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Polysaccharides from mushroom as potential prebiotics with their antioxidant activities

Yousra A. El-Maradny[1], Esmail M. El-Fakharany[2], Amr S. Abouakkada[3]

[1]Microbiology Department, High Institute of Public Health, Alexandria University,
[2]Therapeutic and Protective Proteins Laboratory, Protein Research Department, Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications (SRTA-City), [3]Alexandria Food Inspection Laboratories-Alexandria Port, Agricultural Research Center (ARC), Alexandria, Egypt.

01 INTRODUCTION

- The modulation of the intestinal microbiome or bacteriotherapy using probiotics, prebiotics or synbiotics has a great impact on the reduction of inflammation and combating against the infection and colonization with pathogenic bacteria.
- In this study, we compared the prebiotic properties and antioxidant effect of the crude and polysaccharide extracts of two types of edible mushrooms;
  - *Agaricus bisporus* (Brown)
  - *Pleurotus ostreatus* (Oyster)
- With four strains of Lactobacillus:
  - ✓ *L. acidophilus*
  - ✓ *L. pentosus*
  - ✓ *L. plantarum*
  - ✓ *L. paracasei*

02 METHODS

Mushroom extract

For extraction of polysaccharide:

1. Mushrooms were dried at 105°C overnight, then blended.
2. Samples were extracted by adding 30 mL of a mixture of distilled water and ethanol (95% volume per volume, v/v) at a ratio of 1:4 v/v, respectively.

For crude extract:

1. Mushrooms fruiting bodies were homogenized and extracted with phosphate-buffered saline (PBS), pH7.4, overnight.
2. The homogenate was centrifuged at 4000 rpm for 30 min and the resulting supernatant was collected and filtered.

Total carbohydrate determination

01 By using the phenol sulfuric acid method. (Dubois et al, 1956).

02 0.25 mL of a sample and 1.25 mL concentrated sulfuric acid (95% v/v) and 0.25 mL phenol (5% v/v).

03 The mixture was heated at 100°C for 5 min.

04 The total carbohydrate content was determined using colorimetry at 490 nm absorbance.

Total reducing sugar determination

A By using 3,5-dinitrosalicylic acid (DNS) assay according to (Miller, 1959)

B 1 mL sample was mixed with 1 mL DNS reagent and incubated at 100°C for 5 min.

C The reducing sugar was measured using spectrophotometry at 540 nm absorbance.

Prebiotic properties

Lactobacillus strains were cultured at 37°C for 48 hr in MRS broth.

Compared with culture medium supplemented with 2.5 & 5mg/mL of each mushroom extract & a commercial prebiotic compound as inulin.

Measuring the optical cell density using spectrophotometry at 620nm (Siragusa et al., 2009)

Probiotic growth stimulation

Pathogenic growth inhibition

Supernatant samples of MRS broth containing Lactobacillus & mushroom extract were collected separately.

Pathogenic bacteria (*L. monocytogenes, E.coli, S. dysenteriae, S. aureus*, MRSA) were cultured in Muller-Hinton agar (MHA) at 37°C for 24 hr.

20 µL from the supernatant of each probiotic culture was dropped onto the sterilized filter paper and placed onto the pathogenic bacteria plates.

Diameter of the clear zone was compared (Rousseau et al., 2005).

Antioxidant Activity

Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

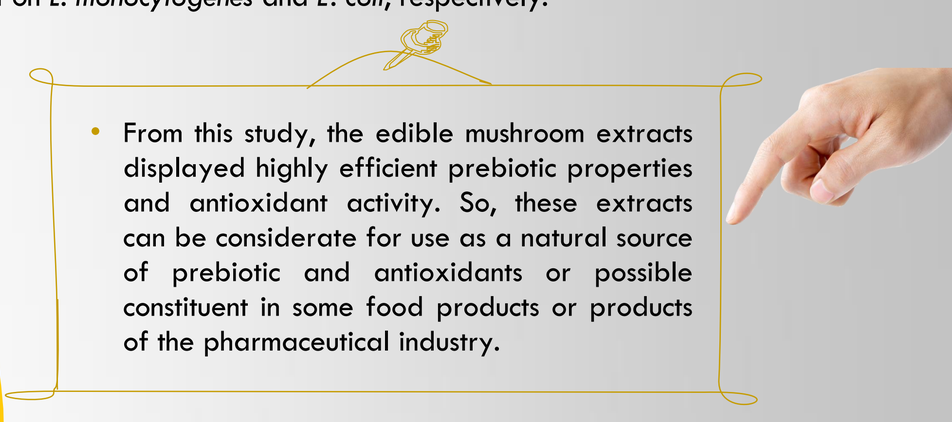
- The scavenging activity of the ethanol and water extracts from mushrooms on DPPH radicals was measured according to the method of (Chu et al., 2000) which produces a decrease in absorbance at 515 nm.

Scavenging activity on 2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) radical cation

- Free radical scavenging activity of mushroom samples was determined by ABTS radical cation decolorization assay. (Re et al., 1999) The relative ability of hydrogen-donating antioxidants to scavenge ABTS radical can be measured spectrophotometrically at 734 nm.

03 RESULTS

1. From this study, two different methods of extraction used with two edible mushrooms.
2. The total carbohydrate, total reducing sugar amounts, and antioxidant activity were determined.
3. *P. ostreatus* polysaccharide had the highest total carbohydrate and total reducing sugar
4. Oyster crude extract had the highest probiotic growth stimulation for *L. acidophilus* & *L. pentosus*, while brown mushroom crude extract showed the highest probiotic growth stimulation for *L. plantarum* and *L. paracasei*.
5. Polysaccharide from brown mushroom showed the most potent radical scavenging activity using DPPH and ABTS radicals.
6. The cultivation of *L. acidophilus* and *L. paracasei* using brown polysaccharide extract produced the highest inhibition effect on *L. monocytogenes* and *E. coli*, respectively.



04 CONCLUSIONS

- From this study, the edible mushroom extracts displayed highly efficient prebiotic properties and antioxidant activity. So, these extracts can be considerate for use as a natural source of prebiotic and antioxidants or possible constituent in some food products or products of the pharmaceutical industry.

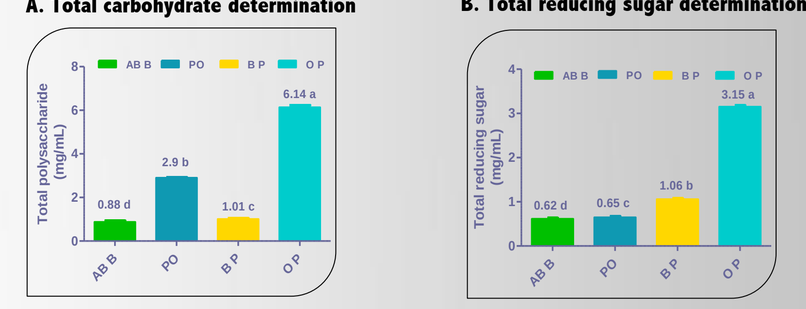


Figure (1): (A) Total carbohydrate and (B) total reducing sugar of *Agaricus bisporus* brown (AB B), *Pleurotus ostreatus* (PO), Brown polysaccharide (B P), and Oyster polysaccharide (O P). Values are presented as mean ± SE (n=3) and different letters specify the significance at $p < 0.05$.

Antioxidant Activity

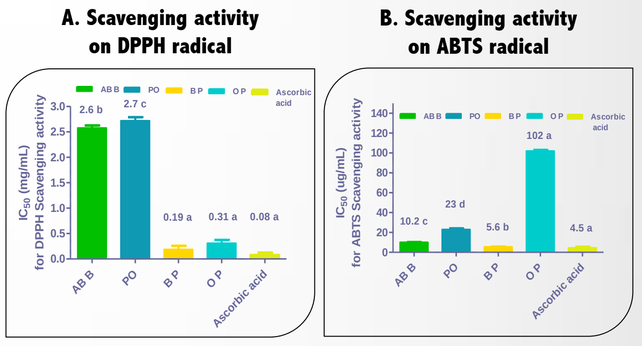


Figure (2): Antioxidant activities of *Agaricus bisporus* brown (AB B), *Pleurotus ostreatus* (PO), Brown polysaccharide (B P), and Oyster polysaccharide (O P) in comparison with ascorbic acid. a) DPPH scavenging activity, b) ABTS scavenging activity Values are presented as mean ± SE (n=3) and different letters specify the significance at $p < 0.05$.

Prebiotic growth stimulation

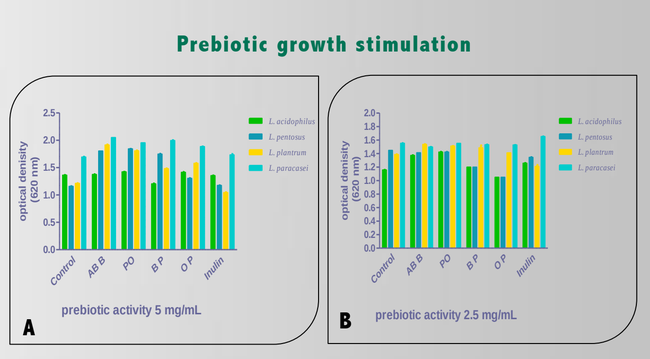


Figure (3): Growth of *L. acidophilus*, *L. pentosus*, *L. plantarum*, and *L.paracasei* cultivation in media with (A) 5 mg/mL, and (B) 2.5 mg/mL of mushroom extract and commercial prebiotic supplements, at 37°C for 48 hr determined using the optical density at 620 nm (OD620). Values are presented as mean ± SE (n = 3).

Pathogenic growth inhibition

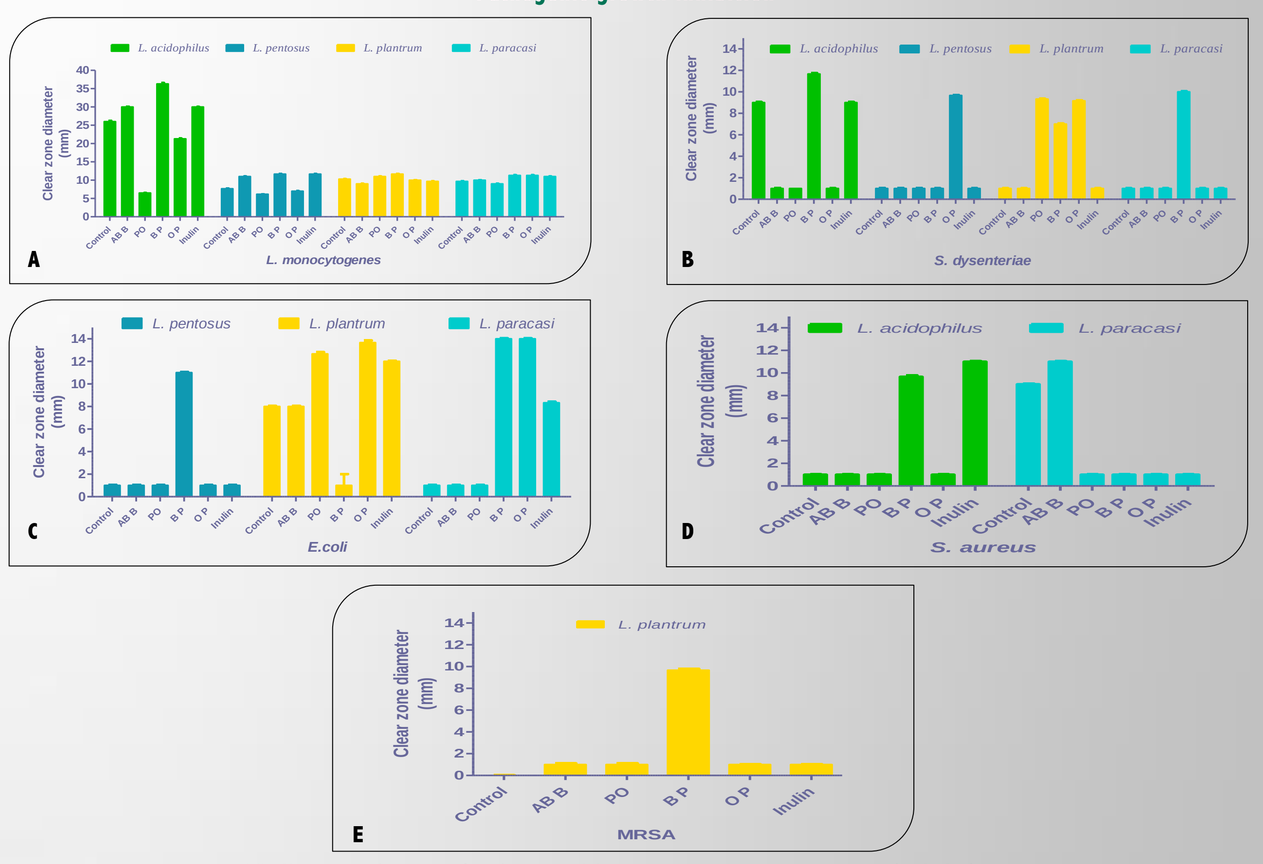


Figure (4): Clear zone diameter of pathogenic inhibition; (A) *L. monocytogenes*, (B) *E. coli*, (C) *S. dysenteriae*, (D) *S. aureus*, and (E) MRSA from; *L. acidophilus*, *L. pentosus*, *L. plantarum*, and *L. paracasei* cultivated in media with mushroom extract and commercial prebiotic supplement, where *Agaricus bisporus* brown (AB B), *Pleurotus ostreatus* (PO), Brown polysaccharide (B P), and *Oyster polysaccharide* (O P). Values are presented as mean ± SE (n = 3).

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