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Growth of probiotic bacteria in trypticase phytone yeast medium supplemented with crude polysaccharides from *Ganoderma lucidum*

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ABSTRACT

Ganoderma lucidum is a fungus usually used in traditional Chinese medicine. The high value of *G. lucidum* is related to its polysaccharides content. Crude polysaccharides from *G. lucidum* (GLCP) were obtained using hot water extraction method. There is about 0.57 g of GLCP in 1 g crude of *G. lucidum*. The prebiotic potential of GLCP was tested against probiotic bacteria namely: *Bifidobacterium longum* BB536, *Bifidobacterium pseudocatenulatum* G4, *Lactobacillus acidophilus* and *Lactobacillus casei* Shirota. The prebiotic potentials were studied in 10 mL basal Trypticase Phytone Yeast (abbreviated as bTPY) medium (without glucose) supplemented with various concentrations of GLCP (abbreviated as bTPYglcp) (0.5%, 1.0%, 1.5% and 2.0%). bTPY medium supplemented with glucose (abbreviated as bTPYglu) and inulin (abbreviated bTPYinu) were used as comparison. Viable cell counts of the bacteria and the pH of the medium were determined during anaerobic incubation period of 0 h, 12 h, 24 h and 48 h at 37 °C. In the presence of carbohydrate source, cultures showed various degree of growth increment. With regards to the growth supporting property: bTPYglu, bTPYglu+glcp, bTPYglcp and bTPYinu were ranked first, second, third and fourth respectively. Interestingly, in bTPYglcp medium, bacterial growth increased with increasing GLCP concentrations when incubated until 24 h. *B. longum* BB536 was ranked first (10.53 log cfu/mL) in term of their growth in this medium. Growth of *B. pseudocatenulatum* G4 was ranked second with 10.40 log cfu/mL. This study shows that, GLCP could support the growth of the bacteria tested.

Keywords: probiotic, bifidobacteria, *Ganoderma lucidum*

INTRODUCTION

Ganoderma lucidum, a basidiomycete mushroom is a popular chemopreventive mushroom in oriental countries usually used in traditional Chinese medicine. Many bioactive components have been identified from its fruiting bodies, mycelia, spores, and culture media (Lin *et al*., 2003). *G. lucidum* has been used traditionally for the prevention and treatment of various types of diseases, such as cancer, hepatopathy, arthritis, hypertension, neurasthenia and chronic hepatitis. However, the most attractive property of *G. lucidum* is its anti-tumour effect (Gao *et al*., 2005) which has been demonstrated to be mainly associated with its polysaccharides fraction by mediating immune system mechanisms (Chen *et al*., 2004). Chemical composition investigation on fruiting bodies, spores and mycelia revealed that they contain various bioactive substances including polysaccharides, proteins, nucleosides, fatty acids, terpenoids, sterols, and cerebrosides (Yeung *et al*., 2004). The polysaccharides of *G. lucidum* are the major source of its biological activities and therapeutic use. Polysaccharides from *G. lucidum* have gained wide popularity as a health food supplement in Japan and China because of its perceived health benefits (Tang and Zhong, 2003). However, its effects on intestinal microbiota are not well understood.

Among more than 400 species of bacteria present in the intestine of adult human being, probiotic microorganisms are considered to be the most beneficial to human health. Fuller (1989) defined probiotic as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.

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An increase in number and activity of probiotic microorganism in the colon of human hosts is therefore likely to be desirable. Genera *Bifidobacterium* and *Lactobacillus* are common probiotic microorganisms that give beneficial to host health. One of the approaches employed to achieve the increase number of probiotic microorganism in the ecosystem of the human gastrointestinal tract is via the use of prebiotics. Prebiotics are mainly oligosaccharides which are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, which can improve host health (Gibson and Roberfroid, 1995). Prebiotic oligosaccharides can be produced by either extraction from plant sources, enzymatic hydrolysis of polysaccharides or fermentation by *Leuconostoc mesenteroides* B-742 to yield α-glucooligosaccharides (Morgan *et al*., 1992; Chesson, 1993; Chung and Day, 2002).

Therefore, the objective of this study is to determine the ability of total polysaccharides from *G. lucidum* to promote the growth of bifidobacteria as a potential prebiotic.

MATERIALS AND METHODS

Microorganisms and culture conditions

B. pseudocatenulatum (A50, C25, D22, Z86, F112, F117 and G4) were local isolates obtained from Probiotic Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia. They were previously isolated from breast-fed infant stools (Shuhaimi *et al*., 2001a; 2001b; 2002). *B. pseudocatenulatum* JCM 1200 and *B. longum* JCM 1260 were purchased from Japan Collection of Microorganisms, Tokyo, Japan. *B. longum* BB536 was obtained from Morinaga Milk Industries, Japan. *L. acidophilus* and *L. casei* Shirota were isolated from commercial fermented drinks Vitagen and Yakult respectively. All strains were routinely grown in TPY broth (Scardovi, 1986). After two successive growths of the strains in TPY broth at 37 °C for 16 to 18 h anaerobically, the activated cultures were properly diluted and serves as inocula. TPY broth without glucose (bTPY) was used as the basal medium to examine the effect of crude polysaccharides from *G. lucidum*.

Media preparation

Trypticase Phytone Yeast medium without glucose (bTPY) was used as a basal medium and control in these experiments. bTPY was supplemented with different concentrations of carbohydrate sources (0.5%, 1.0%, 1.5% and 2.0%, w/v). The media were generally designated as basal TPY medium supplemented with glucose (bTPYglu), glucose with GLCP (bTPYglu+glcp), GLCP (bTPYglcp) and inulin (bTPYinu). The prepared medium was sterilized by autoclaving at 121 °C for 15 min. The pH of all the media were set at pH 6.8 prior to autoclaving. No significant pH changes were recorded after autoclaving.

Extraction of crude polysaccharides from *G. lucidum*

Crude polysaccharides from *G. lucidum* namely (GLCP) were extracted using hot water method. Fresh *G. lucidum* fruiting bodies were supplied by Malimas Healthcare Sdn. Bhd. Other chemicals used in this study were purchased from Sigma Chemical unless specified. Fresh fruiting bodies (50 g) were shredded using a super chopper for polysaccharide extraction. The GLCP in the shredded samples was extracted 3 times with boiling water (250 mL) in which each extraction took 2 h. In order to remove protein content from the extracts, 20% of Trichloroacetic acid (TCA) was added to the extracts and further dialyzed against tap water and distilled water for 2 days and 1 day, respectively. The extracts were pooled and concentrated using a rotor evaporator (Buchi Rotavapor R-200, Switzerland). Four volumes of 95% (v/v) ethanol were added to precipitate the crude polysaccharides. GLCP was collected by centrifugation (1500 x *g*) and washed sequentially with ethanol. The crude product was freeze-dried and gave brownish powder as also observed by Bao *et al*. (2001).

Survival of probiotic bacteria in basal TPY medium supplemented with GLCP

Experiments were conducted to determine the ability of GLCP to support the growth of test bifidobacteria. In this study, test microorganisms were grown in bTPY medium supplemented with 0.5% GLCP (bTPYglcp 0.5%). All bacterial cultures previously stored in glycerol were revived and subcultured in TPY medium. The cultures were subcultured twice before starting the experiment. After twice subculturing, the bacteria were cultivated in TPY agar for 24 h. To obtain the initial counts of approximately 6.60 log cfu/mL for each strain tested, one colony with typical morphology was selected from a TPY agar plate that has been incubated anaerobically using Anaerocult® A (Merck, Germany) in an anerobic jar for overnight and inoculated into the 10 mL TPY broth for another 24 h.

Test microorganisms (0.1 mL) were inoculated into 9.9 mL broth of bTPY medium and bTPYglcp medium. bTPY medium was used as a control. After 24 h incubation, a 0.1 mL culture was inoculated onto TPY agar plates and incubated anaerobically at 37 °C for at least 24 h. The colonies developed after incubation were counted to evaluate the effect of GLCP on the growth of test microorganisms and then compared to the basal medium. The pH of the culture medium was measured with a pH meter (Toledo 320). The mean values and standard deviations were calculated from the data obtained with triplicate trials.

Growth of selected microorganisms in bTPY, bTPYglcp, bTPYglu, bTPYglu+glcp and bTPYinu media

In this study, growth of selected probiotic bacteria and pH changes in bTPY, bTPYglcp, bTPYglu, bTPYglu+glcp and bTPYinu at different concentrations (0.5%, 1.0%, 1.5% and 2.0%, w/v) were investigated. The bacteria used were *B. longum* BB536, *B. pseudocatenulatum* G4, *L. casei* Shirota and *L. acidophilus*. Cultures of test microorganisms were prepared as mentioned earlier. Briefly, a 0.1 mL of the entire test microorganisms were inoculated into 9.9 mL of bTPY, bTPYglcp (0.5%, 1.0%, 1.5% and 2.0%), bTPYglu (0.5%, 1.0%, 1.5% and 2.0%), bTPYglu+glcp (1:1 ratio of glucose and GLCP to obtain final concentration of glucose and GLCP of 0.5%, 1.0%, 1.5% and 2.0%) and bTPYinu (0.5%, 1.0%, 1.5% and 2.0%). bTPY was used as a control. After 12, 24 and 48 h incubation, a 0.1mL of the culture was inoculated onto TPY agar plates and incubated anaerobically at 37 ℃ for 24 h. The colonies developed after incubation were counted to evaluate the effect of GLCP on the growth of test microorganisms and compared with the other medium. The pH of the culture media was measured with a pH meter (Toledo 320). The mean values and standard deviations were calculated from the data obtained with triplicate trials.

Statistical analysis

Data were expressed as mean ±SD. Statistical significance were analyzed by two way ANOVA test using MINITAB statistical software (Release 14 for Windows, 2006, Minitab Inc, USA) followed by Dunnett multiple comparisons to compare treatment groups with control or the reference strain. Probability levels of less than 0.05 were considered significant (p<0.05).

RESULTS AND DISCUSSION

Extraction of crude polysaccharides from *G. lucidum*

One of the common methods to extract the crude polysaccharides from *G. lucidum* is by hot water extraction (Lai and Yang, 2007). In this study, this method was performed to obtain the crude polysaccharides. Hot water extraction performed on the fruiting bodies of *G. lucidum* yielded 3.2% of GLCP (Table 1). This value was in agreement with data by Bao *et al.* (2001) which has reported an extraction of 3.8% brownish crude polysaccharide from *G. lucidum*. TCA was used to remove protein in the GLCP. No absorbance at 280 nm was observed and negative response to Bradford assay indicated that the GLCP contained no protein.

In order to determine the carbohydrate content of crude extract, phenol-sulphuric acid method was performed. Measurement of carbohydrate contents in a variety of samples is a basic analytical operation in many phases of biosciences. Among many calorimetric methods for carbohydrate determination, the phenol-sulphuric acid method is the easiest and most reliable method for measuring neutral sugars in oligosaccharides, proteoglycans, glycoprotein and glycolipids (Masuko *et al*., 2005). The phenol-sulphuric acid is used widely because of its sensitivity and simplicity. Simple sugar, oligosaccharides, polysaccharides and their derivatives give a stable orange colour when reacted with phenol and concentrated sulphuric acid.

Table 1: Extraction of crude polysaccharides from *G. lucidum*

	Replicate			Average
	1	2	3	
Total weight of *G. lucidum* fruiting bodies (g)	50	50	50	50
Total H_2O used (mL)	750	750	750	750
Total extracts after hot water extraction (mL)	700	685	715	700
Total extracts after evaporate (mL)	15.0	13.6	14.2	42.8
Total extracts after freeze dry (g)	1.70	1.51	1.63	1.61
Percentage of crude extracts (%)	3.40	3.02	3.26	3.23
Total carbohydrate content in 1g of crude polysaccharides (g)	0.58	0.52	0.58	0.56

Studies on the growth of test microorganisms in bTPYglcp medium

A total of 14 potential probiotic bacteria were evaluated for their ability to grow in bTPYglcp medium as compared to control (bTPY). Initial pH values were set at 6.8 for all media tested. Table 2 shows the growth of microorganisms and pH changes in bTPY and bTPYglcp after 24 h incubation at 37 ℃. Populations of the test bacteria were determined by pour plate count using TPY agar. The results showed that GLCP had a good growth supporting effect on all the bacteria tested as compared to their growth in the control medium. Generally, all bacteria tested were able to ferment GLCP and showed a numerically significant growth from 6 to 8 log cfu/mL after 24 h incubation.

Among the *Bifidobacterium* sp., *B. longum* BB536 showed the highest viable count in bTPYglcp medium from 6.63 to 8.63 log cfu/mL, after 24 h incubation. The pH value decreased from 6.80 to 4.50 after 24 h. These were followed by *B. pseudocatenulatum* G4 (6.63 to 8.61 log cfu/mL) and *B. pseudocatenulatum* F112 (6.63 to 8.55 log cfu/mL). The final pH values for *B. pseudocatenulatum* G4 and *B. pseudocatenulatum* F112 were 4.52 and 4.59 respectively. The lowest growth count was observed in the medium cultured with *B. pseudocatenulatum* A50 with growth from 6.63 to 8.02 log cfu/mL. The difference in the viable count of bifidobacteria observed in bTPYglcp may

Table 2: Growth of microorganisms and pH changes in bTPY and bTPYglcp media

Microorganisms	Hours	bTPY		bTPYglcp 0.5%	
		Log cfu/mL	pH	Log cfu/mL	pH
B. pseudocatenulatum F112	0	6.63±0.05[a]	6.80±0.00[a]	6.63±0.04[a]	6.80±0.00[a]
	24	7.31±0.08[b]	5.80±0.03[b]	8.55±0.08[b]	4.59±0.04[b]
B. pseudocatenulatum F117	0	6.62±0.03[a]	6.80±0.00[a]	6.72±0.06[a]	6.80±0.00[a]
	24	7.30±0.08[b]	5.79±0.02[b]	8.51±0.07[b]	4.57±0.03[b]
B. pseudocatenulatum G4	0	6.63±0.04[a]	6.80±0.00[a]	6.63±0.05[a]	6.80±0.00[a]
	24	7.32±0.0.9[b]	5.77±0.03[b]	8.61±0.08[b]	4.52±0.03[b]
B. pseudocatenulatum C25	0	6.64±0.04[a]	6.80±0.00[a]	6.62±0.06[a]	6.80±0.00[a]
	24	7.27±0.07[b]	5.80±0.04[b]	8.42±0.05[b]	4.54±0.03[b]
B. pseudocatenulatum Z86	0	6.64±0.05[a]	6.80±0.00[a]	6.63±0.03[a]	6.80±0.00[a]
	24	7.25±0.09[b]	5.81±0.03[b]	8.48±0.08[b]	4.56±0.04[b]
B. pseudocatenulatum JCM1200	0	6.63±0.03[a]	6.80±0.00[a]	6.63±0.04[a]	6.80±0.00[a]
	24	7.30±0.08[b]	5.82±0.02[b]	8.50±0.09[b]	4.56±0.03[b]
B. pseudocatenulatum A50	0	6.62±0.04[a]	6.80±0.00[a]	6.63±0.04[a]	6.80±0.00[a]
	24	7.27±0.06[b]	5.79±0.03[b]	8.02±0.07[b]	4.59±0.04[b]
B. pseudocatenulatum D22	0	6.63±0.05[a]	6.80±0.00[a]	6.63±0.03[a]	6.80±0.00[a]
	24	7.26±0.08[b]	5.83±0.02[b]	8.11±0.08[b]	4.59±0.03[b]
B. longum JCM1260	0	6.63±0.04[a]	6.80±0.00[a]	6.69±0.04[a]	6.80±0.00[a]
	24	7.26±0.09[b]	5.80±0.02[b]	8.40±0.09[b]	4.56±0.04[b]
B. longum BB536	0	6.63±0.04[a]	6.80±0.00[a]	6.63±0.03[a]	6.80±0.00[a]
	24	7.31±0.07[b]	5.77±0.02[b]	8.63±0.07[b]	4.50±0.04[b]
L. casei shirota	0	6.67±0.04[a]	6.80±0.00[a]	6.65±0.04[a]	6.80±0.00[a]
	24	7.30±0.08[b]	5.81±0.03[b]	8.56±0.09[b]	4.59±0.02[b]
L. acidophilus	0	6.66±0.04[a]	6.80±0.00[a]	6.63±0.03[a]	6.80±0.00[a]
	24	7.30±0.07[b]	5.80±0.04[b]	8.60±0.08[b]	4.58±0.03[b]

bTPY was TPY medium without glucose
bTPYglcp was bTPY medium supplemented with 0.5% GLCP
The mean values and the standard deviation (±SD) were calculated from the data obtained with triplicate trials
[a b] mean significantly different among hour (p<0.05)

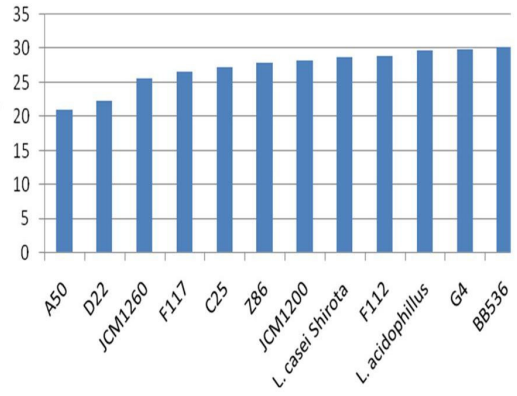

Figure 1: Percent growth increments of the test bacteria in bTPY medium supplemented with 0.5% GLCP (w/v)

be attributed to the difference in the nutritional requirement among strains (Desjardins *et al*., 1990). Fermentation by these bacteria produced acidic condition. The lowest pH value was in medium containing *B. longum* BB536 with the pH value of 4.50. These were followed by *B. pseudocatenulatum* G4, *B. pseudocatenulatum* C25 and *B. pseudocatenulatum* Z86 with the pH values of 4.52, 4.54 and 4.56 respectively after 24 h incubation. The decrease in pH values indicating that the bacteria tested was growing in the medium. Both *Lactobacillus* sp. tested also showed good growth in this bTPYglcp 0.5% medium. The growth of *L. acidophilus* and *L. casei* Shirota were from 6.63 to 8.60 and 6.65 to 8.56 log cfu/mL respectively after 24 h incubation. Fermentation by *Lactobacillus* sp. also created acidic condition with the pH values decreased over time.

Figure 1 shows the growth percentage of bifidobacteria in bTPYglcp 0.5% medium. Highest growth percentage was observed for *B. longum* BB536 with 30.2% increment. These were followed by *B. pseudocatenulatum* G4 and *B. pseudocatenulatum* F112 with increased growth percentage of 29.9% and 29.0%, respectively. The results also proved that the GLCP could promote the growth of the other tested bacteria at least more than 20% increment with the lowest growth percentage was in the medium containing *B. pseudocatenulatum* A50 with 21.0% increment. These results indicated that the GLCP could support the growth of bacteria tested. Discrepancies in capability to metabolize GLCP *in vitro* may be attributed to the differences in the nutritional requirement of the test organisms (Desjardins *et al*., 1990).

Table 3: The growth of *B. longum* BB536 in different media supplemented with various concentrations carbon sources

Media	Time (h)	Log cfu/mL				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.86±0.05[a]	6.86±0.55[a]	6.87±0.11[a]	6.87±0.10[a]
	12		8.61±0.07[c]	8.75±0.09[c]	8.82±0.11[b]	8.62±0.11[b]
	24		9.34±0.09[d]	9.88±0.11[d]	10.24±0.10[c]	10.73±0.11[d]
	48		7.85±0.06[b]	8.13±0.11[b]	8.78±0.11[b]	9.31±0.09[c]
bTPYglu+glcp	0		6.86±0.11[a]	6.86±0.11[a]	6.86±0.12[a]	6.86±0.11[a]
	12		8.72±0.10[c]	8.78±0.11[c]	8.84±0.09[b]	8.69±0.10[b]
	24		9.37±0.12[d]	10.19±0.09[d]	10.65±0.09[c]	10.82±0.09[d]
	48		7.84±0.09[b]	8.36±0.10[b]	8.86±0.08[b]	9.26±0.09[c]
bTPYglcp	0		6.86±0.11[a]	6.86±0.11[a]	6.86±0.11[a]	6.86±0.11[a]
	12		8.52±0.10[c]	8.64±0.10[c]	8.61±0.09[c]	8.62±0.11[b]
	24		9.37±0.12[d]	9.75±0.10[d]	10.23±0.11[d]	10.53±0.10[c]
	48		7.42±0.10[b]	7.90±0.11[b]	8.44±0.11[b]	8.83±0.12[b]
bTPYinu	0		6.86±0.11[a]	6.86±0.11[a]	6.86±0.12[a]	6.86±0.11[a]
	12		8.52±0.09[c]	8.60±0.11[c]	8.73±0.11[b]	8.53±0.09[b]
	24		9.28±0.11[d]	9.83±0.11[d]	10.12±0.10[c]	10.65±0.09[d]
	48		7.62±0.11[b]	8.00±0.13[b]	8.56±0.11[b]	9.07±0.12[c]
bTPY	0	6.87±0.12[a]	NT	NT	NT	NT
	12	8.48±0.10[d]	NT	NT	NT	NT
	24	7.71±0.10[c]	NT	NT	NT	NT
	48	7.14±0.11[b]	NT	NT	NT	NT

bTPY = TPY medium without carbohydrate source (basal medium)
bTPYglu = basal medium supplemented with glucose
bTPYglu+glcp = basal medium supplemented with glucose and GLCP
bTPYglcp = basal medium supplemented with GLCP
bTPYinu = basal medium supplemented with inulin
The mean values and the standard deviation (±SD) were calculated from the data obtained with triplicate trials
NT = Not Tested
[a b c d]mean significantly different among hours (p<0.05) for the respective medium

Growth of selected microorganisms in bTPY, bTPYglu, bTPYglu+glcp, bTPYglcp and bTPYinu media

In this experiment, the bacteria were tested to ferment the GLCP from *G. lucidum* in comparison to control, glucose and inulin. Mixtures of glucose and GLCP in the basal YPY medium (bTPYglu+glcp) were also tested. Table 3 shows the mean log cfu/mL of the *B. longum* BB536 population in bTPY, bTPYglu, bTPYglu+glcp, bTPYglcp and bTPYinu with various concentrations and incubation times. The starting count of the bacterium in all media was approximately 6.86 log cfu/mL. The results showed that the population of *B. longum* BB536 increased with increasing concentrations of carbon sources tested. The highest bacterial count was shown at 2.0% concentration of carbon sources and at 24 h incubation. The populations of *B. longum* BB536 in bTPYglu 2.0%, bTPYglu+glcp 2.0%, bTPYglcp 2.0% and bTPYinu 2.0% were 10.73, 10.82, 10.53 and 10.65 log cfu/mL, respectively after 24 h and decreased to 9.31, 9.26, 8.83 and 9.07 after 48 h incubation. The highest population count of *B. longum* BB536 was achieved in bTPYglu+glcp, followed by bTPYglu, bTPYinu and bTPYglcp. This suggested that GLCP was also taken by *B. longum* BB536 cells even though glucose was present to support their growth. This suggested that GLCP might have a synergistic effect with glucose in the supporting the growth of *B. longum* BB536. However, further research needs to be conducted to confirm this relationship.

Table 4 shows the mean log cfu/mL of the *B. pseudocatenulatum* G4 population in bTPYglu, bTPYglu+glcp, bTPYglcp and bTPYinu with different concentrations and incubation times. The growth pattern of this bacterium was similar to *B. longum* BB536. High viability of bacterium was shown in bTPYglu+glcp 2.0% with growth from 6.89 to 10.68 log cfu/mL after 24 h and decreased to 9.24 log cfu/mL after 48 h incubation. Growth of this bacterium in bTPYglcp indicated that, GLCP was also capable of supporting their growth. High population was shown in 2.0% concentration with 10.40 log cfu/mL after 24 h. The pH values for all media decreased from 6.80 to more acidic because of fermentation by this bacterium produced acidic condition.

Table 5 and 6 illustrate the results of the pH for *B. longum* BB536 and B. *pseudocatenulatum* G4 in all media measured at 0, 12, 24, and 48 h incubation. At 0 h, the pH for all media was set at 6.8. Generally, after 48 h incubation, the pH values slightly decrease to around 4.30 due to fermentation by *B. longum* BB536 which produced lactic and acetic acids (Krieg, 1984). When fermented by both strains of bifidobacteria, bTPY exhibited only a slight decrease in pH during the entire fermentation period. The lowest pH values reached by *B. longum* BB536 and *B.*

pseudocatenulatum G4 were in bTPYglu+glcp 2.0% with 4.23 and 4.24 respectively after 48 h fermentation. The lowering of pH was probably due to the organic acids produced from each of these carbohydrates by microbial fermentation. Bifidobacteria mainly produce acetic and lactic acids as end products from the fermentation of various sugars (Krieg, 1984). However the fermentation

Table 4: The growth of *B. pseudocatenulatum* G4 in different media supplemented with various concentrations of carbon sources

Media	Time (h)	Log cfu/mL				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.90±0.13^{a}	6.90±0.13^{a}	6.90±0.13^{a}	6.90±0.13^{a}
	12		8.53±0.12^{c}	8.60±0.13^{c}	8.46±0.13^{b}	8.60±0.10^{b}
	24		9.13±0.11^{d}	9.63±0.12^{d}	10.25±0.10^{c}	10.55±0.10^{d}
	48		7.61±0.10^{b}	8.04±0.13^{b}	8.63±0.09^{b}	9.10±0.09^{c}
bTPYglu+glcp	0		6.87±0.10^{a}	6.87±0.06^{a}	6.89±0.09^{a}	6.89±0.09^{a}
	12		8.65±0.07^{c}	8.66±0.12^{c}	8.76±0.09^{b}	8.66±0.10^{b}
	24		9.30±0.09^{d}	9.95±0.10^{d}	10.52±0.11^{c}	10.68±0.10^{d}
	48		7.67±0.10^{b}	8.13±0.10^{b}	8.70±0.11^{b}	9.24±0.11^{c}
bTPYglcp	0		6.89±0.09^{a}	6.89±0.09^{a}	6.89±0.09^{a}	6.89±0.09^{a}
	12		8.53±0.09^{c}	8.57±0.10^{c}	8.60±0.11^{c}	8.63±0.11^{b}
	24		9.27±0.08^{d}	9.67±0.08^{d}	10.17±0.09^{d}	10.40±0.08^{c}
	48		7.37±0.11^{b}	7.83±0.09^{b}	8.31±0.09^{b}	8.70±0.09^{b}
bTPYinu	0		6.89±0.09^{a}	6.89±0.09^{a}	6.87±0.06^{a}	6.89±0.09^{a}
	12		8.49±0.11^{c}	8.58±0.11^{c}	8.66±0.09^{c}	8.49±0.09^{b}
	24		9.16±0.10^{d}	9.65±0.09^{d}	10.17±0.09^{d}	10.47±0.07^{d}
	48		7.41±0.08^{b}	7.89±0.11^{b}	8.42±0.09^{b}	8.89±0.10^{c}
bTPY	0	6.89±0.11^{a}	NT	NT	NT	NT
	12	8.47±0.12^{c}	NT	NT	NT	NT
	24	7.66±0.09^{b}	NT	NT	NT	NT
	48	6.96±0.11^{a}	NT	NT	NT	NT

Table 5: The pH changes of *B. longum* BB536 in different media supplemented with various concentrations of carbon sources

Media	Time (h)	pH				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}
	12		5.77±0.04^{b}	5.77±0.04^{b}	5.70±0.03^{b}	5.65±0.03^{b}
	24		4.65±0.03^{c}	4.59±0.03^{c}	4.45±0.03^{c}	4.29±0.03^{c}
	48		4.64±0.03^{c}	4.58±0.03^{c}	4.34±0.03^{c}	4.28±0.04^{c}
bTPYglu+glcp	0		6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}
	12		5.78±0.03^{b}	5.72±0.03^{b}	5.64±0.03^{b}	5.59±0.03^{b}
	24		4.60±0.03^{c}	4.54±0.03^{c}	4.29±0.03^{c}	4.25±0.04^{c}
	48		4.59±0.03^{c}	4.53±0.03^{c}	4.29±0.02^{c}	4.24±0.03^{c}
bTPYglcp	0		6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}
	12		5.89±0.04^{b}	5.86±0.02^{b}	5.81±0.03^{b}	5.76±0.03^{b}
	24		4.72±0.03^{c}	4.68±0.03^{c}	4.51±0.03^{c}	4.36±0.03^{c}
	48		4.71±0.02^{c}	4.67±0.04^{c}	4.51±0.03^{c}	4.35±0.03^{c}
bTPYinu	0		6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}	6.80±0.00^{a}
	12		5.93±0.03^{b}	5.88±0.03^{b}	5.79±0.03^{b}	5.74±0.04^{b}
	24		4.70±0.02^{c}	4.66±0.03^{c}	4.49±0.04^{c}	4.34±0.03^{c}
	48		4.69±0.03^{c}	4.65±0.02^{c}	4.45±0.02^{c}	4.32±0.04^{c}
bTPY	0	6.80±0.00^{a}	NT	NT	NT	NT
	12	5.90±0.03^{b}	NT	NT	NT	NT
	24	5.80±0.03^{c}	NT	NT	NT	NT
	48	5.75±0.02^{c}	NT	NT	NT	NT

Table 6: The pH changes of *B. pseudocatenulatum* G4 in different media supplemented with various concentrations of carbon sources

Media	Time (h)	pH				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.65±0.03[b]	5.61±0.03[b]	5.54±0.03[b]	5.52±0.02[b]
	24		4.56±0.02[c]	4.47±0.02[c]	4.39±0.01[c]	4.29±0.02[c]
	48		4.55±0.03[c]	4.48±0.03[c]	4.34±0.03[c]	4.28±0.03[c]
bTPYglu+glcp	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.60±0.02[b]	5.58±0.03[b]	5.51±0.03[b]	5.48±0.02[b]
	24		4.51±0.03[c]	4.41±0.02[c]	4.29±0.02[c]	4.25±0.03[c]
	48		4.50±0.02[c]	4.40±0.02[c]	4.29±0.03[c]	4.24±0.03[c]
bTPYglcp	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.81±0.03[b]	5.77±0.03[b]	5.65±0.02[b]	5.60±0.03[b]
	24		4.65±0.03[c]	4.59±0.02[c]	4.51±0.03[c]	4.39±0.02[c]
	48		4.64±0.03[c]	4.58±0.02[c]	4.49±0.02[c]	4.38±0.03[c]
bTPYinu	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.70±0.02[b]	5.65±0.03[b]	5.59±0.02[b]	5.57±0.03[b]
	24		4.59±0.03[c]	4.53±0.02[c]	4.45±0.02[c]	4.35±0.03[c]
	48		4.58±0.03[c]	4.52±0.02[c]	4.44±0.03[c]	4.34±0.03[c]
bTPY	0	6.80±0.00[a]	NT	NT	NT	NT
	12	5.89±0.02[b]	NT	NT	NT	NT
	24	5.77±0.02[c]	NT	NT	NT	NT
	48	5.76±0.04[c]	NT	NT	NT	NT

bTPY = TPY medium without carbohydrate source (basal medium)
bTPYglu = basal medium supplemented with glucose
bTPYglu+glcp = basal medium supplemented with glucose and GLCP
bTPYglcp = basal medium supplemented with GLCP
bTPYinu = basal medium supplemented with inulin
The mean values and the standard deviation (±SD) were calculated from the data obtained with triplicate trials
NT = Not Tested
[a b c d]mean significantly different among hours (p<0.05) for the respective medium

activity differed according to the oligosaccharides used as supplement. Gibson and Roberfroid (1995) described similar relationship in the *in vitro* fermentation of various carbon sources by anaerobic slurries of human faecal bacteria.

Tables 3 and 4 showed that GLCP could support the growth of bifidobacteria tested. *B. longum* BB536 showed better growth than *B. pseudocatenulatum* G4 did in TPYglcp. The difference in the growth of bifidobacteria observed in all the media tested may be due to the difference in the nutritional requirement and the sensitivity to oxygen of the test organisms as suggested in the previous studies (Desjardins *et al*., 1990; Shimamura *et al*., 1992). Desjardins *et al*. (1990) reported different growth of various bifidobacteria in skim milk while Dubey and Mistry (1996) observed the same effect when bifidobacteria were grown in infant formulas. Moreover, according to Chou and Hou (2000), the growth response of bifidobacteria to the various carbon sources is species dependent.

The viable count of *L. acidophilus* and *L casei* Shirota in bTPY, bTPYglu, bTPYglu+glcp, bTPYglcp and bTPYinu with various concentrations are presented in Tables 7 and 8. Among the various media tested, bTPYglu+glcp resulted in the highest viable count in both bacteria suggested that the combination of glucose and GLCP could highly support the growth of the bacteria tested. This was followed by bTPYglu, bTPYinu, bTPYglcp and bTPY media. High population of both bacteria was shown after 24 h incubation in all media excluding in bTPY. *L. acidophilus* grew better than *L. casei* Shirota in all media. High population of bacteria in bTPYglu+glcp was exhibited in 2.0% concentration. *L. acidophilus* grew from 6.86 to 10.68 log cfu/mL and decreased to 9.21 log cfu/mL in this media. On the other hand, *L. casei* Shirota showed high count after 24 h with 10.60 log cfu/mL and decreased to 9.18 log cfu/mL after 48 h incubation.

As shown in Tables 7 and 8, the growth of both lactobacilli was better in bTPYglcp in comparison to their growth in bTPY and exhibit even better growth in bTPYglu+glcp when compared to glucose and inulin alone. Based on the comparison of their growth in bTPYglcp and bTPY, it is clearly proven that GLCP itself is able to support the growth of both lactobacilli even though not as good as glucose and inulin. High concentration of GLCP causes high population of bacteria. At 24 h incubation, the highest population of *L. acidophilus* was in 2.0% concentration with 10.34 log cfu/mL and the lowest population was in 0.5% concentration with 9.30 log cfu/mL. Meanwhile, for *L. casei* Shirota, the highest.

Table 7: The growth of *L. acidophillus* in different media supplemented with various concentrations of carbon sources

Media	Time (h)	Log cfu/mL				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.86±0.06[a]	6.86±0.05[a]	6.87±0.10[a]	6.87±0.10[a]
	12		8.73±0.09[c]	8.68±0.12[c]	8.75±0.11[c]	8.83±0.11[b]
	24		9.23±0.13[d]	9.76±0.10[d]	10.32±0.09[d]	10.59±0.11[c]
	48		7.67±0.09[b]	7.99±0.08[b]	8.40±0.09[b]	8.93±0.12[b]
bTPYglu+glcp	0		6.86±0.06[a]	6.87±0.10[a]	6.87±0.10[a]	6.86±0.06[a]
	12		8.76±0.08[c]	8.72±0.09[c]	8.79±0.11[c]	8.88±0.11[b]
	24		9.32±0.10[d]	9.84±0.08[d]	10.47±0.10[d]	10.68±0.10[d]
	48		7.70±0.11[b]	8.05±0.09[b]	8.47±0.09[b]	9.21±0.10[c]
bTPYglcp	0		6.86±0.06[a]	6.86±0.06[a]	6.87±0.10[a]	6.87±0.10[a]
	12		8.64±0.10[c]	8.66±0.11[c]	8.68±0.12[c]	8.67±0.13[b]
	24		9.30±0.13[d]	9.64±0.10[d]	10.10±0.11[d]	10.34±0.10[c]
	48		7.38±0.10[b]	7.78±0.11[b]	8.22±0.11[b]	8.64±0.11[d]
bTPYinu	0		6.87±0.10[a]	6.87±0.10[a]	6.87±0.10[a]	6.86±0.06[a]
	12		8.58±0.11[c]	8.61±0.09[c]	8.72±0.11[c]	8.80±0.10[b]
	24		9.39±0.11[d]	9.71±0.10[d]	10.27±0.10[d]	10.49±0.11[c]
	48		7.50±0.09[b]	7.84±0.10[b]	8.33±0.10[b]	8.77±0.10[b]
bTPY	0	6.86±0.06[a]	NT	NT	NT	NT
	12	8.54±0.09[b]	NT	NT	NT	NT
	24	7.68±0.11[d]	NT	NT	NT	NT
	48	7.10±0.09[c]	NT	NT	NT	NT

Table 8: The growth of *L. casei* Shirota in different media supplemented with various concentrations of carbon sources

Media	Time (h)	Log cfu/mL				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.89±0.10[a]	6.89±0.10[a]	6.89±0.10[a]	6.88±0.11[a]
	12		8.64±0.10[c]	8.78±0.11[c]	8.83±0.10[c]	8.87±0.11[b]
	24		9.19±0.09[d]	9.75±0.13[d]	10.25±0.10[d]	10.50±0.11[c]
	48		7.54±0.10[b]	7.83±0.11[b]	8.30±0.13[b]	8.90±0.09[b]
bTPYglu+glcp	0		6.87±0.10[a]	6.88±0.11[a]	6.88±0.11[a]	6.88±0.11[a]
	12		8.74±0.10[c]	8.85±0.10[c]	8.89±0.09[c]	8.92±0.11[b]
	24		9.30±0.12[d]	9.80±0.09[d]	10.28±0.10[d]	10.60±0.11[d]
	48		7.67±0.11[b]	7.96±0.11[b]	8.38±0.11[b]	9.18±0.11[c]
bTPYglcp	0		6.88±0.11[a]	6.89±0.10[a]	6.89±0.10[a]	6.80±0.09[a]
	12		8.50±0.12[c]	8.54±0.11[c]	8.59±0.11[c]	8.61±0.11[b]
	24		9.25±0.10[d]	9.50±0.11[d]	10.04±0.11[d]	10.26±0.11[c]
	48		7.35±0.10[b]	7.63±0.11[b]	8.13±0.11[b]	8.57±0.10[b]
bTPYinu	0		6.89±0.10[a]	6.88±0.11[a]	6.88±0.10[a]	6.89±0.10[a]
	12		8.56±0.09[c]	8.70±0.09[c]	8.78±0.10[c]	8.84±0.10[b]
	24		9.40±0.11[d]	9.68±0.10[d]	9.93±0.11[d]	10.40±0.11[c]
	48		7.45±0.10[b]	7.75±0.10[b]	8.12±0.12[b]	8.64±0.10[b]
bTPY	0	6.88±0.08[a]	NT	NT	NT	NT
	12	8.42±0.11[d]	NT	NT	NT	NT
	24	7.75±0.10[c]	NT	NT	NT	NT
	48	7.27±0.09[b]	NT	NT	NT	NT

Table 9: The pH changes of *L. acidophilus* in different media supplemented with various concentrations of carbon sources

Media	Time (h)	pH				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.73±0.04[b]	5.70±0.02[b]	5.63±0.04[b]	5.59±0.03[b]
	24		4.68±0.03[c]	4.60±0.03[c]	4.52±0.02[c]	4.36±0.02[c]
	48		4.66±0.02[c]	4.59±0.03[c]	4.50±0.03[c]	4.35±0.03[c]
bTPYglu+glcp	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.64±0.03[b]	5.60±0.04[b]	5.54±0.04[b]	5.50±0.03[b]
	24		4.63±0.02[c]	4.55±0.03[c]	4.46±0.03[c]	4.26±0.04[c]
	48		4.59±0.04[c]	4.53±0.02[c]	4.45±0.02[c]	4.25±0.03[c]
bTPYglcp	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.89±0.04[b]	5.83±0.03[b]	5.77±0.03[b]	5.63±0.03[b]
	24		4.73±0.02[c]	4.67±0.03[c]	4.61±0.02[c]	4.46±0.02[c]
	48		4.70±0.03[c]	4.65±0.03[c]	4.60±0.03[c]	4.45±0.02[c]
bTPYinu	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.78±0.03[b]	5.74±0.04[b]	5.68±0.03[b]	5.60±0.03[b]
	24		4.70±0.02[c]	4.63±0.03[c]	4.57±0.04[c]	4.40±0.03[c]
	48		4.68±0.03[c]	4.62±0.02[c]	4.55±0.03[c]	4.39±0.03[c]
bTPY	0	6.80±0.00[a]	NT	NT	NT	NT
	12	6.21±0.03[b]	NT	NT	NT	NT
	24	5.61±0.04[c]	NT	NT	NT	NT
	48	5.60±0.03[c]	NT	NT	NT	NT

Table 10: The pH changes of *L. casei* Shirota in different media supplemented with various concentrations of carbon sources

Media	Time (h)	pH				
		0%	0.5%	1.0%	1.5%	2.0%
bTPYglu	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.70±0.04[b]	5.67±0.03[b]	5.54±0.03[b]	5.47±0.03[b]
	24		4.66±0.03[c]	4.57±0.03[c]	4.29±0.03[c]	4.25±0.02[c]
	48		4.64±0.04[c]	4.56±0.04[c]	4.28±0.03[c]	4.24±0.03[c]
bTPYglu+glcp	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.60±0.04[b]	5.58±0.03[b]	5.50±0.03[b]	5.48±0.02[b]
	24		4.59±0.02[c]	4.52±0.03[c]	4.47±0.02[c]	4.28±0.02[c]
	48		4.58±0.03[c]	4.51±0.02[c]	4.46±0.04[c]	4.27±0.04[c]
bTPYglcp	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.84±0.03[b]	5.78±0.04[b]	5.63±0.03[b]	5.58±0.04[b]
	24		4.66±0.03[c]	4.63±0.02[c]	4.37±0.03[c]	4.33±0.02[c]
	48		4.65±0.03[c]	4.62±0.03[c]	4.36±0.03[c]	4.32±0.03[c]
bTPYinu	0		6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]	6.80±0.00[a]
	12		5.73±0.03[b]	5.69±0.04[b]	5.59±0.03[b]	5.51±0.03[b]
	24		4.68±0.04[c]	4.60±0.03[c]	4.34±0.03[c]	4.28±0.02[c]
	48		4.67±0.03[c]	4.59±0.02[c]	4.33±0.03[c]	4.27±0.02[c]
bTPY	0	6.80±0.00[a]	NT	NT	NT	NT
	12	6.35±0.04[b]	NT	NT	NT	NT
	24	5.82±0.03[c]	NT	NT	NT	NT
	48	5.81±0.03[c]	NT	NT	NT	NT

bTPY = TPY medium without carbohydrate source (basal medium)
bTPYglu = basal medium supplemented with glucose
bTPYglu+glcp = basal medium supplemented with glucose and GLCP
bTPYglcp = basal medium supplemented with GLCP
bTPYinu = basal medium supplemented with inulin
The mean values and the standard deviation (±SD) were calculated from the data obtained with triplicate trials
NT = Not Tested
[a b c d]mean significantly different among hours (p<0.05) for the respective medium

growth of 10.26 log cfu/mL was achieved in bTPYglcp 2.0% and the lowest bacterial count of 9.25 log cfu/mL was achieved in bTPYglcp 0.5%.

The pH values of both lactobacilli are show in Table 9 and 10. The pH values in all media were decreased against time to the lowest around 4.20 from the initial pH medium of 6.80 because the ability of the bacteria to convert sugars to lactic acid makes an acidic condition (Gibson, 1998). Lowest pH was shown in bTPYglu+glcp 2.0% for both bacteria. The pH values were correlated with the bacterial growth in the medium.

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