrena unicolor* presented the highest and wide antimicrobial activity in the form of submerged mycelium, as shown in Table 2. Submerged mycelium extracts of this strain were active against both bacteria and yeasts. These extracts were the most active to inhibit the growth of *Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus faecium*. Other fungal species showed generally moderate or weak activity in submerged mycelia. In other studies, bioactive metabolites in culture fluids were produced by different fungus species such as *Hericium* [25], *Ganoderma* [26], *Hebeloma* [27], *Lentinus* [28, 29], *Flammulina* [8] and *Stereum* [9].

Antimicrobial activities of *L. strigosus* and *C. truncatus* basidiocarps were higher than the other mushroom strains tested. Especially heptane, chloroform and dichloromethane extracts of *L. strigosus* showed higher antimicrobial activity as compared to the controls. The chloroform extract of *C. truncatus* basidiocarps presented wide antimicrobial activity against both bacteria and yeasts. Antimicrobial activities against tested bacteria were much less common against yeasts. But, it is apparent that most of the studied extracts showed activity against *C. glabrata* (Table 1-2).

In the present study, exopolysaccharides of *Cerrena unicolor, Ganoderma carnosum* (D 21 and M 88), *Lenzites betulina* (S 2) and *Polyporus arcularius* (T 438) showed activity against some of the studied test microorganisms (Table 3). The activities of *Ganoderma carnosum* (D 21) exopolysaccharides were higher than positive control (vancomycine or fluconazole) against *Micrococcus luteus*, *Enterococcus faecium* and *Candida albicans.* The activities of *Cerrena unicolor* and *Polyporus arcularius* exopolysaccharides against *Enterococcus faecium, Staphylococcus aureus* and *Micrococcus luteus* were the same with the positive control. Exopolysaccharides of other basidiomycetes strains did not show any activity against the test cultures.

Antimicrobial activity of a basidiomycetes strain varied according to the basidiocarp, submerged mycelia and exopolysaccharide. These data suggest that compounds responsible for the antimicrobial activity may be different in same species which is in accordance with the findings for other fungi [4, 10]. For instance, Suay et al. (2000) reported that two different *C. unicolor* strains showed the highest activities against *B. subtilis* [10]. But, in the present study, our *C. unicolor* strain did not show any activity against *B. subtilis*. Similarly, antimicrobial activities of *P. arcularius* and *L. sulphureus* strains seem entirely different in these studies [10]. While basidiocarps of *Oudemensiella mucida* do not exhibit the antibiotic activity in submerged culture, the mycelial cultures of this fungus produces a compound, mucidin, which inhibits fungi [6]. On the other hand, *C. unicolor* showed antimicrobial activity in their all used forms. These combine activity of antimicrobials increase the chance of the mushroom for medicinal purposes.

The fact that the Basidiomycetes have been insufficiently investigated coupled with the broad range of structural types of antibiotics. However, basidiomycetes may be a source of new and useful bioactive compounds. To our knowledge, no investigation has been performed for comparing antimicrobial activity potential of basidiomycetes strains in different life forms. Further studies on isolation and identification of the active compounds may provide a better source for developing new therapeutic agents.

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