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Evaluation of Prebiotic Property in Edible Mushrooms

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Abstract: Mushroom are increased interest as a source of potential prebiotic substrate. This study was aimed to evaluate prebiotic property of edible mushroom. Five samples of commercial mushroom, *Auricularia auricula-judae*, *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus abalonus, and Volvariella volvacea* were used in the experiment. All mushroom samples were extracted separately to obtained soluble and insoluble polysaccharides. The bifidogenic effect using 4 strains of *Bifidobacterium* sp. (*B. bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. longum* TISTR 2194 and*B. animalis* TISTR 2195) were tested with all mushrooms. The selected mushrooms which could enhance growths of bifidobacteria were measured for prebiotic index. Fluorescent in situ hybridization technique (FISH) was used to enumerate specific bacteria in the fecal culture fermentation. The results showed that *Pleurotus sajor-caju* had the highest PI value followed by *Pleurotus abalonus*. These two mushrooms could stimulate the growths of bifidobacteria and lactobacilli and could suppress the growth of harmful bacteria in human gut model.

Keywords: prebiotic, mushroom, bifidobacteria

1. Introduction

Mushrooms are considered as a delicacy with high nutritional and functional value. They have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber, poor fat but with excellent important fatty acids content. Mushrooms also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) [1]. Moreover, mushrooms contain a huge diversity of biomolecules with medicinal properties. Due to these properties, they have been recognized as functional foods, and as a source for the development of medicines and nutraceuticals. Fruiting bodies, mycelia, and spores accumulate a variety of bioactive metabolites with immunomodulatory, cardiovascular, liver protective, antifibrotic, anti-inflammatory, antidiabetic, antiviral, antioxidant, antitumor, and antimicrobial properties [2, 3] Thus, they could be an excellent source of many different nutraceuticals and

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might be used directly in human diet to promote health for the synergistic effects of all the bioactive compounds.

Mushrooms are also rich in polysaccharides which are chitin, hemicelluloses, β-glucan, mannans and xylans. Different mushroom produces different types of polysaccharides which could be either water soluble or insoluble. Non-digestible mushroom polysaccharides are potential source of prebiotics as they may prevent viral or bacterial infection by enhancing the growth of probiotic bacteria in the large intestine [4-8]. The aim of this study was to evaluate the prebiotic properties of 5 edible mushrooms. The ability of bifidogenic effect was tested using 4 strains of *Bifidobacterium* sp. The potential mushrooms which could enhance growths of bifidobacteriawere measured further for prebiotic index.

2. Materials and Methods

Samples of fruit bodies

Five mushroom samples of *Auricularia auricula-judae*, *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus abalonus, and Volvariella volvacea* were purchased from supermarket in Phatumthani provinve. All mushroom samples were cleaned and blended using food processor (Model Cucina, Philips, China). The blended samples were kept at 20°C.

Extraction of mushroom fraction

Five blended samples of *Auricularia auricula-judae*, *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus abalonus, and Volvariella volvacea* were extracted separately to obtain mushroom fraction according to the modified method of Wasser [9] The blended samples were washed with 95% (w/w) ethanol, then washed with distilled water. All mushroom fractions were lyophilized to obtain the dried fractions and kept at 4°C until used.

Evaluation of bifidogenic effect

Four strains of bifidobacteria consisting of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 were used in this study. Each strain isolated from MRS agar plate were precultured twice in MRS liquid broth at 37ºC, first for 24 h and then for 18 h, to ensure that all the cells were harvested from the early stationary phase. The bacterial suspensions were then used to inoculate the testing media at 1% (v/v). In all cases, the initial microbial concentration was approximately 7.5 log cfu/ml.

The testing media were LB medium adding 1**%** of extracted mushroom sample instead of glucose and adjusted pH to 6.8. The 400 ml testing media were added into 500 ml screw-capped glass bottles and sterilized at 120°C for 15 min.

The fermentations were performed in triplicate. The testing media were inoculated with a 1% (v/v) inoculum and incubated at 37°C under anaerobic condition. Samples were regularly taken every 3 hours for total cell counting during 48 h.

Viable cells were enumerated using the method of Miles and Misra [10]. Decimal dilutions of fermentation broths were prepared using sterile normal saline solution. The 10 µl of diluted sample was dropped onto MRS agar plates and then incubated at 37°C for 2 days. Viable cell counts were calculated as Log colony forming units per ml.

Evaluation of prebiotic index (PI)

Faecal batch culture fermentation

The extracted mushrooms were evaluated for PI value by using faecal batch culture fermentation. The method was modified from the method of Mandalari et al [11]. The 500 ml water-jacketed fermenters were filled with 360 ml presterilised basal growth medium at pH 7.0. The fermenters were inoculated with 40 ml of faecal slurry. This faecal slurry was prepared by homogenizing 10% (w/v) fresh faeces from a healthy donor, who had not taken antibiotics for 3 months beforehand, in 0.1 M phosphate buffered saline, pH 7.0. Each 2 g extracted mushroom was added to give a final concentration of 0.5% (w/v). Each vessel was magnetically stirred with temperature controlled at 37°C. Culture pH was controlled at pH 6.6-6.8. Anaerobic conditions were maintained by sparing the vessels with oxygen-free nitrogen gas at 15 ml/min. Samples (5 ml) were taken from the fermenters at the start and at intervals over a 24-h incubation period. The fermentation experiments were performed in triplicate for each substrate using faecal inoculum from three volunteers.

Enumeration of bacteria

Enumeration of bacterial populations was performed by Fluorescence in situ hybridization (FISH) using synthetic oligonucleotide probes designed to target specific diagnostic regions of 16S rRNA and labelled with the fluorescent Cy3 dye (Sigma Aldrich Ltd., Singapore) as described by Daims et al [12] and Rycroft et al. [13]. The probes used consisted of Bif 164 for *Bifidobacterium* spp. [14], His 150 specific for *Clostridium histolyticum* subgroup [15], Bac 303 specific for *Bacteroides* spp. [16], Erec 482 specific for

Clostridium coccoides-Eubacterium rectale group [15], Ato 291 specific for *Atopobium* spp. and Lab 158 specific for *Lactobacillus/Enterococcus* spp. [17, 18] and 4′,6-diamidino-2- phenylindole (DAPI) for total cell counts.

Prebiotic index equation

The PI was used as a general quantitative comparative measure of the selectivity of fermentation. The PI is calculated from changes in the proportions of organisms generally considered to be beneficial and those generally considered detrimental in relation to the initial population [19-21].

To quantify the prebiotic effect (selective fermentation), a prebiotic index was calculated as follows:

PI = (Bif/Total) +(Lac/Total)+(Erec/total)–(Bac/Total)– (Ato/Total)-(Clos/Total)

Where Bif = bifidobacterial numbers at sample time/number at inoculation;

Lac = lactobacilli numbers at sample time/number at inoculation

Erec = *Clostridium coccoides-Eubacteriumrectale* group numbers at sampletime/number at inoculation

Bac = bacteroides numbers at sample time/numbers at inoculation

Ato = atopobium numbers at sample time/numbers at inoculation

Clos = clostridia numbers at sample time/number at inoculation

Total = total bacteria numbers at sample time/numbers at inoculation.

3. Results and Discussion

The bifidogenic effect

To investigate the prebiotic potential, the bifidogenic effect of different mushroom fractions was carried out. The growths of four strains of bifidobacteria consisting of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 were compared under the same fermentation conditions. The population changes were monitored throughout a 48-h fermentation period.

Figure 1 shows the growths of four bifidobacteria in the medium of *Auricularia auricula-judae*. In all cases, bacterial growths started to decrease from the early period of fermentation except the growth of *B.*

animalis which is relatively stable before 12 h. Its growth turned to an exponential phase at 24 h after which it decreased sharply after 30 h.

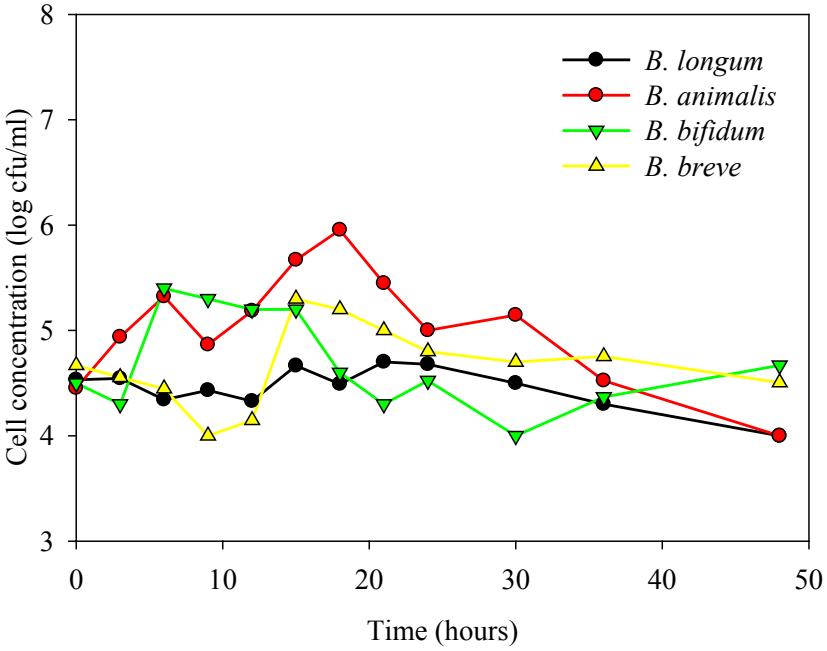

Figure 1. The growths of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 in the medium of *Auricularia auricula-judae*.

Figure 2 and 3 shows the growths of four bifidobacteria in the media of *Pleurotus ostreatus* and *Pleurotus sajor-caju*. The cell concentration of *B. animalis* was highest followed by *B. breve, B. bifidum* and *B. longum* in both media. However, the maximum cell concentration was obtained from the medium of *Pleurotus sajor-caju*.

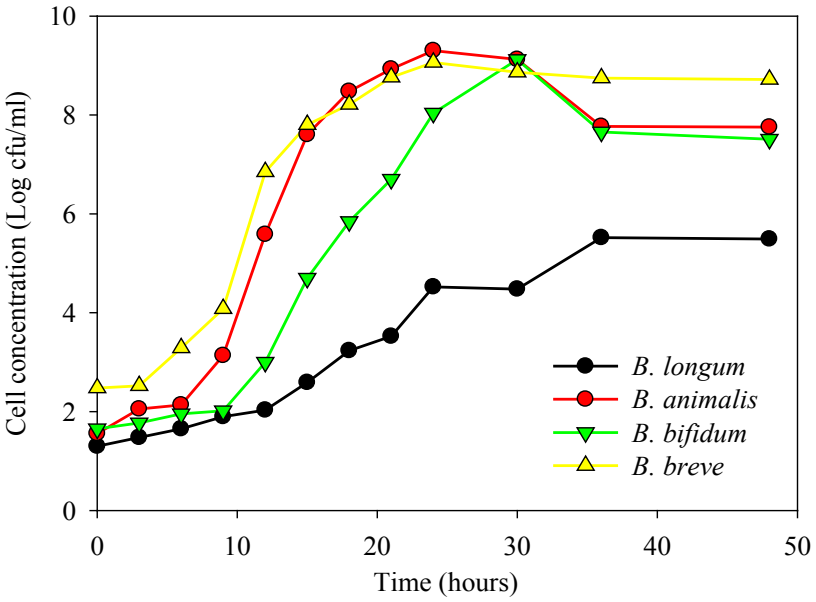

Figure 2. The growths of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 in the medium of *Pleurotus ostreatus.*

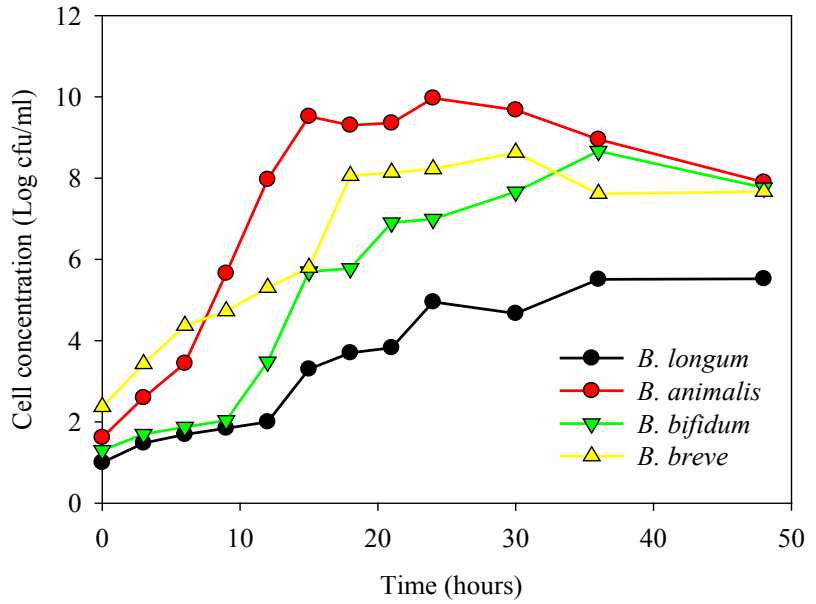

Figure 3. The growths of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 in the medium of *Pleurotus sajor-caju.*

Figure 4 shows the growths of four bifidobacteria in the media of *Pleurotus abalonus*. The highest cell concentration was *B. breve.* The growths of other bifidobacteria were similar. It seemed that all bacterial growths reached to stationary phase after 12-18 h after which the cell concentration slightly decreased till the end.

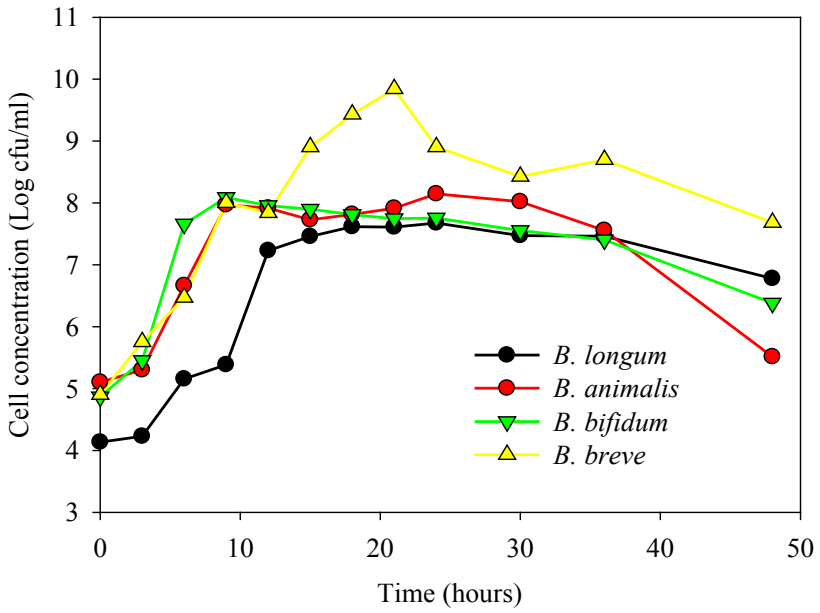

Figure 4. The growths of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 in the medium of *Pleurotus abalonus*

Figure 5 shows the growths of four bifidobacteria in the media of *Volvariella volvacea*. Similar growth of all bifidobacteria tested were represented from the fermentation. All growths started to approach exponential phase after 6 h. The cell concentrations increased sharply until early stationary phase at 12 h. Then, all bacterial growth turned to be relatively constant until 30 h. After that all bacterial growths started to decrease gradually through the end of fermentation.

From these results, it seem that three mushrooms consisting of *Pleurotus ostreatus, Pleurotussajor-caju*and *Pleurotus abalonus* can stimulate the growth of bifidobacteria better than the other two mushrooms (*Auricularia auricula-judae* and *Volvariella volvacea*). Thus, these three mushrooms are selected and used for further study.

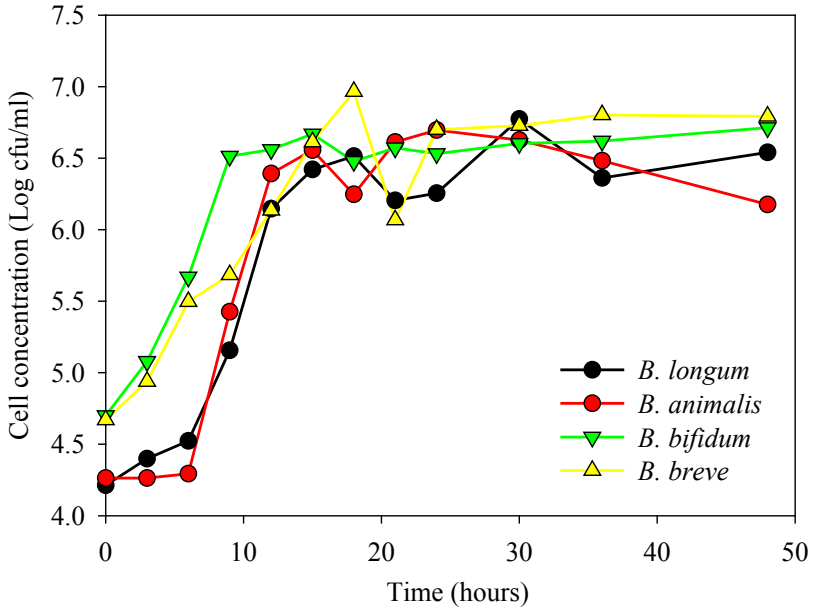

Figure 5. The growths of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194 in the medium of *Volvariella volvacea*

Evaluation of Prebiotic index

To evaluate the prebiotic activity, it is necessary to analyse the evolution of the bacterial populations in the presence of the substrate being tested. The behaviour of different bacteria in the fermentation could be monitored in mixed cultures due to synergistic, antagonistic and/or competitive effects [11]. The fermentation process in the gut is a complex process whereby many metabolic pathways are carried out by different groups of bacteria. The end products from one group could be metabolised by others that cannot directly metabolise the original source substrate [22]. Therefore, in this study the mushroom fractions were tested to evaluate the potential prebiotic using mixed faecal cultures. The fermentation conditions were maintained anaerobically at 37°C and pH 6.6-6.8. These conditions are set similarly to the human colon [13, 20]. Samples were taken at intervals in order to monitor the levels of different bacterial groups.

Results showed that an increase in the level of total bacteria was obtained in all batch cultures from 6 h until 24 h. The number of *Bifidobacterium* spp. obviously increased, especially in the cultures of *Pleurotus sajor-caju* followed by *Pleurotus ostreatus* and *Pleurotus abalonus* respectively. Increments of *Lactobacillus* spp. And *Entercococcus* spp. were slightly and observed in each culture. In addition, an increase in the

bacteroides was obtained slightly in all batch cultures. The population of *Clostidium histolyticum* and *Atopobium* spp. notably decreased in each culture after faecal inoculation at 12 h.

A PI was calculated for each mushroom to obtain a quantitative measure of the degree of selectivity of fermentation. PI values obtained from each mushroom at the incubation time of 6, 12, 18 and 24 h were variable. *Pleurotus sajor-caju* revealed the highest PI values while *Pleurotus abalonus* presented the lowest PI values. However, all mushroom could stimulate the growth of beneficial bacteria (bifidobacteria, lactobacilli and *Clostridium coccoides-Eubacterium rectale* group) rather than the growth of photogenic bacteria (bacteroides, *Clostidium histolyticum* and *Atopobium* spp.).

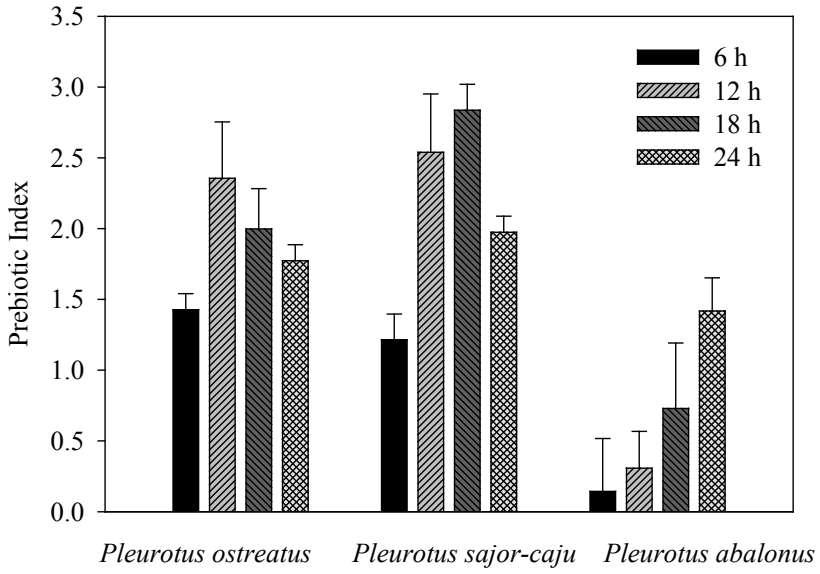

Figure 6. Prebiotic index of *Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus abalonus*after 6, 12, 18 and 24 h of anaerobic fermentation at 37°C and pH 6.6-6.8

4. Conclusion

In this study, mushroom could be a potential source of prebiotic which can activate probiotics that sustain in the gastrointestinal tract. The selected mushrooms, *Pleurotus ostreatus, Pleurotus sajor-caju* and *Pleurotus abalonus* represent the bifidogenic effect which can stimulate the growths of *Bifidobacterium bifidum* TISTR 2129, *B. breve* TISTR 2130, *B. animalis* TISTR 2195 and *B. longum* TISTR 2194. When evaluation of prebiotic index, *Pleurotus sajor-caju* has the highest PI amongst all mushrooms tested. Thus, this mushroom can be considered as a potential prebiotic source in future.

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