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In vitro Prebiotic Activity of Polysaccharides Extracted from Edible / Medicinal Macrofungi Species

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Abstract: In this study, the extracted polysaccharides of *Agaricus bisporus* (Kültür mantarı; white and cream lines), *Boletus edulis* (Çörek mantarı), *Cantharellus cibarius* (Sarıkız mantarı), *Ganoderma lucidum* (Reyşi), *Pleurotus ostreatus* (İstiridye mantarı) and *Trametes versicolor* (Hindi kuyruğu) were investigated in terms of their *in vitro* prebiotic potential. For this purpose, the growth of *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Escherichia coli* were observed as nephalometrically in presence of polysaccharides at 0.25%, 0.5%, 1.0% and 2% concentrations for 24 hours. *Trametes versicolor* and *Ganoderma lucidum* have presented the highest (5.59%) and lowest (0.31%) polysaccharide yields, respectively. The higher polysaccharide concentrations have inhibited to proliferation of all bacterial strains. Among the polysaccharides, efficient ones as potential prebiotics were *Agaricus bisporus* (white line), *Trametes versicolor*, *Cantharellus cibarius*, *Boletus edulis*, and *Pleurotus ostreatus*.

Keywords: Macrofungi, Prebiotic, Microbiota, Nephelometre, *In vitro*.

Yenebilir / Tıbbi Önemi Olan Makrofunguslardan Ekstrakte Edilen Polisakkaritlerin *in vitro* Prebiyotik Aktivitesi

Öz: Bu çalışmada *Agaricus bisporus* (Kültür mantarı; beyaz ve kestane formu), *Boletus edulis* (Çörek mantarı), *Cantharellus cibarius* (Sarıkız mantarı), *Ganoderma lucidum* (Reyşi), *Pleurotus ostreatus* (İstiridye mantarı) ve *Trametes versicolor* (Hindi kuyruğu) türlerinden ekstrakte edilen polisakkaritler *in vitro* prebiyotik potansiyelleri açısından araştırılmıştır. Bu amaçla %0.25, %0.5, %1 ve %2 konsantrasyonlarda polisakkarit içeren ortamda *Lactobacillus plantarum*, *Lactobacillus acidophilus* ve *Escherichia coli* bakterilerinin üremesi 24 saat boyunca nefalometrik olarak belirlenmiştir. *Trametes versicolor* ve *Ganoderma lucidum* en yüksek (%5.59) ve en düşük (%0.31) polisakkarit verimi veren türler olmuştur. Yüksek polisakkarit konsantrasyonlarının tüm bakterilerin üremesini ihhibe ettiği görülmüştür. *Agaricus bisporus* (beyaz form), *Trametes versicolor*, *Cantharellus cibarius*, *Boletus edulis* ve *Pleurotus ostreatus* türlerinden elde edilen polisakkaritlerin potansiyel prebiyotik olarak kullanılabileceği belirlenmiştir.

Anahtar kelimeler: Makrofungus, Prebiyotik, Mikrobiyota, Nefalometre, *In vitro*.

Introduction

Prebiotics are oligosaccharides and polysaccharides that can not be digested by digestive system enzymes or can not be metabolized by the host (Prathumpai et al., 2019). Therefore, they can reach to the colon, can stimulate the growth of probiotic bacteria, and can prevent the growth of non-probiotic or pathogenic microorganisms such as *Escherichia coli* and *Salmonella* spp. in the gastrointestinal track (Pompei et al., 2008). Thus, the prebiotics can regulate the microbiota balance in favor of the host live. Different plants and/or their products such as garlic, wheat bran, bananas, onions, leeks, almond, lentil, and grain legumes such as lupin and chickpea are well known prebiotic sources (Swennen et al., 2006; Dwivedi et al., 2014).

Macrofungi are also contain non-digestible fibers such as chitin, α and β glucans, xylans, mannans, and galactans (Aida et al., 2009) Among them particularly β glucans are of considerable interest because of their immune-enhancing, anticancer, antibacterial, antifungal and antioxidant effects (Khan et al., 2018). In addition, it is reported by Nowacka-Jechalke et al. (2018) that the growth of *Lactobacillus* strains can be stimulated by fungal polysaccharides better than commercially available prebiotics like inulin or fructooligosaccharides (FOS) and macrofungal polysaccharides subjected to artificial human gastric juice have remained undigested in more than 90%. It means that these polysaccharides can be a good candidate as prebiotic and can maintained their properties to stimulate the beneficial probiotic microrganisms. *In vitro* prebiotic activities of polysaccharidic fractions of different macrofungi species have been reported in last years such as *Auricularia nigricans* (Karakulak mantarı, Nasution et al., 2018), *Cyclocybe cylindracea* (Çizgili metelik; Mitsou et al., 2020), *Hericium erinaceus* (Tülübüzük; Mitsou et al., 2020), *Lactifluus volemus* (Tirmit; Huang et al., 2020), *Lignosus rhinocerus* (Gao et al., 2009), *Ophiocordyceps dipterigena* (Prathumpai et al., 2019), *Ophiocordyceps sinensis* (Song et al., 2019), *Pleurotus* spp (İstiridye mantarı; Synytsya et al., 2009, Zhao and Cheung, 2011, Nasution et al., 2018, Mitsou et al., 2020 , Ogidi et al., 2020), and *Wolfiporia cocos* (Gao et al., 2009). Besides *in vitro* prebiotic activity of polysaccharide fractions of 53 wild growing macrofungi species has presented by Nowak et al. (2018). In the presented study, *in vitro* prebiotic potential of polysaccharide fractions of fruiting body samples of six edible/medicinal macrofungi species was reported.

Material and Metod

Materials

The fruiting body samples of *Agaricus bisporus* (Kültür mantarı; white line, AB(W)), *Agaricus bisporus* (cream line; AB(C)) and *Pleurotus ostreatus* (İstiridye mantarı; PO), was purchased from MÜPA (Kocaeli). *Trametes versicolor* (Hindi kuyruğu; TV) and *Ganoderma lucidum* (Reyşi; GL) fruiting body samples were obtained from AGROMA (Denizli). *Boletus edulis* (Çörek mantarı; BE) fruiting body samples were obtained from Çalışkan Mantar (Denizli) *Cantharellus cibarius* (Sarıkız mantarı; CC) fruiting body samples were donated by Aysun Pekşen (Ondokuz Mayıs Üniversity, Samsun, Turkey).

Escherichia coli (ATCC 25922) and two *Lactobacillus* species (*L. acidophilus* (B 4495) and *L. plantarum* (B 227) from Agricultural Research Service Culture Collection, NRRL, USA) were used as test organisms. Inulin was purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). Ethyl alcohol, trichloroacetic acid, and all culture media were purchased from Merck KGaA (Darmstadt, Germany).

Polysaccharides extraction of macrofungi materials

The studied macrofungi samples were cleaned, dried overnight at 60°C, and blended. The prepared samples were pulverized, and boiled twice in distilled water at 1:10 (w/v) for 1 h. Then, the extract solutions were treated with an equal volume of 0.8 M trichloroacetic asid at 4°C for 3 h to deproteinization. The obtained solutions were centrifuged for 10 min at 5000 g. The polysaccharide fraction in the supernatant was precipitated with 95% cold ethanol at a ratio of 1:4 (v/v) and kept overnight at 4°C. The obtained precipitates were collected by centrifugation for 10 min at 5000 g and lyophilized using a shelf freeze-drying model (Christ Alpha 1-2 LD). The percentage polysaccharide yields (%) were calculated by the following equaition (Chou et al., 2013);

Yield (%;w/w) = (Weight of extracted polysaccharide / Weight of dried macrofungi material) ×100

Preparation of bacterial suspension and culture conditions

Lactobacillus strains (*L. acidophilus* (B 4495), *L. plantarum* (B 227)) as probiotic bacteria and *E. coli* ATCC 25922 as pathogenic bacterium were used in this study. The cultures of *E. coli* and *Lactobacillus* spp. were grown in Luria Bertani broth and MRS broth and incubated at 37°C, for 24 h at anaerobic and aerobic conditions, respectively. For culture control of *Lactobacillus* bacteria, the catalase test and gram strains were performed. The suspensions of bacteria were prepared according to McFarland standard 0.5 as inoculum that contains 1.5 × 10^8 CFU/mL.

Determination of macrofungi polysaccharides on growth of the test microorganisms

The tested bacteria were cultivated in presence of the fungal polysaccharides as carbon source in comparison to glucose and inulin. The bacterial growth was monitored by microplate laser nephelometry (MLN) using the NEPHELOstar Galaxy (BMG, Offenburg, Germany) (Finger et al., 2013). A 100 µl appropriate double strenght medium for the test microorganisms were put in triplicate into the wells of sterile 96-well microplate (GreinerBioOne, Frickenhausen, Germany). The media were supplemented with 100 µl polysaccharide fraction at different concentrations (0.25, 0.5, 1, and 2%). Each well was inoculated with 5 µl bacterial suspension. Microplates were covered with a clear adhesive film and placed to the microplate laser nephelometer, and incubated 37°C, for 24 hours. The used nephelometer posses a 635-nm laser as radiating source with a laser beam focus 2 mm. The plates were shaken in 3 mm orbital shake witdth. Each well was measured for 1.0 s during the hourly measurement. The reproduction of bacteria is expressed in the RNU (Relative Nefalometric Unit) unit (Finger et al., 2012). Density adjusted to McFarland 0.5 was accepted as 0 (zero) in the initial RNU. Commercial prebiotic inulin and glucose were used as controls. The medium including polysaccharide was used as blank for each polysaccharide concentrations.

Statistical analysis

Statistical analysis of polysaccharide fractions of the macrofungi species on growth of the test microorganisms were performed after incubation period. Three independent experiments were performed and each sample was measured in three replicates. Data are presented as mean ± standard deviation. The obtained results were evaluated by analysis of variance. The differences were statistically significant when p≤0.05.

Results and Discussion

Extraction Yield

Apparent differences were found between the polysaccharide yields of the macrofungi species in this study (Table 1). The highest and lowest polysaccharide yields were obtained in *Trametes versicolor* and *Ganoderma lucidum*, respectively. Since a standard method was used in the polysaccharide production stage (Chou et al., 2013), it is thought that these yield differences are due to the difference in the specific polysaccharide ratio, which is included in the macrofungi species/strain rather than deviations from the method. The differences between the polysaccharide yields of macrofungi basidiocarps can result from the difference in polysaccharide solubility, boiling time, temperature, the water/solid ratio, the number of extraction, and especially macrofungi species/strain. For example, soluble polysaccharide yield of *Ganoderma lucidum* by hot water extraction could vary from 0.4% (Chang and Lu, 2004) to 3.7% (Pang et al., 2007). On the other hand, following the hot water extraction, very low (0.022%) and moderate (3.7%) polysaccharide yield values were obtained from *Trametes versicolor* (Kozarski et al., 2012; Nowak et al., 2018).

Table 1. The polysaccharide yields of macrofungus species

Macrofungi species	Yields (%; w/w)
*Agaricus bisporus (*white line)	0.81
*Agaricus bisporus (*cream line)	3.17
Boletus edulis	2.40
Cantharellus cibarius	1.39
Ganoderma lucidum	0.31
Pleurotus ostreatus	1.41
*Trametes versicolo*r	5.59

Determination of macrofungi polysaccharides on growth of the test microorganisms

Investigating the fermentability of potential prebiotics in human or animal model is time consuming, labor-intensive, expensive, and difficult *in vivo* conditions. Therefore, *in vitro* screening studies are presents valuable data for the determination of novel prebiotics. In this study, the effect of polysaccharide fractions obtained

from fruiting body samples of 7 strains belonging to 6 edible/medicinal macrofungus species on the proliferation of 3 bacterial species was investigated nephelometrically.

To promote and to inhibit the growth of Lactobacilli and *E.coli*, respectively, the ability of the seven macrofungi polysaccharides was investigated in four different concentrations during incubation period. In general, the growth of all reference microbial strains has decreased dose dependently in presence of almost all macrofungi polysaccharides. The microbial growth in presence of higher polysaccharide concentrations in all experimental polysaccharide groups were lower than those of negative and positive controls, glucose and inulin, respectively (Figures 1-3). It means that the studied macrofungi polysaccharides have antimicrobial activities in higher concentrations and should be used as prebiotic in lower concentrations such as 0.25% for the studied macrofungi species.

The influence of fungal polysaccharides at four concentrations on the proliferation of *L. plantarum* after 24 hours is presented in Fig 1. The maximum growth of the bacterium was obtained at the 0.25% concentration in all fungal polysaccharides. In this concentration, five of the studied polysaccharides (TV, PO, CC, BE, AB(W)) have stimulated the growth of the *L. plantarum* according to glucose control (p≤0.05). This situation confirms the potential of these polysaccharides as prebiotics and could be good substrate for *Lactobacillus plantarum*. However, none of them did not stimulate the growth of *L. plantarum* as inulin (p≤0.05). The growth inhibiting activity of GL and AB(C) polysaccharides for *L. plantarum* were distict in all concentrations.

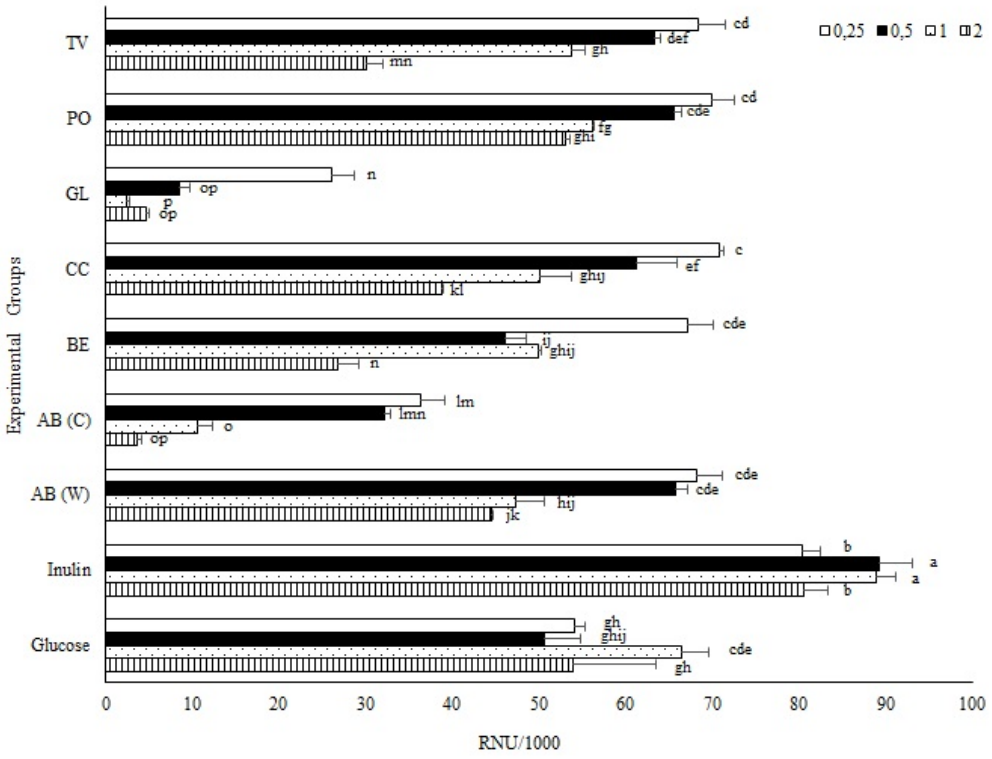

Fig 1. The influence of fungal polysaccharides on the growth of *Lactobacillus plantarum*. Same letters denote statistical equivalence between groups

Only two (PO and AB(W)) polysaccharides at the 0.25% concentration have increased the growth intensity of the other *Lactobacillus* species, *L. acidophilus*, in statistically significant level (p≤0.05) (Fig 2) according to inulin. Similar to *L. plantarum*, GL and AB(C) have presented the lowest growth rates in *L. acidophilus*, also.

Four (TV, PO, CC, BE) and two (GL, AB(C)) of the seven macrofungi polysaccharides were found to be statistically significant in enhanging and decreasing the growth of the *E. coli* according to glucose at the concentration of 0.25%, respectively. The only polysaccharide presented similar growth with glucose was AB(W) (p≤0.05) (Fig 3). The influence of TV, CC, and BE polysaccharides on the growth of *E. coli* was statistically similar to inulin (p≤0.05). On the other hand, the growth of *E.coli* was lower in presence of AB(W) polysaccharide according to inulin.

The time dependent proliferation studies with *Lactobacillus plantarum* demonstrated that AB(W), TV, CC, and BE polysaccharides after 24 hours were very similar which were lower and higher than inulin and glucose, respectively ($p<0.05$). The highest proliferative effects on the bacterium was presented by the polysaccharides of TV and CC during almost all incubation period which were higher or very similar to that of inulin except last 4 hours (Fig 4). These results indicates that all of the polysaccharide fractions of the tested macrofungi species could be good substrates for *Lactobacillus plantarum* than glucose and could be compete with inulin. The positive control, inulin, and the most of the macrofungi polysaccharides demonstrated similar prebiotic activity on *L. acidophilus* after 24-h of incubation (Fig 5). It is worth mentioned that only AB(W) resulted in significantly higher proliferative effect compared to inulin during in all incubation period. On the other hand, glucose was the best substrate for *L. acidophilus*. Besides the proliferation of *E. coli* was not promoted by AB(W) during 24-h of incubation period (Fig 6). Commercial prebiotic, inulin, had lower inhibition activity for *E. coli* than most of the studied polysaccharides.

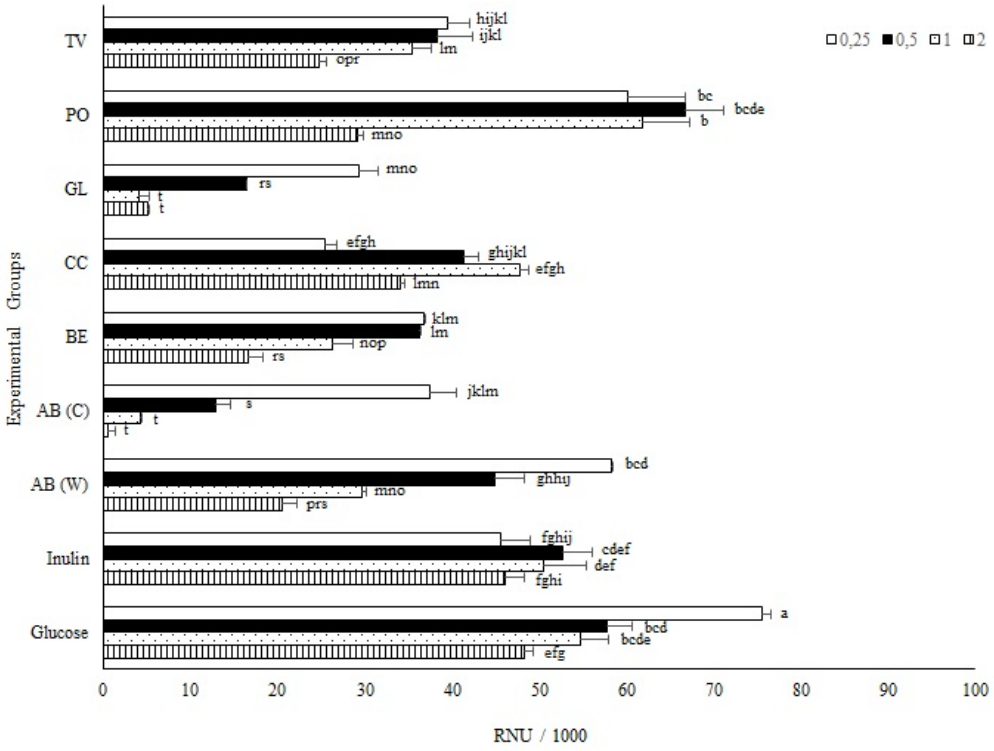

Fig 2. The influence of fungal polysaccharides on the growth of *Lactobacillus acidophilus*. Same letters denote statistical equivalence between groups.

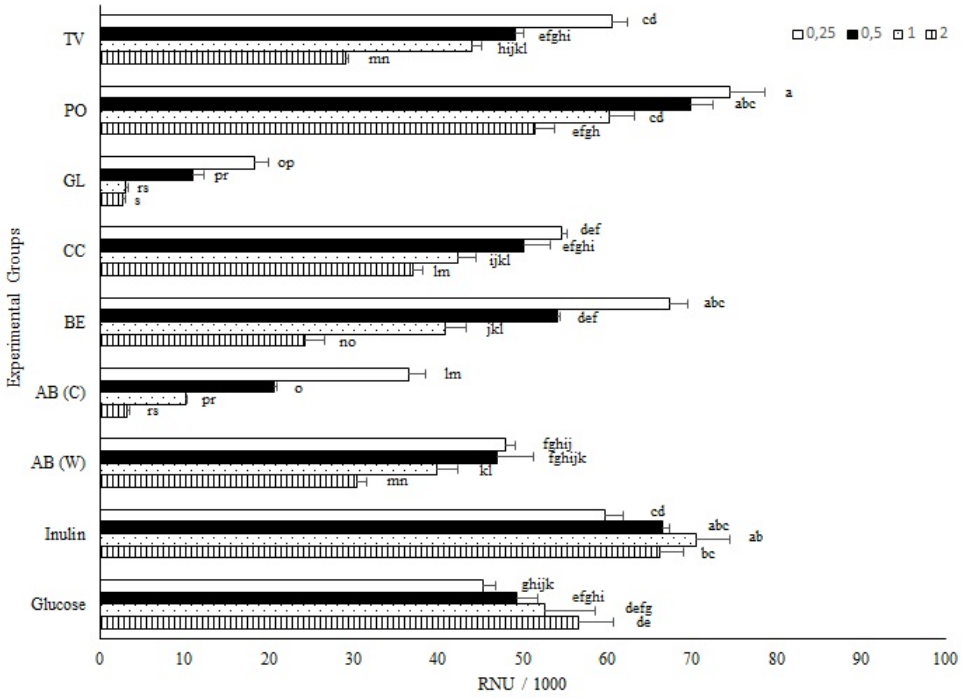

Fig 3. The influence of fungal polysaccharides on the growth of *Escherichia coli*. Same letters denote statistical equivalence between groups.

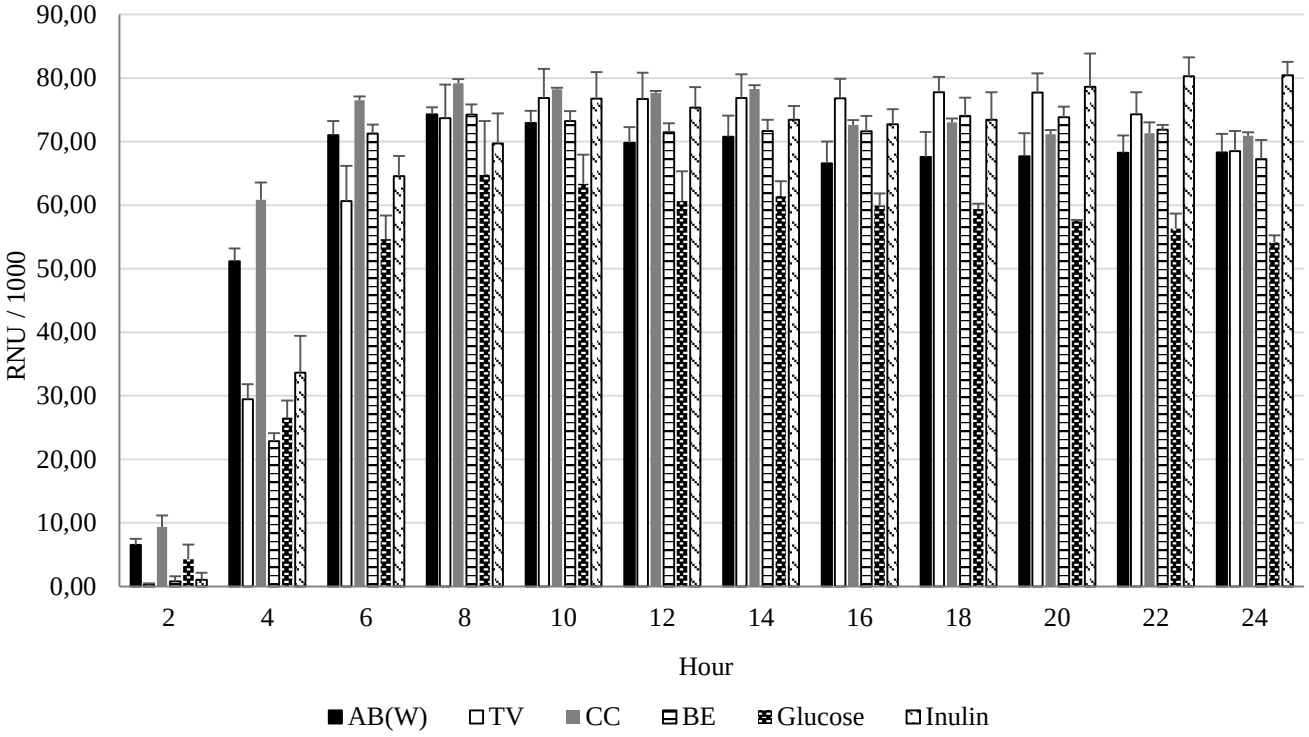

Fig 4. Time dependent growth of *Lactobacillus plantarum* in presence of 0.25% fungal polysaccharides.

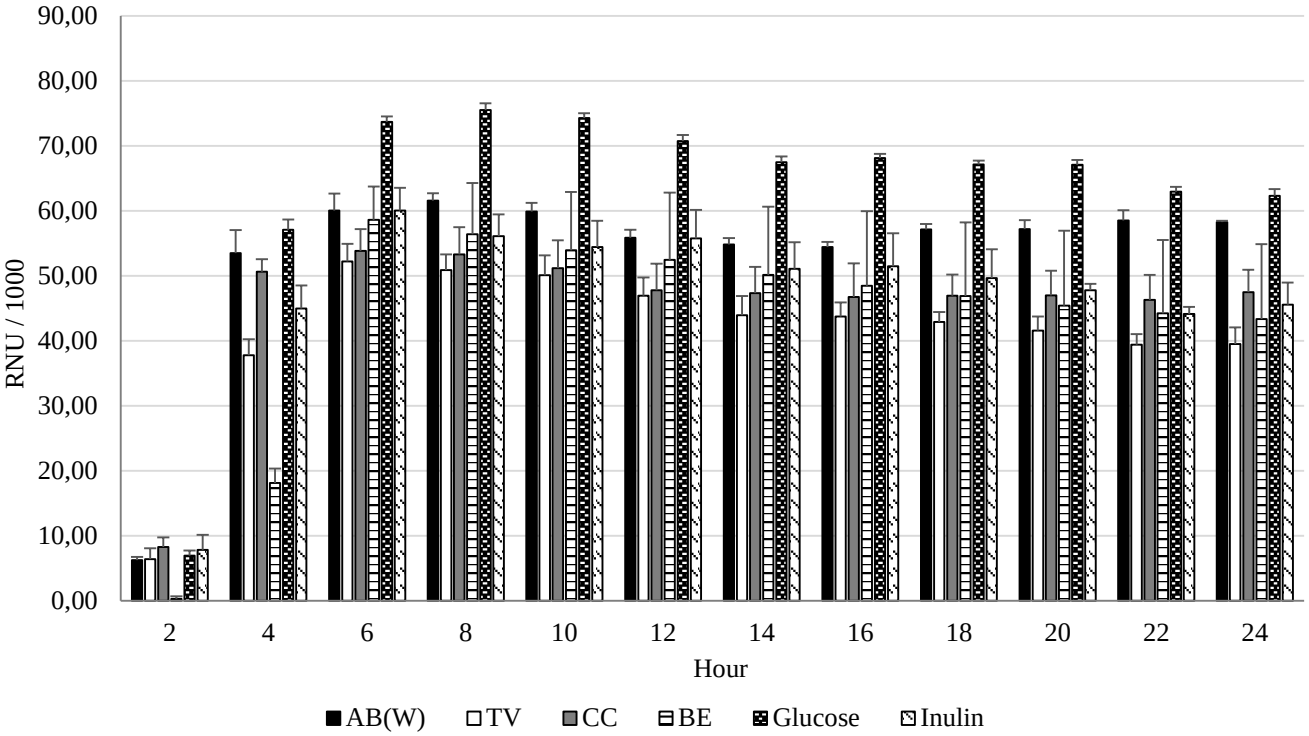

Fig 5. Time dependent growth of *Lactobacillus acidophilus* in presence of 0.25% fungal polysaccharides

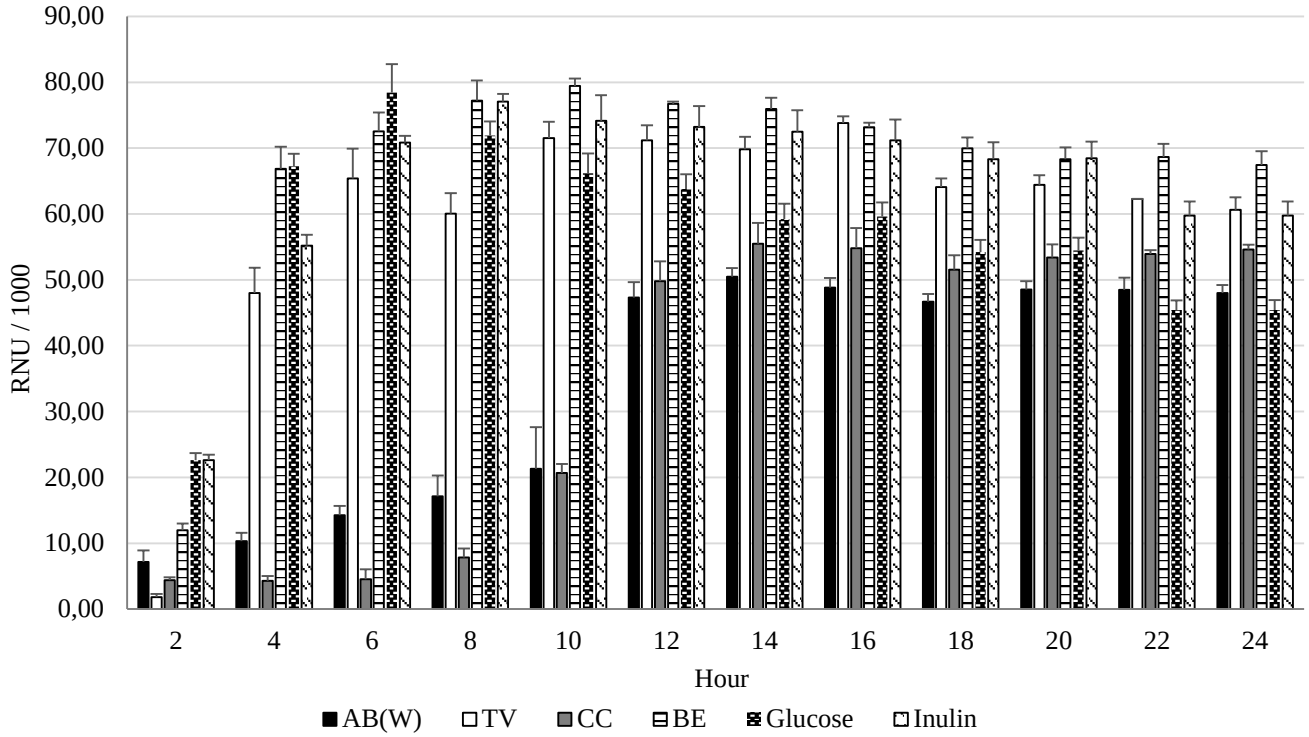

Fig 6. Time dependent growth of *Escherichia coli* in presence of 0.25% fungal polysaccharides

As a result of detailed examination of the data presented in Figure 4, it was determined that *Agaricus bisporus* (white form) polysaccharide (ABP) increased the growth of *L. plantarum* at 0.25% concentration according to glucose, but lower than the inulin used as a positive control. On the other hand, 0.25% ABP increases the growth of *L. acidophilus* compared to inulin, although it offers lower values than glucose. Briefly, the stimulation of probiotic bacterial strains in the presence of AB(W) supports the fact that AB(W) has a prebiotic feature which is in agreement with Giannenas et al. (2010). As an important finding, it was determined that growth of *E. coli* is not stimulated in the presence of AB(W). The enhanging of the *Lactobacillus* spp. and *E. coli* amouth in gastrointestinal track is and is not demanded, respectively. Therefore, it can be argued that AB(W) polysaccharide has presented the highest prebiotic activity and in particular AB(W) has potential as prebiotic and nutraceutical. TV, CC, and BE polysaccharides have also prebiotic activity potential. Among them, the prebiotic activity of CC was confirmed by our group at *in vivo* conditions (Türsen Uthan et al., 2021)

Agaricus bisporus (white form) is a fungal species cultivated worldwide. It has high polysaccharide and fiber content. In addition, its protein, vitamin and mineral ratio is high (Giannenas et al., 2010). It is emphasized that this macrofungus polysaccharide has prebiotic activity due to heteropolysaccharide, D-galactose, D-mannose, D-xylose, L-fructose, L- (or D) -arabinose, xylose, fructose, glucose, sucrose and trehalose (Aida et al., 2009).

Mitsou et al. (2020) confirmed that there are limited number of study examined the *in vitro* prebiotic effets of edible muhroom species. The previous reports have demonstrated that prebiotic activities of the macrofungi are species/strain dependent, very variable (Table 2), and only some of them can be used as nutraceuticals.

Based on the results, it could be speculated that the polysaccharide of especially AB(W), TV, CC, and BE could be used as substrate to promote the growth of the *Lactobacillus* species, selectively. Nevertheless, our further *in vivo* studies will be focused on to clarify the exact mechanism amd to perform microbiome analysis.

Table 2. Growth stimulation activities of different macrofungi species for probiotic bacteria

Species	Polysaccharide	Source	PS (%, w/v)	Probiotic taxon	Growth Conditions	Medium	GOS	FOS	İnulin	Glucose	Reference
Agaricus bisporus (Cream line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	82.04 24.48 8.31 1.30 [1]	60.00 22.35 7.66 1.24 [2]	This study
Agaricus bisporus (Cream line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	45.12 36.15 11.92 4.47 [1]	67.02 63.74 15.93 6.66 [2]	This study
Agaricus bisporus (White line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	84.89 73.70 53.19 55.40 [1]	93.53 77.93 54.01 42.39 [2]	This study
Agaricus bisporus (White line)	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	127.88 85.35 58.59 44.50 [1]	126.11 129.94 71.09 82.56 [2]	This study
Agaricus bisporus	Crude PS	Fruiting body	3.12 6.25 12.50 25.00	*Lactobacillus casei*	37 °C 24 h	MRS Broth	ND	ND	ND	4.51 29.65 58.28 192.44 [2]	Nasution et al., 2018
Auricularia nigricans	Crude PS	Fruiting body	3.12 6.25 12.50 25.00	*Lactobacillus casei*	37 °C 24 h	MRS Broth	ND	ND	ND	64.69 80.56 103.89 110.91 [2]	Nasution et al., 2018
Boletus edulis	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	80.50 68.88 51.86 36.29 [1]	58.88 62.89 47.81 34.57 [2]	This study

Boletus edulis	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	83.52 51.68 56.12 33.29 [1]	124.08 91.11 74.99 49.61 [2]	This study
Cantharellus cibarius	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	55.93 78.37 94.42 73.99 [1]	40.91 71.55 87.04 70.48 [2]	This study
Cantharellus cibarius	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	88.12 68.71 56.46 48.20 [1]	130.91 121.15 75.44 71.83 [2]	This study
Cantharellus cibarius	Crude PS	Fruiting body	1.50	*L. acidophilus ATCC 4356*	48 h	Rogosa Broth without glucose	ND	11.53 [2]	11.34 [2]	20.3 [2]	Nowacka-Jechalke et al., 2018
Cantharellus cibarius	Crude PS	Fruiting body	1.50	*L. rhamnosus-1*	48 h	Rogosa Broth without glucose	ND	11.71 [2]	12.72 [2]	33.85 [2]	Nowacka-Jechalke et al., 2018
Cantharellus cibarius	Crude PS	Fruiting body	1.50	*L. rhamnosus-2*	48 h	Rogosa Broth without glucose	ND	8.87 [2]	11.34 [2]	0.00 [2]	Nowacka-Jechalke et al., 2018
Ganoderma lucidum	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	64.27 31.31 8.06 10.93 [1]	47.01 28.59 7.43 10.41 [2]	This study
Ganoderma lucidum	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	32.44 9.64 2.70 5.84 [1]	48.19 17.00 3.61 8.70 [2]	This study
Ganoderma lucidum	Crude PS	Fruiting body		*Bifidobacterium spp.*	37 °C 24 h	Human feces	ND	15.28	ND	4.17 [4]	Yamin et al., 2012

Ganoderma lucidum	PS Fraction	Fruiting body		*Bifidobacterium spp.*	37 °C 24 h	Human feces	ND	15.28	ND	9.72 [4]	Yamin et al., 2012
Ganoderma lucidum	Crude PS	Fruiting body		*Lactobacillus spp.*	37 °C 24 h	Human feces	ND	27.69	ND	10.77 [4]	Yamin et al., 2012
Ganoderma lucidum	PS Fraction	Fruiting body		*Lactobacillus spp.*	37 °C 24 h	Human feces	ND	27.69	ND	15.38 [4]	Yamin et al., 2012
Hypholoma capnoides (Al sarıpapak)	Crude PS	Fruiting body	1.50	*L. rhamnosus 1*	37 °C 72 h	Rogosa broth	ND	11.71 [2,5]	12.72 [2,5]	56.63 [2,5]	Nowak et al., 2018
Hypholoma capnoides	Crude PS	Fruiting body	1.5	*L. rhamnosus 2*	37 °C 72 h	Rogosa broth	ND	11.34 [2,5]	8.87 [2,5]	17.84 [2,5]	Nowak et al., 2018
Lactifluus volemus	Crude PS	Fruiting body	0.50 1.00 1.50	*Lactobacillus plantarum DMDL 9010*	4 °C 28 day	Yoghurt	ND	ND	ND	9.83 10.91 13.07 [2]	Huang et al., 2020
Lactifluus volemus	Crude PS	Fruiting body	0.50 1.00 1.50	*Lb. casei subsp. rhamnosus 6013*	4 °C 28 day	Yoghurt	ND	ND	ND	6.85 9.02 9.89	Huang et al., 2020
Lactifluus volemus	Crude PS	Fruiting body	0.50 1.00 1.50	*Lactobacillus acidophilus 1.1878*	4 °C 28 day	Yoghurt	ND	ND	ND	6.67 8.61 10.22 [2]	Huang et al., 2020
Lignosus rhinocerus	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Bifidobacterium longum (JCM 1217)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	14.3 [4]	Gao et al., 2009
Lignosus rhinocerus	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Lactobacillus brevis (JCM 1059)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	18.9 [4]	Gao et al., 2009
Ophiocordyceps sinensis	Crude EPS	Submerged culture	0.50	*Bifidobacterium adolescentis (CICC 6070),*	4 °C 28 day	Water	0.70 3	ND	25.30 [3]	49.90 [3]	Song et al., 2019
Ophiocordyceps sinensis	Crude EPS	Submerged culture	0.50	*Bifidobacterium infantis (CICC 6069)*	4 °C 28 day	Water	0.40 3	ND	-1.30 [3]	65.80 [3]	Song et al., 2019

Ophiocordyceps sinensis	Crude EPS	Submerged culture	0.50	*Bifidobacterium infantis (R33)*	4 °C 28 day	Water	35.40 3	ND	34.60 [3]	71.80 [3]	Song et al., 2019
Ophiocordyceps dipterigena	(1, 3)-β-D-glucan	Submerged culture	1.00	*Lactobacillus acidophilus BCC 13938*	37 °C 150 rpm 48 h	MRS Medium	400 [2]	400 [2]	ND	ND	Prathumpai et al., 2019
Ophiocordyceps dipterigena	(1, 3)-β-D-glucan	Submerged culture	1.00	*Bifidobacterium animalis ATCC 25527*	37 °C 150 rpm 48 h	Reinforced Clostridial Medium	0 2	0 2	ND	ND	Prathumpai et al., 2019
Paxillus involutus (Ağulu iztutan)	Crude PS	Fruiting body	1.50	*Lactobacillus acidophilus ATCC 4356*	37 °C 72 h	Rogosa broth	ND	11.53 [2,5]	11.34 [2,5]	37.96 [2,5]	Nowak et al., 2018
Pleurotus cystidiosus	Crude PS	Fruiting body	3.12 6.25 12.50 25.00	*Lactobacillus casei*	37 °C 24 h	MRS Broth	ND	ND	ND	65.31 134.81 244.50 287.32 [4]	Nasution et al., 2018
Pleurotus ostreatus	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	131.83 126.57 122.33 63.19 [1]	103.58 115.56 112.77 60.19 [2]	This study
Pleurotus ostreatus	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	86.88 73.65 63.32 65.93 [1]	129.06 129.84 84.61 98.24 [2]	This study
P. pulmonarius (Yaz istiridyesi)	Crude EPS	Submerged culture	1.00	*Lactobacillus delbrueckii*	37 °C 48 h	MRS Broth	ND	23.41 [2]	ND	26.98 [2]	Ogidi et al., 2020
P. pulmonarius	Crude EPS	Submerged culture	1.00	*Streptococcus thermophiles*	37 °C 48 h	MRS Broth	ND	19.81 [2]	ND	20.77 [2]	Ogidi et al., 2020
P. tuber-regium	β-glucan	Sclerotium	0.50	*B. infantis (JCM 1222)*	37 °C 100 rpm 24 h	Medium for colonic bacteria (MCB)	ND	ND	ND	67.06 [5]	Zhao and Cheung, 2011

P. tuber-regium	β-glucan	Sclerotium	0.50	*B. longum (JCM 1217)*	37 °C 100 rpm 24 h	Medium for colonic bacteria (MCB)	ND	ND	ND	30.02 [4]	Zhao and Cheung, 2011
P. tuber-regium	β-glucan	Sclerotium	0.50	*B. adolescentis (JCM 1275)*	37 °C 100 rpm 24 h	Medium for colonic bacteria (MCB)	ND	ND	ND	51.36 [4]	Zhao and Cheung, 2011
Trametes versicolor	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus acidophilus (B_4495)*	37 °C 24 h	MRS Broth	ND	ND	86.64 72.68 70.07 53.67 [1]	63.37 66.35 64.60 51.13 [2]	This study
Trametes versicolor	Crude PS	Fruiting body	0.25 0.50 1.00 2.00	*Lactobacillus plantarum (B_227)*	37 °C 24 h	MRS Broth	ND	ND	85.01 71.07 60.50 37.37 [1]	126.29 125.30 80.85 55.68 [2]	This study
Trametes versicolor	Polysaccharopeptide (PSP)	Cultured mycelium	0.50	*Bifidobacterium spp.*	37 °C 24 h	Sythetic medium	ND	29.66 [2]	ND	23.28 [2]	Yu et al., 2013
Trametes versicolor	Polysaccharopeptide (PSP)	Cultured mycelium	0.50	*Lactobacillus spp.*	37 °C 24 h	Sythetic medium	ND	17.57 [2]	ND	41.89 [2]	Yu et al., 2013
Wolfiporia cocos	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Bifidobacterium longum (JCM 1217)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	6.74 [4]	Gao et al., 2009
Wolfiporia cocos	β-glucan rich nondigestible carbohydrates	Sclerotium	1.00	*Lactobacillus brevis (JCM 1059)*	37 °C 100 rpm 24 h	Basal medium	ND	ND	ND	11.9 [4]	Gao et al., 2009

PS: Polysaccharide, EPS: Exopolysaccharide, FOS: Fructooligosaccharide, GOS: Galactooligosaccharide

[1] Growth stimulation activity (%) according to inulin (taken as 100%).

[2] Growth stimulation activity (%) according to glucose (taken as 100%).

[3] Reduction of bacterial death rate (%)

[4] Ratio of number of bacterial populations at 24 hours compared to that at 0 hours.

[5] The highest value among 53 wild-growing macrofungi for the studied probiotic bacterium.

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