International Food Research Journal 19 (3): 1199-1205 (2012)

Effect of *Ganoderma lucidum* polysaccharides on the growth of *Bifidobacterium* spp. as assessed using Real-time PCR

[1]Yamin, S., [1,5,*] Shuhaimi, M, [2]Arbakariya, A., [3]Fatimah, A.B., [6]Khalilah, A.K., [7]Anas, O. and [4]Yazid, A.M.

[1]*Microbiology Department, Faculty of Biotechnology and Biomolecular Sciences,*
[2]*Bioprocess Technology Department, Faculty of Biotechnology and Biomolecular Sciences*
[3]*Food Science Department, Faculty of Food Science and Technology*
[4]*Food Technology Department, Faculty of Food Science and Technology*
[5]*Halal Products Research Institute*
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[6]*Microbiology Department, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM)*
40450 Shah Alam, Selangor, Malaysia.
7Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor

Abstract: The use of component from *Ganoderma lucidum* as prebiotic source is interesting as the *G. lucidum* itself was known for more than a decade in the traditional Chinese medicine. In this work, *Ganoderma lucidum* crude polysaccharides (GLCP) and Polysaccharide-fraction number 2 (PF-2) were used as carbon sources in the fermentation with *Bifidobacterium* sp. The results showed the potential of prebiotic effect of the *G. lucidum* extract in batch-culture fermentation based on increment in the growth of bacteria used (0.4 – 1.5 log10 CFU/mL) after 18h fermentation. Fermentation was further done using faecal materials as bacterial inocula and bacterial growth changes were examined using real-time PCR. The results showed the ability of GLCP and PF-2 to support the growth of *Bifidobacterium* genus with 0.3 and 0.7 log10 cells/ml increased, respectively. Interestingly, *Lactobacillus* which is known as beneficial bacterial genus also showed growth increment with 0.7 and 1 log10 cells/ml increased. The competition for carbon sources thus inhibits the growth of potentially harmful genus, *Salmonella* (0.3 and 0.5 log10 cells/ml) in comparison to the control.

Keywords: *Ganoderma lucidum* crude polysaccharides, polysaccharide-fraction number 2, *Bifidobacterium* spp., prebiotic

Introduction

Over the centuries, food for keeping a healthy gastrointestinal track (GIT) has evolved and risen into a very important issue among the public and researchers, in particular. Many people have gone beyond looking for food simply to maintain normal health and are seeking to optimise performance and wellness in addition to reducing the risk of some diseases such as cardiovascular disease, cancer and osteoporosis. This has set the scene for increased demand and development of functional foods (Delphine *et al*., 2009).

One of the functional foods that is increasing in demand is prebiotic, which has been proven through scientific research for its beneficial effects. Prebiotics are defined as "non-digestible dietary food ingredients that, when passing through the colon will benefit the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria *in situ*" (Gibson and Roberfroid, 1999). Prebiotics provide food for the growth of lactobacilli and bifidobacteria. These non-fermentable carbohydrates can be found naturally not only in cereals such as wheat and oat, but even in garlic, chicory and artichokes. However, their quantity is present in small amounts. Fruit and vegetables are among the most common, available and easily obtained prebiotic in the diet. Although it is preferable to obtain prebiotics from natural sources, this may not always be practical. Prebiotics can be incorporated into the diet through foods such as cereals and dairy products. Direct consumption of prebiotics is already available in the market in the capsule form.

New research is being proposed to find and establish new prebiotics synchronised with the demands of the market and people. The potential of polysaccharides from mushroom is a growing interest among researchers. Polysaccharides from *Pleurotus ostreatus* and *Pleurotus eryngii* were reported to be able to support the growth of *Lactobacillus* sp. (Andriy *et al.,* 2009). Thus, interest was increased on the potential of *Ganoderma lucidum*, the well-known medical fungi as prebiotic. The *G. lucidum* mushroom

*Corresponding author.
Email: *shuhaimi@biotech.upm.edu.my*
Tel.: +603 8943 6711; Fax: +603 8943 0913

contains pharmacologically active variables and the constituents of *G. lucidum* include polysaccharides (including beta-D-glucans, heteropolysaccharides and glycoproteins), triterpines, germanium, essential and non-essential amino acids, sterols, lipids, anti-oxidants, vitamins B1, B2, B6, iron, calcium, and zinc (Huie, 2004; McKenna, 2002). This mushroom has been reported to be effective in the treatment of chronic hepatopathy, hypertension, hyperglycaemia and neophasia (Franz, 1989; Furusawa *et al*., 1992; Shiao *et al*., 1994). The polysaccharides were also reported to have anti-tumour properties (Miyazaki and Nishijima, 1981; Wang *et al*., 1997) and hypoglycaemic activities (Hikino *et al.*, 1985; Tomoda *et al*., 1986).

Thus, this study was conducted in order to determine the ability of oligosaccharides from *Ganoderma lucidum* crude polysaccharide (GLCP) extract and second fraction of polysaccharide fractions (PF-2) to selectively promote the growth of *Bifidobacterium* strains and reveal the effect of GLCP and PF-2 as prebiotic towards the bacterial microflora in human faeces.

Materials and Methods

Ganoderma lucidum extract preparation

The dried fruit bodies of *G. lucidum* were purchased from Malimas Healthcare Sdn. Bhd. (Selangor, Malaysia). The polysaccharides from fruiting bodies were extracted and fractionated according to Bao et al. (2002) with some modifications. Briefly, the fruiting bodies were boiled and fractionated with DEAE-cellulose column, eluted with H2O and followed by 0.005, 0.2, 0.5 M NaCl solutions at a flow rate of 1.0 mL/min. The fractions were collected and separated based on the NaCl solutions concentration to have four fractions namely; PF-1, PF-2, PF-3 and PF-4. Fractionation was performed for several times and fractions with the same elution times were pooled together. PF-2 was the only fraction used to further the tests because of its potential in supporting bifidobacterial growth (Mohd Hamim, 2009). PF-2 was kept in 4°C. Fructooligosaccharides (FOS) was used as prebiotic control obtained from Orafti (Teinen, Belgium).

Proximate analysis of GLCP

Proximate analysis of GLCP was carried out according to AOAC method (1995). Moisture, protein, ash and crude fiber contents were analysed. Total carbohydrate contents were determined in percent by the formula, [100% − (%protein + %fat + %ash + %moisture)].

Determination of growth rates and survival of bacteria in GLCP and PF-2

The growth of *B. pseudocatenulatum* G4, *B. longum* BB536 and *B. breve* ATCC 15700 on glucose, GLCP and PF-2 was examined using batch culture fermentation. The bacteria were grown in the 1L bioreactor (B. Braun, France) for 24h in 37ºC. Sampling was done for every 6h. Growth rates and the ability of bacteria to grow in GLCP and PF-2 were observed. Glucose was used as the control.

Determination of prebiotic ability of GLCP and PF-2 in faecal culture

Fresh voided faecal samples were collected in sterile plastic bags, and kept cool in insulated boxes until they were transferred to the laboratory where they were immediately analysed. Faecal slurry inoculum was prepared 1h prior to fermentation. Fresh faecal samples were collected from three healthy people who practiced normal Malaysian diet and did not take any probiotic supplements or any antibiotics for 3 months before sampling. Faecal samples (10 g) from each person were mixed and homogenised in 300 mL of 0.1 M phosphate-buffered saline (PBS), pH 7.0 (10% w/v). Then, the faecal slurry was filtered using metal sieve to discard food and solid particles from inoculum suspension. All the procedures were done in laminar flow to prevent any contamination from undesirable outside microorganisms. Four types of carbon sources namely glucose, FOS, GLCP and PF-2 were added to anaerobic faecal batch cultures separately and ran for 24h fermentation. The effects upon major groups of the microbiota namely *Bifibacterium, Lactobacillus, Bacteroides* and *Salmonella* genus were evaluated using real-time PCR. The temperature of bioreactor was maintained for 37°C along the fermentation process to simulate human body temperature for optimum bacterial growth.

Quantitative real-time PCR

Cell concentration in fermentation samples was determined using molecular method. Duplication samples from fermentation bottles (2 mL each) were taken after 12 h and 24 h of fermentation and were stored at −20°C. After the extraction of DNA, 4 genera and species of predominant bacteria in human GIT namely *Bifidobacterium, Lactobacillus, Bacteroides*, and *Salmonella* were detected and quantified using quantitative real-time PCR method. The cell concentration in the samples was determined using standard curves generated from known concentration of plasmid DNA that carried the targeted gene for each genus and species. Quantification of four genera of bacteria in fermentation products was

achieved by using standard curves made from known concentrations of plasmid DNA containing the respective amplicon for each set of primers (Fite *et al*., 2004). The real-time PCR machine generated standard curve automatically by using the standard plasmid DNA and specific primers for each bacterial genus and species. Standard curves for quantification of number of cells in samples were generated using known concentration of plasmid DNA containing target gene. Plasmid DNA that was previously extracted from competent cells was measured for its concentration (g/µL) using spectrophotometry method at the absorbance of 260 nm. Then, the plasmid DNA was serially diluted (10-fold) until five times before 5 µL from each dilution were mixed with PCR mixture. The target genes were amplified by real-time PCR using the same primers in triplicate. The concentration of plasmid DNA was converted from g/µL in stock solution to copies/µL in PCR mixture. PCR amplification were performed using primers Forward 5'-CTCCTGGAAACGGGTGG-3' and Reverse 5'-GGTGTTCTTCCCGATATCTACA-3' for *Bifidobacterium* genus (Matsuki *et al*., 2003), Forward 5'-TGGAAACAGGTGCTAATACCG-3' and Reverse 5'-CCATTGTGGAAGATTCCC-3' for *Lactobacillus* genus (McOrist *et al*., 2002), Forward 5'-TCCACCTGGGGAGTACGCCG-3' and Reverse 5'-TATGGCACTTAAGCCGACACC-3' for *Bacteriodes* genus (McOrist *et al*., 2002) and Forward 5'- ACAGCAAAATGCGGATGCTTT-3' and Reverse 5'-GAGCGCTCAGTGTAGGACTC -3' for *Salmonella* genus (Carlson et al., 1999), to amplify 16S rRNA genes in this selected bacteria. Determination of specificity for each primer set and the optimum annealing temperature was done using a BiometraTM T Gradient thermocycler (Biometra, Goettingen, Germany). The primers were synthesised commercially by First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia). Real-time PCR amplification for the detection and enumeration of bacterial DNA was performed in a computer-operated Rotorgene 3000 real-time PCR system from Corbett Research, Australia. SYBR green master mix (Qiagen, USA) was used as a reporter dye in real-time PCR assay. The DNA template that had already been extracted from fermentation sample was mixed with other real-time PCR components in one 0.2 mL PCR tube before analyzed using real-time PCR machine.

Organic acid analysis

Profiles of SCFA were analysed by high-performance liquid chromatography (HPLC) (Shimadzu LC-10AS Liquid Chromatography, Japan) with a Shimadzu SPD-10AV UV-VIS detector. An organic column packed with 9 µm of polystyrene divinylbenzene ion exchange resin (Aminex HPX-87H; 300 mm × 7.8 mm, Bio-Rad Laboratories, USA) and maintained at 65°C was used. The UV detector was set at 220 nm and the mobile phase was 0.009 N sulphuric acid with a flow rate of 0.7 ml/min.

Statistical analysis

Data analysis was done using MINITAB version 14 (Minitab Inc., PA, United States). Differences between growth rate constants were checked by ANOVA for pure culture fermentation. Differences between bacterial counts at 0, 18 and 24 h of fermentation for each batch culture, treatments, and concentration of organic acids were checked for significance by paired t-test, assuming equal variances and considering both sides of distribution. A probability of $P<0.05$ was used as criterion for statistical significance.

Result and Discussion

The composition analysis of the GLCP

The yield of hot water extracts for G. lucidum shows that the carbohydrates (polysaccharides) was the highest (82.2%), while ash was the lowest (1.5%). The yield of protein, fat and moisture were 3.6, 4.3 and 8.4 percent, respectively. The fractionation process produced four types of fractions known as polysaccharides fraction one (PF-1), polysaccharides fraction two (PF-2), polysaccharides fraction three (PF-3) and polysaccharides fraction four (PF-4) as shown in Figure 1. However, based on the previous experiment, PF-2 was the only fraction selected for further experiment because of its ability to maintain and support the growth of Bifidobacterium sp. in the previous studies (Mohd Hamim, 2009).

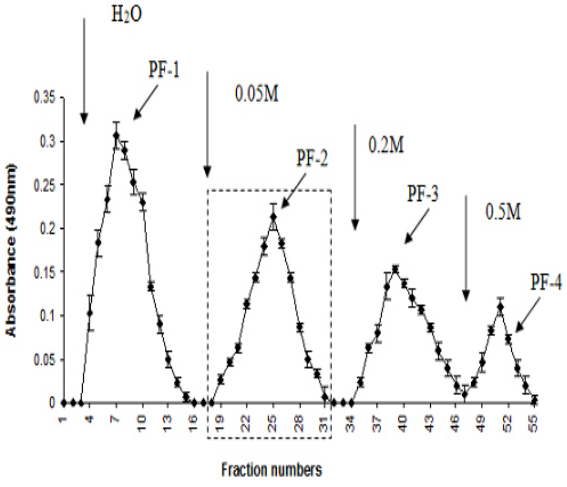


Figure 1. DEAE–cellulose elution profile of the polysaccharides present in the aqueous extract of Ganoderma lucidum fruit bodies. The column was eluted stepwise with H2O, 0.05, 0.1, 0.2 and 0.5 M NaCl solutions. PF-2 was used in the further fermentations (marked with the box).

Fermentation of GLCP and PF-2 in pure culture

Polysaccharides from mushroom were found to be able to induce the growth of probiotic bacteria. Andriy *et al*. (2009) reported that polysaccharides from *Pleurotus ostreatus* and *Pleurotus eryngii* supported the growth of *Lactobacillus* strains. The research has proven the ability of polysaccharides from mushroom to act as prebiotics. In this study, polysaccharides from *Ganoderma lucidum* extract were able to increase the growth of three selected *Bifidobacterium* strains. GLCP are first polysaccharides produced through extraction and purification. Based on Fig. 2, bacterial were shown of optimum growth at 18h and slightly reduced after 24h. At 18h, GLCP was shown slight increase in the bacterial growth for *B. pseudocatenulatum* G4, *B. longum* BB536, B. breve ATCC 15700 with 0.69, 0.59 and 0.53 log10 CFU/ml, respectively when compared to initial inoculums. The increase was because of the present of simple sugar such as glucose, in the crude extract (GLCP) itself. The present of simple sugar was also found in crude extract from several previous researches such as Saidou et al. (2011) with 22-36 percent of glucose from local African legumes. In the algal researches by Attachai and Anong (2010), glucose ranged within 0.09 to 0.45 percent was detected. The natural quantity after being extracted in the crude extract was the cause for the differences in glucose composition. As for PF-2, the sugar was produced through fractionation process. Fermentation of PF-2 was shown increase in the growth of Bifidobacterium strains (1.42, 1.61 and 1.73 log10 CFU/ml) compared to the initial inoculums. In comparison to FOS, the difference in bacterial growth for PF-2 at 18h was between 0.9 – 1.6 log10 CFU/ml for those three types of Bifidobacterium strains.

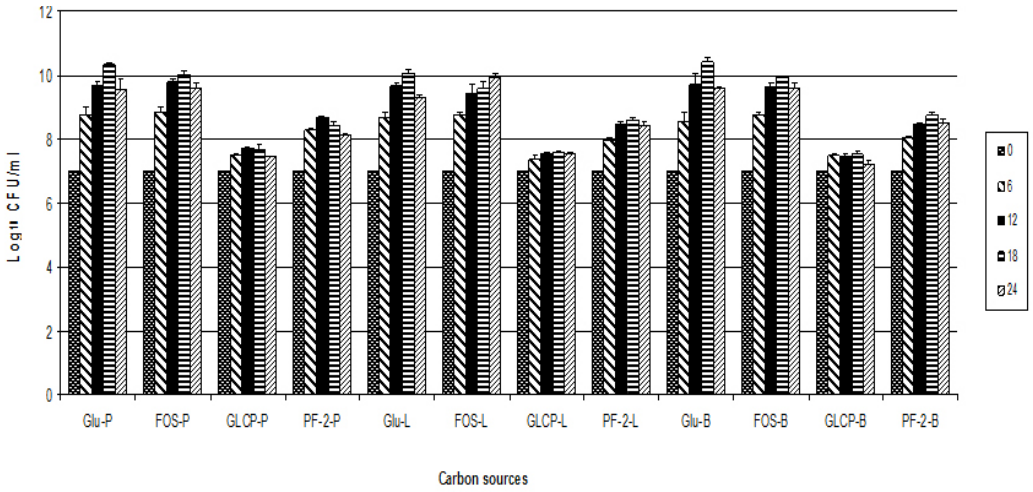
As for commercial prebiotic, FOS seems to have the ability to increase the growth of *Bifidobacterium* strains higher than GLCP and PF-2 with maximum growth at 18 h with range 2.6 to 3 log10 fold bacterial count. As for *B. longum* BB536, when the fermentation reached 18 h, the growth of this bacterium seemed to increase and managed to reach 9.94 log10 CFU/ml in FOS, respectively at the end of 24 h fermentation. Extended fermentation might be necessary to observe carbon source depletion point for this particular bacterium. Based on the growth profiles of *Bifidobacterium* strains, all strains were able to consume and degrade the component of crude GLCP and PF-2. As for *G. lucidum*, polysaccharides that are beneficial to human are also beneficial to Bifidobacterium strains from the perspective of this research.

From the Figure 2, all of the *Bifidobacterium* strains used possessed a different growth rate in the fermentation process with glucose, FOS, GLCP or PF-2 as their carbon sources. The growth rates of Bifidobacterium strains were determined by the ability to ferment each carbon sources and degree of polymerisation (DP) structure as *Bifidobacterium* sp. preferred to use low-DP carbohydrates (Cummings et al., 2001). From the three strains assessed, the best growth rates belonged to B. pseudocatenulatum G4 in FOS. FOS has the lowest DP structure among these four carbon sources. Even though the *Bifidobacterium* strains gave different log numbers of bacteria when grown in different types of prebiotics and carbon sources, the growth patterns were almost the same as they consumed the carbon sources on the same biochemical pathway.

Fermentation of GLCP and PF-2 in human faecal culture

Fermentation of polysaccharides in faecal cultures was conducted to show the prebiotic capabilities on all *Bifidobacterium* strains. The changes in cell number of bacteria were monitored using real-time PCR analysis. The fermentations were performed in the bioreactor with faecal slurry as the bacterial inoculum. Initial numbers of bacteria were different from each other with the highest being *Bacteriodes* with 7.4 log10 cells/ml, followed by *Bifidobacterium, Lactobacillus* and *Salmonella* with concentrations at 7.2, 6.5, and 2.8 log10 cells/ml, respectively. In these fermentations, the ability of bacteria to use carbon sources was shown with differences in the increment of bacterial cell numbers. Using real-time PCR analysis, the numbers of bacteria will be shown merely increment in the results as the machine will calculate not only alive but also dead bacteria.

In the human gut, bacterial composition changes according to daily diet taken by the host. FOS was proven to be able to increase *Bifidobacterium* genus (Bouhnik *et al*., 1996) thus giving a better gut health to the host. Based on Table 1, all selected carbon sources were able to increase the number of *Bifidobacterium* genus with the range between 0.3 log10 cells/ml to 1.1 log10 cells/ml. In the prebiotic treatment, the increase in the growth of the bacteria tested could be seen with significant numbers ranging from 0.6 log10 cell/ml to 1.8 log10 cell/ml after 24 h of fermentation. GLCP and PF-2 that were chosen to be prebiotics candidates were able to increase the growth of total *Bifidobacterium* genus by 0.3 log10 cell/ml and 0.7 log10 cells/ml, respectively. The increase of *Bifidobacterium* genus showed significant difference ($P \leq 0.05$) in PF-2 at 24 h when compared to the initial



Glu-P, FOS-P, GLCP-P, PF-2-P = Carbon sources used for fermentation of B. pseudocatenulatum G4; Glu-L, FOS-L, GLCP-L, PF-2-L = Carbon sources used for fermentation of B. longum BB536; Glu-B, FOS-B, GLCP-B, PF-2-B = Carbon sources used for fermentation of B. breve ATCC 15700. Data are expressed as mean±standard error (N=3).

Figure 2. Growth of Bifidobacterium spp. in different types of carbon sources in faecal batch culture fermentation

count. However, no significant difference (P>0.05) could be seen for GLCP and PF-2 when compared to the control. On the other hand, the increase of cell number for *Lactobacillus* genus was interestingly higher compared to *Bifidobacterium* genus with 0.7 log10 cells/ml for GLCP and 1 log10 cells/ml for PF-2. *Lactobacillus* is known for its beneficial effects and most of the probiotics come from this genus. The increase of *Lactobacillus* was significantly different (P≤0.05) when compared to the initial count of cell numbers with the highest growth at 1.8 log10 cells/ml in FOS. The increase was even higher as compared to *Bifidobacterium* genus thus showing their ability to be better at fermenting polysaccharides. *Bacteriodes* also showed an increase in FOS, GLCP and PF-2. However, the increase was far too low when compared to the control. Thus, it could be concluded that Bacteriodes does not prefer to use a FOS, GLCP and PF-2 as a carbon source. The inhibition of *Salmonella* was clearly shown in the FOS and PF-2 treatment. The inhibition of potential pathogenic bacteria fulfilled one of the prebiotic criteria. As the increase of *Bifidobacterium* and *Lactobacillus* in GLCP and PF-2 media was not as significantly (P>0.05) high as in FOS, the ability of GLCP and PF-2 to support the growth of those bacteria increases the expectation for a new prebiotic source.

Organic acids production

Acetic acid and lactic acid are two types of main organic acids produced by *Bifidobacterium* genus in ratio 3 to 2. In this research, the ability of *Bifidobacterium* genus in fermenting GLCP and PF-2 were shown by quantity of organic acid produced along their fermentation process (Table 2). The production of organic acids was in line with the

Table 1. Changes in bacterial population (log10 cells/ml) in batch cultures after 12 h and 24 h of incubation using real-time PCR based on total number of cells (viable and dead).

Group	Time (h)	Glucose (control)	FOS (prebiotic control)	GLCP	PF-2
Bifidobacterium	N_0	7.2 ± 0.4	7.2 ± 0.4	7.2 ± 0.4	7.2 ± 0.4
	12	7.6 ± 0.4^{a}	8.2 ± 0.3^{a,A}	7.4 ± 0.3	7.5 ± 0.3
	24	8.0 ± 0.4^{a}	8.3 ± 0.3^{a,A}	7.5 ± 0.2^{B}	7.9 ± 0.1^{a}
Lactobacillus	N_0	6.5 ± 0.8	6.5 ± 0.8	6.5 ± 0.8	6.5 ± 0.8
	12	7.3 ± 0.6^{a}	8.0 ± 0.7^{a,A}	6.9 ± 0.7^{A}	7.1 ± 0.7^{a}
	24	7.9 ± 0.5^{a}	8.3 ± 0.6^{a,A}	7.2 ± 0.6^{a,B}	7.5 ± 0.7^{a}
Bacteroides	N_0	7.4 ± 0.3	7.4 ± 0.3	7.4 ± 0.3	7.4 ± 0.3
	12	7.9 ± 0.4^{a}	7.7 ± 0.3^{a}	7.5 ± 0.3	7.6 ± 0.3^{a}
	24	8.1 ± 0.4^{a}	7.9 ± 0.4^{a}	7.6 ± 0.2^{a,B}	7.7 ± 0.3^{a,B}
Salmonella	N_0	2.8 ± 0.8	2.8 ± 0.8	2.8 ± 0.8	2.8 ± 0.8
	12	3.1 ± 0.6	2.8 ± 0.9	2.9 ± 0.8	2.9 ± 0.9
	24	3.4 ± 0.5	2.9 ± 0.9	3.1 ± 0.8	2.9 ± 0.9

N0 = Initial inoculum
a Significantly different from initial count (P≤0.05) at 0h
A Significantly different from glucose (P≤0.05) with higher value
B Significantly different from glucose (P≤0.05) with lower value
Values are mean log10 cells/ml culture ± SD for three replicates.

Table 2. Production of lactic acid and acetic acid in batch cultures fermentation of human faecal microbiota and significant difference between GLCP and PF-2 with glucose (control) and FOS (prebiotic control).

Carbon sources	Hours	Lactic acid (μmol/ml)	Acetic acid (μmol/ml)
Glucose (control)	0	3.0 ± 0.8	5.3 ± 0.4
	12	20.2 ± 1.4	26.7 ± 0.7
	24	29.3 ± 0.9	38.5 ± 1.2
FOS (prebiotic control)	0	3.0 ± 0.8	5.3 ± 0.4
	12	28.5 ± 2.1^{a}	36.2 ± 1.1^{a}
	24	39.6 ± 0.7^{a}	46.8 ± 1.4^{a}
GLCP	0	3.0 ± 0.8	5.3 ± 0.4
	12	6.9 ± 1.6^{bA}	10.1 ± 0.9^{bA}
	24	8.2 ± 1.3^{bA}	13.3 ± 1.2^{bA}
PF-2	0	3.0 ± 0.8	5.3 ± 0.4
	12	11.4 ± 0.7^{bA}	15.7 ± 1.3^{bA}
	24	15.2 ± 1.1^{bA}	21.1 ± 0.6^{bA}

^{a} Significantly different from Control (P≤0.05) with higher value
^{b} Significantly different from Control (P≤0.05) with lower value
^{A} Significantly different from FOS (P≤0.05) with lower value

growth of *Bifidobacterium* genus which shown the high numbers of this bacteria in faecal batch fermentation. Organic acid also one of the factors identified in inhibiting pathogenic bacteria in several researches. In the research by Midolo *et al*. (2005), the high concentration of organic acid was reported to be able to inhibit the growth of pathogenic bacteria in the gut such as *Helicobacter pylori*. In this study, the primers used for quantification using real-time PCR assay were counting both the dead and alive bacteria thus only increases of bacteria could be seen. Therefore, the inhibition of *Salmonella* genus only could be seen by comparing the growth of *Salmonella* genus in glucose medium with the other carbon sources containing media. From Table 1, the cell number of Salmonella genus was slightly increased or in same numbers as initial inoculums. When compared to the glucose (control), the difference was between 0 to 0.1 log10 cells/ml which resulted from the inability of Salmonella genus to grow in GLCP and PF-2 and thus can be concluded that the Salmonella growth was inhibited. The inhibition of Salmonella sp. by Bifidobacterium sp. was also shown by Araya-Kojima et al. (1995) by administration of B. longum BB536.

Conclusion

Fermentation of *Ganoderma lucidum* extracts have shown prebiotic ability of polysaccharides in increasing the number of bifidobacteria. The increase in the number of *Lactobacillus*, which is also well-known for its probiotic capability, should be considered as part of success in this study. The production of organic acid during prebiotic treatments showed domination of Bifidobacterium species throughout the fermentation process. The pattern of its production and inhibition of potential pathogenic bacteria suggest that the organic acids might directly confer antimicrobial action.

Acknowledgements

The authors are grateful for the financial support from Malaysian Government (FRGS Grant No. 01-11-08-663FR) to support this project.

References

Aida, F.M.N.A., Shuhaimi, M., Yazid, M. and Maaruf A.G. 2009. Mushroom as a potential source of prebiotics: a review. Trends in Food Science and Technology 20: 567-575.

Andriy, S., Katerˇina, M., Alla, S., Ivan, J., Jirˇí, S., Vladimír, E., Eliška, K. and Jana C. 2009. Glucans from fruit bodies of cultivated mushrooms P*leurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. Carbohydrate Polymers 76: 548–556.

AOAC Official Method of Analysis. 1995. 13th Edition. Association of Official Analytical Chemist, Wanshington, D.C.

Araya-Kojima, T., Yaeshima, T., Ishibashi, N., Shimamura, S. and H. Hayasawa, 1995. Inhibitory effects of *Bifidobacterium longum* BB536 on harmful intestinal bacteria. Bifidobacteria and Microflora 14(2): 59-66.

Attachai, K. and Anong, C. 2010. Components and Antimicrobial Activity of Polysaccharides Extracted from Thai Brown Seaweeds. Kasetsart Journal of Natural Sciences 44: 220-233.

Bao, X.F., Wang, X.S., Dong, Q., Fang, J.N. and Li, X.Y. 2002. Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. Phytochemistry 59: 175-181.

Bouhnik, Y., Flourié, B., Riottot, M., Bisetti, N., Gailing, MF., Guibert, A., Bornet, F. and Rambaud, J.C. 1996. Effects of fructooligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. Nutrition and Cancer 26(1): 21-29.

Carlson, S.A., Bolton, L.F., Briggs, C.E., Hurd, H.S., Sharma, V.K., Fedorka-Cray, P.J. and Jones, B.D. 1999. Detection of multiresistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR. Molecular and Cellular Probes 13(3): 213–222.

Cummings, J.H., Macfarlane, G.T. and Englyst, H.N. (2001). Prebiotic digestion and fermentation. American Society for Clinical Nutrition 73: 415-20.

Delphine, M.A.S., Jennifer, K.S., Glenn, R.G. and James, V. 2009. Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. Current Opinion in Biotechnology 20(2): 135–141.

Fite, A., Macfarlane, G.T., Cummings, J.H., Hopkins, M.J., Kong, S.C., Furrie, E. and Macfarlane, S. 2004. Identification and quantitation of mucosal and faecal desulfovibrios using real-time PCR. International Journal of Gastroenterology and Hepatology 53(4): 523–529.

Franz, G. 1989. Polysaccharides in pharmacy: current applications and future concepts. Planta Medica 55: 493-497.

Furusawa, E., Chou, S.C., Furusawa, S., Hirazami, A. and Dang, Y. 1992. Antitumor activity of *Ganoderma lucidum,* an edible mushroom, on intraperitoneally implanted Lewis Lung Carcinoma in synergenic mice. Phytotherapy research 6(2): 300–304.

Gibson, G.R. and Roberfroid, M.B. 1999. Colonic microbiota, nutrition and health. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Haarman, M. and Knol, J. 2005. Quantitative Real-Time PCR Assays To Identify and Quantify Fecal *Bifidobacterium* Species in Infants Receiving a Prebiotic Infant Formula. Applied and Environmental Microbiology 71(5): 2318-2324.

Hikino, H., Konno C., Mirin, Y. and Hayashi, T. 1985. Isolation and hypoglycemic activity of *Ganoderma* A and B, glycan of *Ganoderma lucidum* fruit bodies. Planta Medica 51(4): 339–340.

Huie, C. and Di, X. 2004. Chromatographic and electrophoretic methods for Lingzhi pharmacologically active components. Journal of Chromatography B 812: 241–57.

Matsuki, T., Watanabe, K. and Tanaka, R. 2003. Genus and species-specific PCR primers for the detection and identification of *Bifidobacteria*. Current Issues in Intestinal Microbiology 4(2): 61-69.

McKenna, D., Jones, K. and Hughes, K. 2002. Reishi. Botanical medicines: The desk reference for major herbal supplements. 2nd Edition. Binghamton, New York: The Haworth Herbal Press 825–855.

McOrist, A.L., Jackson, M. and Bird, A.R. 2002. A comparison of five methods for extraction of bacterial DNA from human faecal samples Journal of Microbiological Methods 50: 183–191.

Midolo, P.D., Lambert, J.R., Hull, R., Luo, F. and Grayson, M.L. 2005. *In vitro* inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. Journal of Applied Bacteriology 79: 475–479.

Miyazaki, T. and Nishijima, M. 1981. Studies on fungal polysaccharides of *Ganoderma lucidum*. Chemical and Pharmaceutical Bulletin 29(12): 3611-3616.

Mohd Hamim, H.M. 2009. Growth of bifidobacteria and other selected intestinal bacteria in TPY media supplemented with crude polysaccharides from *Ganoderma lucidum*. Selangor, Malaysia: Universiti Putra Malaysia, MSc thesis.

Ouwehand, A.C. 1998. Antimicrobial components from lactic acid bacteria. In: Salminen, S. and Von Wright, A. (eds.) Lactic Acid Bacteria: Microbiology and Functional Aspects 2nd Edition. New York: Marcel Dekker Inc. 139-160.

Saidou, C., Tchatchueng, J.B., Ndjouenkeu, R. and Roux, D. 2011. Extraction and Partial Characterisation of Hydrocolloid Gums from Some African Legumes. International Journal of Food Engineering. 7(3): 15.

Shiao, M.S., Lee, K.R., Lee, J.L. and Cheng, T.W. 1994. Natural products and biological activities of the Chinese medicinal fungus Ganoderma lucidum. In: Proceeding of the ACS symposium series on food phytochemicals for cancer prevention II. America chemical society. Washington DC. 547: 342-354.

Tomoda, M., Gonda, R., Kasahara, Y. and Hikino, H. 1986. Glycan structures of *Ganoderma lucidum* B and C, hypoglycemic glycans of Ganoderma lucidum fruit bodies. Phytochemistry 25: 2817–2820.

Vardakou, M., Palop, C.N., Christakopoulos, P., Faulds, C.B., Gasson, M.A. and Narbad A. (2008). Evaluation of the prebiotic properties of wheat arabinoxylan fractions and induction of hydrolase activity in gut microflora. International Journal of Food Microbiology 123: 166– 170.

Wang, S.Y., Hsu, M.L., Hsu, H.C., Tzeng, C.H., Lee, S.S., Shiao, M.S. and Ho, C.K. 1997. The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines reduced from activated macrophages and T lymphocytes. International Journal of Cancer 70(6): 699–705.