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Effects of mushroom consumption on the microbiota of different target groups – Impact of polyphenolic composition and mitigation on the microbiome fingerprint

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A B S T R A C T

This study determined the effect of the consumption of six edible wild mushroom species (*Boletus edulis*, *B. pinophilus*, *B. aureus*, *Armillaria mellea*, *Lactarius piperatus*, *Pleurotus eryngii*, *P. djamor* - positive control, *Amanita rubescens* - negative control) on the microbiota of three target groups: clinically healthy (NM) individuals, individuals with nutritional disorders (ND), and individuals with cardiovascular disease (CVD). The fingerprints of the three microbiota were determined by polymerase chain reaction (PCR). This study focused on investigating the *in vitro* effect of mushroom consumption on the distribution of bifidobacteria, lactobacilli, and entrobacteriacee strains. Some mushrooms exhibited a direct effect by mitigating the microbiome fingerprint among those in the CVD group when compared with those in the ND group. The most stable acid observed was gallic acid, which reached high levels following *Boletus* sp. consumption, while simulation of the ND microbiota presented with low levels of gallic acid, which was correlated with the number of coliforms. The primary conclusion of this study was that there was an increase in – and a higher diversity of – lactic acid bacteria (LAB) based on edible wild mushroom consumption. The presence of gallic acid correlated with positive changes in the microbiome fingerprint of the target groups.

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1. Introduction

Microbiota disorders can affect the symbiotic balance between hosts and microbes, and this may determine the occurrence of various diseases, such as inflammatory bowel diseases, colon cancer, and cardiovascular and neurodegenerative pathologies (Sekirov, Russell, Caetano, Antunes, & Brett Finlay, 2010). Changes in the bacterial composition of various microbiota were previously found to be associated with the development of certain diseases. For example, in patients with colon cancer, the number of *Bifidobacteria* strains is significantly lower when compared with the number of other strains, such as *Streptococcus bovis*, *Helicobacter pylori*, *Bacteroides fragilis*, *Clostridium septicum*, *Fusobacterium* spp., and *Escherichia coli* (Gagnière et al., 2016; Rossi & Amaretti, 2011).

The descending colon has a fully stabilized microbiota; thus, it contains the dominant microbial fingerprint, which may be considered representative of a target group. Samples obtained naturally will offer a representative microbial load for this terminal segment of the colon. It is possible that during gastrointestinal transit, other strains or different ratios of microbial strains (i.e., different microbial fingerprints) exist, but they have not been identified in the collected fecal samples. Changing the pH level, so it becomes more alkaline, occurs during the transition from one level to another; however, interactions with resident strains also lead to their elimination, even if some of those strains (including, for example, *Lactobacillus* strains) exert favorable effects. Of note, the consumption of certain species of edible wild mushrooms has a positive influence on the human microbiota by modulating microbiome fingerprint (Duda-Chodak, Tarko, Satora, & Sroka, 2015).

The aim of this study was to identify the microbial fingerprint in the descending colon following the consumption of edible wild mushrooms. The effects of mushroom consumption on the microbiological structure of two target groups (the microbiota of people

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with nutritional disorders [ND] and of those suffering from cardiovascular diseases [CVD]) were observed and compared to that of a control group (clinically healthy [NM] subjects). The results were obtained from *in vitro* studies that were conducted in the GIS1 system (www.gissystems.ro). The effect of the six aforementioned edible wild mushroom species on the number of bifidobacteria, lactobacilli, and entrobacteriacee was analyzed via polymerase chain reaction (PCR). In this experiment, *P. djamor* was used as a positive control, while *A. rubescens* was the negative control.

2. Materials and methods

2.1. Biological materials

The six mushroom species (*B. edulis, B. pinophilus, B. aureus, A. mellea, L. piperatus, P. eryngii*) were harvested from different regions of Romania (Table 1). Samples of whole mushrooms were cleaned of vegetable product residues after harvesting. They were dried in an oven (Memmert GmbH + Co.KG, Schwabach, Germany) at 50 °C, until they reached a constant weight. The voucher specimen for each species was deposited into a new herbarium (a dried mushroom collection) at the Faculty of Biotechnology, UASVM Bucharest. The corresponding number for each specimen is specified in Table 1. In addition, *P. djamor* was used as a positive control and *A. rubescens* served as the negative control.

2.2. Sample preparation for the in vitro tests

2.2.1. Digestion in the stomach and small intestine

All samples were subjected to the successive actions of the low pH of the stomach (pH 2) for 2 h, and of pancreatin (0.1%) and bile salts (0.5%) for 4 h (pH 6–7) (Khadhri et al., 2017). The samples were introduced into a sterile volume of 200 mL for each level. The experiments were conducted in a Duran bottle, made from borosilicate glass, at a temperature of 37 °C. pH was adjusted to the value 2 with 1 M HCl and to the values 6–7 with 1 M Na_2CO_3. All entries were made with a laboratory peristaltic pump, Behrotest, Type PLP 33, flow rate 0.4–2.0 L/h. The Duran bottle was put onto the magnetic stirrer with ceramic heating plate IKA C-MAG HS 7 to keep the contents in constant shaking at 50 rpm (Vamanu, Pelinescu, Marin, & Vamanu, 2012). After the first digestive process, the samples were immediately placed in the next phase (Naim, Messier, Saucier, & Piette, 2004).

Table 1
Variation of gallic acid level (mg/100 mL) after *in vitro* simulations and wild edible mushrooms consumption.

Samples	Microbiotas		
	Control (clinically healthy persons)	Nutritional disorders (diabetes)	Cardiovascular diseases
Boletus edulis (P1) ***01091201 GJ***	0.13 ± 0.01^{b}	0.27 ± 0.03^{b}	0.20 ± 0.02^{b}
Boletus pinophilus(P2) ***25061506 PH***	3.15 ± 0.58^{a}	ND	0.23 ± 0.01
Boletus aureus(P3) ***25071514 PH***	ND	ND	2.37 ± 0.30^{a}
Armillaria mellea(P4) ***20091517IS***	0.05 ± 0.00	ND	0.17 ± 0.00
Lactarius piperatus(P6) ***10081512BT***	ND	ND	ND
Pleurotus eryngii(P8) ***30011615B***	0.49 ± 0.01^{c}	0.41 ± 0.00	0.40 ± 0.00
Pleurotus djamor(C+) ***11051510B***	0.19 ± 0.01^{c}	0.71 ± 0.00	0.36 ± 0.01^{b}
Amanita rubescens (C-) ***11081508BT***	ND	ND	ND

ND—not detected. Different letters mean significant statistical differences($p < 0.05$).

2.2.2. Obtaining a stable microbiota

Feces samples were obtained from a minimum of three healthy volunteers of both sexes (control group – NM). The volunteers were individuals without communicable diseases, who consumed an omnivorous diet, and who were not receiving or had not received any antibiotics or other drugs that could affect the microbiota balance in the last 6 months. The two target groups were divided into those suffering from CVD and those with ND (Charoensiddhi, Conlon, Vuaran, Franco, & Zhang, 2016). Samples were collected in 10% glycerol and stored at −15 °C until use. Following the removal of large particles, the microbiota was reconstituted in peptone water for 7–10 days, and the entire process was carried out *in vitro* in a GIS1 static simulator (www.gissystems.ro).

2.2.3. Colonic in vitro simulation in the GIS1 system

The *in vitro* studies were realized in a single-chamber GIS1 simulator, and the protocol adapted here was described in a previous paper (Vamanu, Pelinescu, Avram, Nita, & Vamanu, 2013). The *in vitro* colonic GIS1 simulator consisted of one Duran bottle (1000 mL capacity) made of borosilicate glass with a removable screw cap. The bottle was maintained at a constant temperature of 37 °C, on a ceramic heating plate, IKA CMAG HS 7, to keep the mixture constantly shaking at 50 rpm, using a magnetic bar. For the entries in the system, four laboratory peristaltic pumps were used: Behrotest, Type PLP 33, flow rate 0.4–2.0 L h^{-1}. The operating conditions were the following: pH was adjusted to the value 5.6–5.9 with 6 N NaOH (pump 4), for a 4 h period (ascending colon); pH was adjusted to the value 6.2–6.5 with 6 N NaOH (pump 4), for an 8 h period (transverse colon); pH was adjusted to the value 6.6–6.9 with 6 N NaOH (pump 4), for a 12 h period (descending colon).

Fresh feces samples, for each microbiota, were used for media inoculation (maximum: 10%), and they were prepared as described in the previous section (2.2.2.). Control fermentation was achieved without the presence of mushrooms, in the same conditions. At the end of each segment simulation, a minimum of three samples were taken for metabolic and microbiological analysis.

2.3. Determination of metabolic activity

The amount of lactate was determined by Lactate assay kit no. MAK064 (Sigma-Aldrich Co., St Louis, MO, USA) using a colorimetric protocol with minor modifications. The reaction mixture consisted of 92 μL of lactate assay buffer, 4 μL of lactate enzyme mix, and 4 μL of the sample (a clear supernatant that was obtained following microbe removal); the reaction mixture was realized directly on 100-well polystyrene micro-well plates (Oy Growth Curves Ab Ltd., Helsinki, Finland). The micro-well plate was incubated at room temperature in a Bioscreen C MBR (Oy Growth Curves Ab Ltd.) for 30 min, and the values were read at 580 nm (Capita, Riesco-Peláez, Alonso-Hernando, & Alonso-Calleja, 2014). The amount of lactate present following the *in vitro* simulation was determined from the standard curve.

The amount of ammonia was determined by an Ammonium Quantofix kit (Macherey-Nagel GmbH & Co. KG, Duren, Germany). The reaction mixture consisted of 5 mL sample (clear supernatant, following the removal of microbes) and 10 drops of NH4+ (sodium hydroxide). The mixture was stirred and placed on a test strip for 5 s. Compared with the color scale, the measured quantity varied from 10 to 400 mg/L of NH_4^+.

Determination of polyphenol carboxylic acids was performed by high-performance liquid chromatography (HPLC), as described previously (Aliyazicioglu et al., 2016; Vamanu, Pelinescu, Avram, &

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