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Effects of protein-bound polysaccharide against some microorganisms (*)

Protein bağlı polisakkaritin bazı mikroorganizmalara karşı etkisi

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SUMMARY

ABSTRACT: Protein-bound polysaccharopeptide (PSP) was extracted from submerged fermentation of Coriolus versicolor subculture and was investigated for in vitro antimicrobial activity. 3 Gram-positive and Gram-negative bacterial species and 1 yeast were analyzed for their susceptibility to PSP based on the Agar Hole Diffusion test. According to the results, 1/100 and 1/250 dilutions of PSP were found to be effective against Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Klebsiella pneumoniae CCM 2318 and Mycobacterium smegmatis CCM 2067.

***Key words:** Antimicrobial activity, Coriolus versicolor, Polysaccharopeptide, Subculture.*

ÖZET

Coriolus versicolor'un subkültürünün batık kültür fermentasyonu ile protein bağlı polisakkaropeptid (PSP) elde edilmiş ve PSP'nin antimikrobiyal aktivitesi araştırılmıştır. 3 Gram pozitif ve Gram negatif bakteri türü ile bir maya türünün agar oluk difüzyon yöntemi ile PSP'ye karşı hassasiyetleri incelenmiştir. Sonuçlara göre, PSP'nin 1/100 ve 1/250 oranlarındaki seyreltmelerinin, Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Klebsiella pneumoniae CCM 2318 ve Mycobacterium smegmatis CCM 2067 bakterilerine karşı etkili olduğu bulunmuştur.

INTRODUCTION

Medicinal mushrooms have an established history of use in traditional oriental therapies. Medicinal effects have been demonstrated for many traditionally used mushrooms (Ooi and Liu,1999), including extracts of *Favolus alveolarius* (Chang et al.,1988), *Phellinus linteus* (Chung et al.,1993; Kim et al.2001), *Lentinus edodes* (Kim and Park,1979; Sugano et al.,1985; Sang et al.,1998) and *Coriolus versicolor* (Kim and Park,1979; Mayer and Drews,1980; Fujita et al.,1988; Li et al.,1990; Yang et al.,1992a; Han et al.,1996; Mao and Gridley,1998; Ng,1998; Ooi and Liu,1999; Chu et al.,2002). Of the mushroom-derived therapeutics, polysaccharopeptides obtained from *C. versicolor* are commercially the best established. The best known polysaccharopeptide of *C. versicolor* is PSP. It is obtained from the extraction of *C. versicolor* mycelia.

PSP is light or dark brown powder that is soluble and stable in hot water. It doesn't have a definite melting point. It is soluble in water but insoluble in methanol, pyridine, chloroform, benzene and hexane. It contains α-1,4 and β-1,3 glucosidic linkages in its polysaccharide moieties. D-glucose is the major monosaccharide present (Ng, TB.,1998).

According to the reviewers, an extremely broad range of physiological effects has been linked with the use of PSP. Some of the main effects include the following; immunopotentiation by inducing production of interleukin-6, interferons, immunoglobulin-G, macrophages and T-lymphocytes; counter immunosuppressive effects of chemotherapy, radiotherapy, and blood transfusion; antagonization of immunosuppression induced by tumors; inhibition of proliferation of various cancers by inducing production of supe-

*(Bu araştırma 2005 tarihinde 18. Ulusal Biyoloji Kongresinde (Aydın/Kuşadası) poster olarak sunulmuştur.)

roxide dismutase (SOD), glutathione peroxidase. In addition, *C. versicolor* polysaccharopeptides are beneficial in the therapy of opportunistic microbial infections that suppress the immune response.

This study is made for investigating the antimicrobial activity of the fermentation product of *C. versicolor*.

MATERIAL AND METHOD

MATERIAL

The submerged culture of mushroom *Coriolus versicolor* ATCC 200801 was obtained from Hacettepe University Faculty of Science and Arts, Department of Biology.

Coriolus versicolor

The visible form of *C. versicolor* is a fan-shaped mushroom with wavy margin and colored concentric zones of varying colors: brown, yellow, gray, greenish or black (Figure 1). *C. versicolor* is an obligate aerobe that is commonly found year-round on dead logs, stumps, tree trunks and branches (Figure 2). 3-5 cm across brackets that are semicircular, flattened, thin and tough. Young brackets are flexible. The mushroom has white spores that are oblong and cylindrical (4-6 x 2-2.5μm) (Chui,J.et al.,2003).

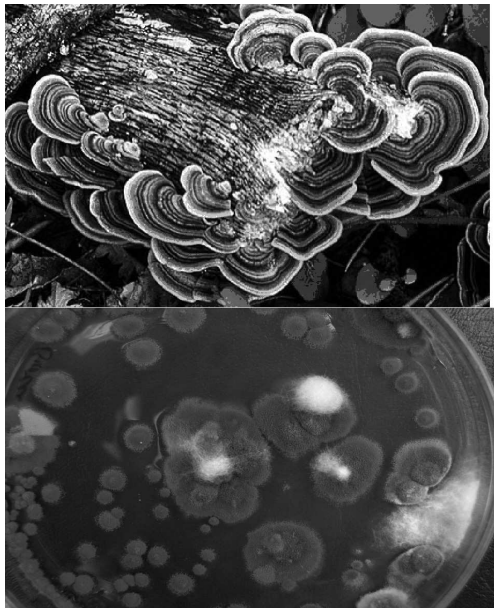

Figure 1. Coriolus versicolor makrofungusu
Figure 2. Subculture of Coriolus versicolor (www.mykoweb.com) (Photograph by ADÜ Microbology Laboratory)

METHOD

Production of C. versicolor polysaccharopeptides

Fermentation

In this study, C. versicolor polysaccharopeptide is extracted from mycelial biomass produced in submerged fermentations (Figure 3). Malt extract agar (Merck) was used for C. versicolor submerged culture and inoculated erlenmeyer flasks (250 ml) were incubated at 270C for 7-10 days under aerobic conditions. The fermentation medium contained; (g/100 ml) 5% glucose, 0,2% pepton, 0,3% yeast extract, 0,1% KH2PO4 ve 0,1% MgSO4.7H2O (Hotta et al.,1981). The fermentation broth (100 ml) was placed in an erlenmeyer flask (500 ml). The solid grown culture of C. versicolor was inoculated into the liquid medium in an proportion of 108cfu/ml. And the inoculated erlenmeyer flask was incubated at 270C for 10 days in static conditions. At the end of incubation, the mycelial mat that formed on the surface of the broth was collected and filtered. The mycelial mat was dried at 800C and the dry weight of mycelial was measured.

The sufficient amount of water for the extraction of mycelial mat was determined according to Chisti and MooYoung (1986). A typical extraction would use 2 kg of biomass (dry weight) in 30 l of water for the first stage (Cui and Chisti, 2003). And the hot water extraction was performed three times at 350C for 2h. ? volume %80 EtOH was added to supernatant and was incubated at 350C for 24h (EtOH precipitation). The precipitate was dissolved in water and desalted by dialysis using a cellulose acetate membrane for 3 days and non-dialysate was lyophilised. Lyophilisate was dissolved in distilled water (10mg/ml) and centrifuged at 15 000g for 10 minutes. And the supernatant, which was known as PSP (Cui,J., Chisti,Y.,2003;

Song,C.H. et al.,1998), was maintained at -200C and used for investigations (Figure 4).

Figure 3. grown in fermentation medium of C. versicolor submerged culture

(Photograph by ADÜ Microbology Laboratuary)

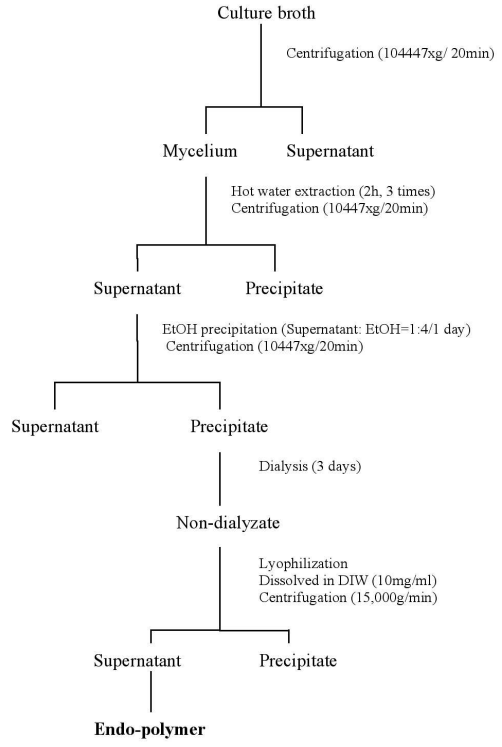

Figure 4. Recovery and purification options for C. versicolor polysaccharopeptides

(Song et al., 1998; Cui and Chisti, 2003)

Determination of PSP Content

The contents of polysaccharides were measured by phenol sulphuric acid method and protein determination was done by Lowry method (Dubois et al.,1956; Lowry et al.,1951). Moreover the contents of polysaccharides were obtained by TLC (thin-layer chromotography) (Fontana et al., 1988).

Test Microorganisms

Test microorganisms were obtained from Ege University, Faculty of Science, Department of Basic and Industrial Microbiology Culture Collection. Following microorganisms were used: Escherichia coli DSMZ 1562, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* CCM 2318, *Mycobacterium smegmatis* CCM 2067 and *Candida albicans*.

Antimicrobial Effects

Seven microorganisms belonging to three Gram-positive and three Gram-negative bacterial species as well as one yeast were tested for their susceptibility to PSP based on the Agar Hole Diffusion Test. The hole diffusion test was performed using Mueller Hinton Agar (Merck). The inoculum was prepared from an overnight culture of microorganisms on Nutrient agar and Malt extract agar in a sterile saline solution (0.85%). The turbidity of the suspension was adjusted with a spectrophotometer to 530 nm for obtaining a final concentration to match that of a 0.5 McFarland standard ($0.5\text{-}2.5\text{x}10^3$). 20 ml of Mueller Hinton Agar were melted, cooled to 55 °C and than inoculated with 200 μl of the organism suspension. The inoculated agar was poured into the assay plate (9 cm in diameter), and allowed to cool down on a leveled surface. Once the me-

dium had solidified, four wells, each 6mm in diameter, were cut out of the agar, and 20 μl of the PSP dilutions (1/5, 1/10, 1/25, 1/50, 1/100, 1/250) were placed into each well. All tests were carried out in triplicate. Reference antibiotics (Tetracycline for bacteria and Nystatin for yeast) were placed into each plate and all plates were incubated at 30-37 ºC for 24 hours except for *Candida albicans* which was incubated at 27ºC for 3 days.

RESULTS

Our results show that 1/100 and 1/250 dilutions of PSP possess antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* CCM 2318, *Mycobacterium smegmatis* CCM 2067 and *Bacillus subtilis* ATCC 6633. Non-diluted PSP and 1/5, 1/10, 1/25 and 1/50 concentrations of PSP did not produce any measurable zones of inhibition. The mean zones of inhibition produced against test bacteria ranged between 7.0 mm and 11.0 mm. The zone diameters of the plates after incubation are given in Table 1. As seen on the table, 1/100 dilution was the most effective concentration by 11 mm zone diameter of inhibition against *Mycobacterium smegmatis* CCM 2067 (Table 1).

Table 1. Inhibition zone diameters

Test Microorganisms	Zones of Inhibition (mm)							Comparison Antibiotic	
	Dilutions of PSP								
	0	**1/5**	**1/10**	**1/25**	**1/50**	**1/100**	**1/250**	***TE**	***Ns**
Escherichia coli DSMZ 1562	*ND	ND	ND	ND	ND	ND	ND	19	NT*
Staphylococcus aureus ATCC 6538	ND	ND	ND	ND	ND	9	7	21	NT
Staphylococcus epidermidis ATCC 12228	ND	ND	ND	ND	ND	ND	ND	19	NT
Bacillus subtilis ATCC 6633	ND	ND	ND	ND	ND	10	7	16	NT
Klebsiella pneumoniae CCM 2318	ND	ND	ND	ND	ND	10	7.5	17	NT
Mycobacterium smegmatis CCM 2067	ND	ND	ND	ND	ND	11	7	21	NT
Candida albicans	ND	ND	ND	ND	ND	ND	ND	NT	14

*NT: Not tested
*ND: Not detected
*TE: Tetracycline
*Ns: Nystatin

CONCLUSION

Antimicrobial drugs have been employed for prophylactic and therapeutic purposes. However, recent occurrence of drug-resistant strains has made treatment difficult. Thus, the antimicrobial activity of various anti-tumor polysaccharides is evaluated in term of their clinical efficacy. Schizophyllan is reported to *Streptococcus sp*. infection (Matsuyama et al., 1992). Lentinan is therapeutically effective against *Mycobacterium tuberculosis* and *Listeria monocytogenes* (Chihara, G. 1992). PSK induces potent antimicrobial activity against *Escherichia coli*, Listeria monocytogenes and *Candida* (Tsukagoshi et al., 1974; Sakagami et al., 1991). In some vivo animal studies, *Coriolus versicolor* extract was observed to display a broad spectrum of antibacterial and antifungal activities against common pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa, Listeria monocytogenes, Staphylococcus aureus, Candida albicans, Klebsiella pneumoniae* and *Streptococcus pneumoniae* (Chu et al., 2002). The observed antimicrobial effects of extract are possibly due to the activation of polymorphonuclear cells and an increased secretion of antimicrobial cytokines.

The fact that crude extracts of this medicinal mushroom produced zones of inhibition against some Gram positive and Gram negative bacteria indicate the presence of potent antibacterial activity which can be developed. Although both the Gram negative and Gram positive bacteria was affected by PSP, the PSP was more effective against Gram positive bacteria in our study.

Candida albicans and *Escherichia coli* DSMZ 1562 was not inhibited by all concentrations of the PSP. This result indicates that the PSP may not be effective in the treatment of Gram negative and yeast infections.

Another important result of our study is the importance of dilution factor for revealing the active effect of PSP. Accordingly to this result, it can be said that the higher dilutions of PSP is facilitating the inhibition process of bacteria.

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