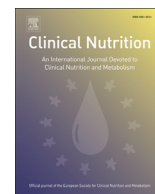


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Randomized control trials

## Efficacy of microbial cell preparation in improving chronic constipation: A randomized, double-blind, placebo-controlled trial<sup>☆</sup>



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### SUMMARY

**Background & aims:** Probiotics is an emerging therapeutic agent which may alleviate the symptoms of constipation. We evaluated the effectiveness of microbial cell preparation (Hexbio<sup>®</sup>) containing fructooligosaccharide, *Bifidobacterium* and *Lactobacillus* in improving stool frequency and symptoms of chronic constipation.

**Methods:** A total of 120 constipated adults diagnosed using Rome III criteria were randomized and given either microbial cell preparation or placebo to be consumed twice daily. Follow-up was done after a 7-day intervention based on a questionnaire which includes an assessment of symptom profile and a stool diary.

**Results:** During the intervention period, the stool frequency was higher ( $p = 0.001$ ) in the treatment group. Subjects experienced less straining ( $p = 0.001$ ) and sensation of incomplete evacuation ( $p < 0.001$ ), as well as improved stool consistency ( $p < 0.001$ ) compared to the placebo group. While a higher proportion of subjects in the treatment group had a reduction in anorectal blockage sensation and having to defecate by manual maneuvers, the differences were not statistically significant.

**Conclusion:** The results suggest that microbial cell preparation is effective in improving stool frequency and stool consistency. Furthermore, it could reduce the symptoms of straining and sensation of incomplete evacuation in adults with chronic functional constipation.

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## 1. Introduction

Constipation is characterized by difficulty in defecation, with dry and hard stools and infrequent defecation of less than three times per week. It is a fairly common condition as recent epidemiological data showed that prevalence of constipation in North America is 3.2–45% (median 16%), Europe 0.7–79% (median 19.2%) and Asia 1.4–32.9% (median 10.8%).<sup>1</sup> The incidence of adult constipation increases after the age of 60 and is more predominant in females.<sup>1</sup> The cause of chronic constipation can be multifactorial and the exact pathophysiology is often elusive.<sup>2</sup> Underlying risk factors are low fiber diet, low water intake, low physical activity, laxative abuse and lifestyle changes.<sup>3,4</sup> Constipation has also been linked with abnormal fecal flora, such as decreased concentrations

of lactic acid bacteria and increased methanogens, potentially pathogenic bacteria and fungi.<sup>5</sup>

Functional constipation is diagnosed when no underlying organic cause can be identified.<sup>6</sup> When constipation is prolonged, it can lead to complications like hemorrhoids, anal fissures and rectal bleeding. Although non life-threatening, chronic constipation has a significant impact on the quality of life, hence the management of the symptoms is important in improving quality of life for the patients.<sup>7</sup>

The best treatment for constipation relies on a clear understanding of the underlying cause, although it is not often found. Changes in lifestyle and dietary habits are usually recommended, for example increasing the intake of fiber and fluid. Besides dietary and lifestyle changes, the standard medication for constipation is laxatives.<sup>8</sup> Recently, other therapeutic options in treating chronic constipation have been investigated. A fairly new approach to treat constipation is the consumption of probiotics and prebiotics. Probiotics are defined as microbial cell preparations or components of microbes that have a beneficial effect on the health and well-being of the host.<sup>9</sup> Probiotic bacteria such as *Lactobacillus* and

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*Bifidobacterium* are producers of organic acids like lactic acid and acetic acid which can lower the pH of the colon, enhancing peristalsis and reducing colonic transit time.<sup>10</sup> Prebiotics are certain types of soluble fibers for example fructooligosaccharides (FOS), galactooligosaccharides (GOS) and inulin that promote the growth and activity of probiotic bacteria in the colon.

This study was carried out to evaluate the effects of a microbial cell preparation (Hexbio<sup>®</sup>) containing FOS, *Bifidobacterium* and *Lactobacillus* on stool frequency, stool consistency and symptoms of chronic constipation in adults.

## 2. Materials & methods

### 2.1. Subjects

Subjects were recruited from the primary day care clinic, medical clinic or surgical clinic at University of Malaya Medical Centre, Malaysia. A total of 132 subjects, aged 18 and above, were screened based on the Rome III diagnostic criteria for having chronic constipation (Table 1).<sup>11</sup> Subjects with irritable bowel syndrome (IBS) or organic constipation (constipation associated with any neoplastic diseases, neuropathy or mechanical obstructions), severe medical complications such as end-stage renal failure, liver cirrhosis, malignancy, chronic congestive heart failure and long term laxative users were excluded. Out of this cohort of patients, 120 subjects were included in the trial (Fig. 1). The protocol was approved by the Institutional Review Board (IRB) of the University Malaya Medical Centre (Reference no: 866.59). The study was explained to all recruited subjects by the clinical researchers and written informed consent was obtained before enrolment into the trial.

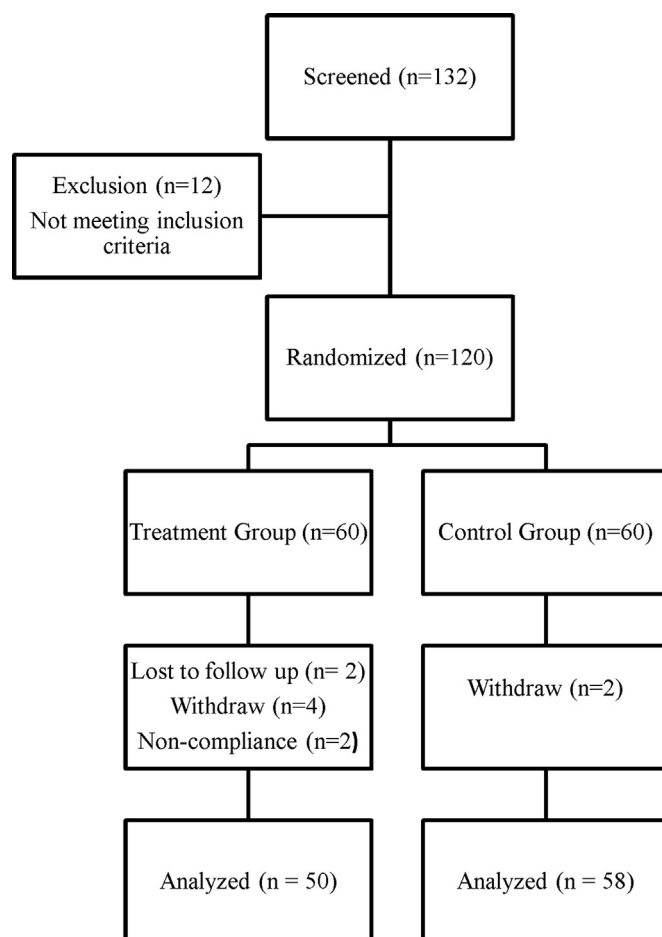
### 2.2. Study design

Subjects were randomized using the sealed envelope method to either the treatment or placebo group. They were given sachets containing either microbial cell preparation or placebo to be consumed twice daily for 7 consecutive days. The treatment sample consisted of an orange flavored, granulated microbial cell preparation (Hexbio<sup>®</sup>), containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Bifidobacterium infantis* with  $3 \times 10^{10}$  colony forming units (cfu) per sachet. The composition includes the strains listed above, granulated fermented milk, lactose, fructooligosaccharide and flavouring. The placebo sample was similar in appearance and composition but it was not fermented and contained no FOS and microbial cells. Nutritional compositions are crude protein (0.16 g/sachet), crude fat (0), crude fiber (0), carbohydrate (2.7 g/sachet) and calories (11.6 kcal/sachet). Hexbio<sup>®</sup> is a granulated formulation

**Table 1**

Rome III diagnostic criteria for functional constipation.

Symptoms of functional constipation
Symptomatic for the last 3 months
Onset $\geq 6$ months prior to diagnosis
$\geq 2$ of following symptoms
- Straining
- Lumpy/hard stools
- Incomplete evacuation
- Anorectal blockage
- Manual maneuvers to aid in defecation
- $< 3$ Smooth bowel movements per week
Loose stools rarely present without laxatives
Insufficient criteria for IBS



**Fig. 1.** CONSORT diagram of patient recruitment and analysis.

for oral consumption. Patients and researchers were blinded to the allocated groups and the treatment allocation was revealed at the end of the research, once analysis was done.

The subjects were advised to continue their normal diet but avoid any other probiotic products, laxatives or fiber supplements during the study period. For baseline assessment, a medical history, demographic details, information on the current defecation pattern and constipation symptoms were recorded. Follow-up was done at the end of the study period based on a questionnaire which includes symptomatic improvement and a stool diary.

### 2.3. Outcome evaluation

The primary outcome measured was frequency of bowel movements per week. The secondary outcomes measured were the self perception on the improvement of symptoms (straining, lumpy or hard stool, sensation of incomplete evacuation, sensation of anorectal blockage and manual maneuvers to aid in defecation) after the 1 week treatment period.

### 2.4. Sample size

Sample size was calculated based on the stool frequency standard difference of 0.66 between the two groups.<sup>12</sup> Therefore, a total sample size of 120 (60 in each group) with 1:1 allocation was determined to be sufficient to expect 95% power with a 5% significance level and 10% attrition rate.

## 2.5. Statistical analysis

Analysis was performed using SPSS V.16.0 statistical software based on the intention-to-treat (ITT) principle. Differences between the two treatment groups at baseline were evaluated using Mann–Whitney and Chi-square tests. Comparison of stool frequency before and after intervention was done using the Kruskal Wallis test and improvement of symptoms was assessed using the Chi-square test. Results with a  $p$ -value of  $<0.05$  was considered statistically significant.

## 3. Results

### 3.1. Baseline characteristics

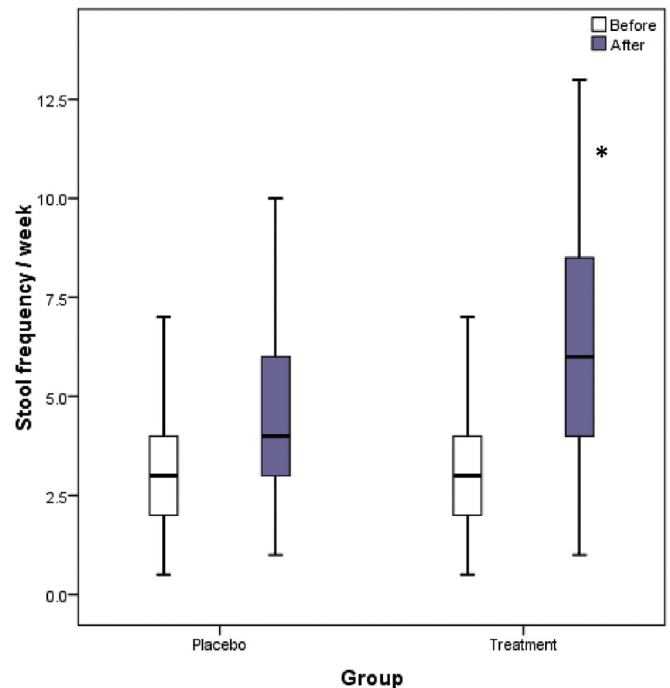
Between March 2011 and December 2011, a total of 120 subjects were recruited but 12 did not complete the study and were considered dropouts. Dropouts were due to loss to follow-up, consent withdrawal and non compliance such as consuming  $< 80\%$  of the test samples, intake of antibiotics, laxatives or other probiotics during the treatment period. Table 2 shows the baseline characteristics for the two groups (treatment and placebo). Overall, the majority of subjects recruited were females (62%) compared to males (38%). On average, the subjects have been suffering from constipation for 1 year or more and showed 3 or more symptoms for constipation. The median ages were 41.5 and 37 years for treatment and placebo groups respectively. There were no significant differences between the two groups in terms of age, gender, ethnic origin, duration of constipation, stool frequency and symptoms. Furthermore, history of abdominal surgery, alcohol consumption, smoking and exercise were also comparable between the two groups. This illustrates that both groups were well randomized and unbiased at baseline.

**Table 2**  
Baseline characteristics of treatment vs placebo groups.

Baseline characteristics	Treatment	Placebo	$p$ Value
Number of subjects ( $n$ )	50	58	
Age, median (range)	41.5 (18–81)	37 (20–78)	0.890 <sup>a</sup>
Gender, $n$ (%)			
Male	17 (34)	24 (41.4)	0.431
Female	33 (66)	34 (58.6)	
Ethnic origin, $n$ (%)			
Malay	25 (50)	30 (51.7)	0.592
Chinese	14 (28)	15 (25.9)	
Indian	11 (22)	11 (19)	
Others	0 (0)	2 (3.4)	
Years of constipation, median (range)	2.0 (0.5–10)	1.0 (0.25–30)	0.644 <sup>a</sup>
Stool frequency, median (range)	3 (0.5–7)	3 (0.5–7)	0.352 <sup>a</sup>
Constipation symptoms, $n$ (%):			
Straining	37 (74)	46 (79.3)	0.648
Lumpy or hard stool	44 (88)	54 (93.1)	0.509
Sensation of incomplete evacuation	42 (84)	43 (74.1)	0.245
Sensation of anorectal blockage	19 (38)	18 (31)	0.543
Manual maneuvers to aid in defecation	8 (16)	9 (15.5)	1.000
$< 3$ Times defecation per week	21 (42)	19 (32.8)	0.424
History of abdominal surgery, $n$ (%)	13 (26)	10 (17.2)	0.268
Alcohol consumer, $n$ (%)	7 (14)	8 (13.8)	0.975
Smoker, $n$ (%)	2 (4)	8 (13.8)	0.080
Regular exercise, $n$ (%)	18 (36)	21 (36.2)	0.982

Unless indicated, other  $p$ -values are obtained using Chi-square test.

<sup>a</sup>  $p$ -Value obtained using Mann–Whitney Test.



**Fig. 2.** Stool frequency of placebo and treatment groups before and after intervention. Boxplots show 25th and 75th percentiles, median and range. \* $p = 0.001$  significantly different from the placebo group using Kruskal Wallis test.

### 3.2. Stool frequency

At baseline, the median defecation frequency for the placebo and treatment groups were both 3 (0.5–7) times/week. There was no significant difference in the stool frequency before intervention in the placebo and treatment groups. During the 7 days intervention, the stool frequency of the treatment group increased to 6 (1–13) while the placebo group was 4 (1–10). Therefore, with intervention, stool frequency in the treatment group was significantly higher ( $p = 0.001$ ) than the placebo group as shown in Fig. 2.

### 3.3. Constipation symptoms

Improvement of constipation symptoms is shown in Table 3. In the treatment group, subjects experiencing less straining during the intervention period was 66.7% compared to 29.8% for the placebo group. Additionally, subjects in the treatment group reported a significant decrease in lumpy hard stools (66.7% vs 29.8%) and sensation of incomplete evacuation (75.6% vs 31.4%) as compared to the placebo. A higher proportion of subjects in the treatment group felt that there was an improvement of anorectal blockage (66.7% vs 39.1%) and reduction in having to defecate by manual maneuvers (77.8% vs 46.7%). However, the differences were not

**Table 3**  
Improvement of constipation symptoms in the treatment vs placebo groups.

Symptoms	Treatment	Placebo	$p$
	% with Improvement		
Straining	66.7	29.8	0.001
Lumpy hard stools	82.9	31.4	$<0.001$
Sensation of incomplete evacuation	75.6	27.9	$<0.001$
Sensation of anorectal blockage	66.7	39.1	0.068
Manual maneuvers to defecate	77.8	46.7	0.134

$p$ -Value obtained using Chi-square test.

statistically significant. No adverse effects were reported in this study.

#### 4. Discussion

The presence of probiotics plays an important role in maintaining a healthy balance in the intestine. Studies have shown that there are differences in the intestinal microbiota between healthy persons and patients with chronic constipation.<sup>13</sup> Patients with chronic constipation have lower concentrations of intestinal *Bifidobacterium* and *Lactobacillus* and higher pathogenic bacteria and fungi.<sup>5,14</sup> Whether this imbalanced microbiota is a factor contributing to constipation or a manifestation of constipation is unknown. This observation may justify the use of probiotics as a therapeutic agent in treating chronic constipation.

Hexbio<sup>®</sup> is a fermented milk product in granule form with FOS and a mixed culture of six strains of *Lactobacillus* and *Bifidobacterium*. This combination is used as *Lactobacillus* tends to colonize the small intestine while *Bifidobacterium* are found predominantly in the colon.<sup>15,16</sup> Additionally, the presence of FOS may encourage the growth of probiotics in the colon. In this study, there may be a variation in the consumption of diet despite our instruction of taking a normal diet, which was not standardized by our dietician. Nevertheless, as the two groups in this randomized trial are well matched at baseline, we anticipate that this confounding effect has been effectively resolved by the process of randomization. During the one week intervention period, the stool frequency of the treatment group increased significantly compared to the placebo group ( $p = 0.001$ ). This demonstrates the efficacy of a microbial cell preparation containing *Bifidobacterium* and *Lactobacillus* on increasing the frequency of bowel movements per week. The production of short-chain fatty acids such as acetate, propionate and butyrate by *Bifidobacterium* and *Lactobacillus* may result in a lower pH in the colon. A lower pH improves the intestinal peristalsis which eventually leads to a decrease in the colonic transit time.<sup>10,17</sup> A study by Krammer et al., 2011 on patients with chronic constipation showed that consumption of *L. casei* Shirota accelerated the colonic transit time and improved defecation frequency.<sup>17</sup>

Subjects treated with Hexbio<sup>®</sup> had a significant improvement in constipation symptoms such as straining, hard stools and sensation of incomplete evacuation. It has been suggested that probiotics soften the stools by increasing the secretion of water and electrolytes.<sup>18</sup> The fermentation process by probiotics in the intestine produces short-chain fatty acids which promote osmotic stimulation.<sup>19</sup> Studies on healthy adults have shown an increase in short-chain fatty acids and improvement in defecation conditions after the administration of probiotics.<sup>20,21</sup> Softer stools and an improved intestinal peristalsis will likely relieve the symptoms of constipation as shown in our study. There was improvement in the anorectal blockage sensation and reduction in having to defecate by manual maneuvers in the treatment group. However, when compared to the placebo group, the differences were not statistically significant. This could be due to the smaller number of subjects having these two complaints at baseline. A total of 37 (34.3%) subjects reported anorectal blockage sensation and only 17 (15.7%) subjects had to use manual maneuvers to aid in defecation. Furthermore, these two symptoms could indicate a more severe spectrum of chronic constipation and 7 days intervention might not be sufficient for any significant changes.

Probiotics has been shown to be effective in increasing stool frequency and improving symptoms in other studies as well.<sup>12,22</sup> However, randomized controlled trials using probiotics were conducted on different strains or single strain, hence a direct

comparison is difficult. In a pilot study involving constipated children, a probiotics mixture containing *Bifidobacterium* and *Lactobacillus* increased stool evacuations but did not show significant improvement in stool consistency.<sup>23</sup> The authors suggested that a larger sample size should be tested as only 35% of the children had hard stools at baseline. Waitzberg et al. assessed the effects of FOS and probiotics mixture of *Lactobacillus* and *Bifidobacterium* on bowel movement frequency and constipation symptoms after a one month intervention.<sup>24</sup> They found an improvement in stool frequency and consistency as well as constipation symptoms in the treatment group.<sup>24</sup> However, these effects were significant after the second and third weeks of treatment while our study showed significance after one week.<sup>24</sup>

Similarly, a study on constipated women showed improvement in stool frequency, defecation condition and stool condition after one week of consuming fermented milk containing *Bifidobacterium lactis*.<sup>12</sup> Although most trials using probiotics showed some positive results (Table 4), a multicentre trial conducted on children produced no significant difference in constipation symptoms and stool frequency in the probiotics *B. lactis* treatment group.<sup>25</sup> These two trials used a similar strain of *B. lactis* in fermented milk and had comparable sample size but achieved opposing outcomes. The different response seen could be due to the different study population or scoring methods used. It is still unclear if multistrain probiotics is better than single strain for treatment of constipation as both have shown various degrees of effectiveness. However, based on a review by Chapman et al., 2011 comparing the use of multi or single strain probiotics for a range of health related outcomes, it was suggested that multistrain mixtures show higher efficacy against vast a range of end points.<sup>26</sup> This might be an effect of synergistic interaction between the multistrains in the probiotic mixtures.<sup>26</sup> The presence of prebiotic, fermentation product and other compositions in the treatment mixture might also affect the results.

A recent review of the safety of *Lactobacillus* and *Bifidobacterium* showed that there was no health damage and adverse effects for the consumers.<sup>27</sup> In accordance to this, there were no adverse effects reported in our study. However, there are concerns about the safety of probiotics in immunocompromised patients. Further studies in this population are necessary to determine whether probiotics are safe in these patients and could be used as a therapeutic agent. Laxative is still the most commonly prescribed medication for constipation although probiotics has gained attention as an alternative treatment. Certain synthetic laxatives like polyethylene glycol could alter colonic flora by decreasing the number of *Bifidobacterium* and short-chain fatty acids in the stool.<sup>28</sup> Therefore, probiotics can either be an adjunct to the conventional use of laxatives or be given alone in the treatment of constipation.

In this trial, we demonstrated that short-term intervention with probiotics improved the symptoms of chronic constipation. However, it may be important to evaluate the outcome after a longer duration of treatment and whether or not these beneficial effects will be maintained after cessation of probiotics intake. Although the constipation symptoms of the probiotics group improved over the treatment period, analysis of the stool was not carried out. The promising results from this trial warrant further investigation such as analysis of stool pH and probiotics content in order to determine the exact mechanism of action. Finally, it would be interesting to study the potential protective effects of long term probiotics consumption on prevention of colon cancer as probiotics and prebiotics were proven to favourably alter colorectal cancer biomarkers.<sup>29</sup> Moreover, constipation and infrequent bowel movements have been linked to a higher risk of colon cancer.<sup>30</sup>

**Table 4**

(a) Studies using probiotics as treatment for constipation in adults. (b) Studies using probiotics as treatment for constipation in children.

Study ID	Probiotic strains	Design	Constipation criteria	Patients (N), age	Duration	Study outcomes (probiotic treatment group)
(a)						
An et al., 2010 <sup>31</sup>	<i>L. acidophilus</i> (LH) CBT, <i>P. pentosaceus</i> (PP) CBT and <i>B. longum</i> SPM1205	Non-RCT, observational study	Laxative usage >1 per week	Nursing home residents (19) Age: 77.1 ± 10.1 years	2 Weeks	Increase in stool frequency, amount of stool excreted and normal stool during defecation, but changes NS. Increase of fecal LAB levels ( $p < 0.05$ ) Decrease in harmful enzymes of intestinal microflora: tryptophanase ( $p = 0.047$ ) and urease ( $p = 0.005$ )
De Milliano et al., 2012 <sup>32</sup>	<i>B. bifidum</i> W23, <i>B. lactis</i> W52, <i>B. longum</i> W108, <i>L. casei</i> W79, <i>L. plantarum</i> W62 and <i>L. rhamnosus</i> W71	Non-RCT, observational study	Rome III	Pregnant women (20) Age: 29.5 ± 5.3 years	4 Weeks	Decrease in: Straining during defecation ( $p = 0.01$ ), hard stools (NS), sensation of incomplete evacuation ( $p < 0.01$ ) Sensation of anorectal obstruction ( $p < 0.01$ ) Manual manoeuvres (NS) Abdominal pain ( $p = 0.01$ ) Reflux episodes ( $p = 0.01$ )
Fateh et al., 2011 <sup>33</sup>	<i>L. casei</i> NCIMB1 30185, <i>L. rhamnosus</i> NCIMB 30188, <i>S. thermophilus</i> NCIMB 30189, <i>B. breve</i> NCIMB 30180, <i>L. acidophilus</i> NCIMB 30184, <i>B. longum</i> NCIMB 30182, <i>L. bulgaricus</i> NCIMB 30186 FOS	RCT, parallel, placebo-controlled	Rome III	Men (31 treatment : 29 control) Age: >18 years	4 Weeks	Stool frequency increased in the synbiotic compared with the placebo group ( $p = 0.02$ ). Improvement of Bristol stool form score (weeks 2 and 4 [ $p = 0.006$ ]) Laxative use and performing manual manoeuvres (NS) Patients perception that the treatment was effective or "partially effective" was higher in the synbiotic group compared to the placebo ( $p = 0.037$ ).
Koebnick et al., 2003 <sup>22</sup>	<i>L. casei</i> Shirota	RCT, parallel, placebo-controlled	Patients with chronic idiopathic constipation	Adults (35 treatment: 35 control) Age: 18–70 years	4 Weeks	Decrease in moderate to severe constipation ( $p = 0.001$ ) and hard and lumpy stools ( $p < 0.011$ ) from week 2 onwards Increase in stool frequency from week 2 ( $p < 0.001$ ) Occurrence of flatulence and bloating (NS)
Riezzo et al., 2012 <sup>21</sup>	<i>L. paracasei</i> IMPC 2.1	RCT, crossover, artichoke vs probiotic-enriched artichoke	Rome III	Adults (10 treatment: 10 control) Age: 38.8 ± 14.4 years	15 Days + 15 days	Preference: Probiotic-enriched artichokes (80%) vs ordinary artichokes (20%) Relief from constipation symptoms higher in probiotic-enriched artichokes ( $p = 0.0014$ ) Improvement of stool consistency ( $p = 0.009$ ) Improvement in GSRS (Gastrointestinal Symptom Rating Scale) constipation score ( $p = 0.032$ ) Higher propionic acid levels in probiotic treatment group ( $p = 0.035$ )
Waitzberg et al., 2012 <sup>24</sup>	<i>L. paracasei</i> (Lpc-37), <i>L. rhamnosus</i> (HN001), <i>L. acidophilus</i> (NCFM) and <i>B. lactis</i> (HN019) FOS	RCT, parallel, placebo-controlled	Rome III	Women (49 treatment: 50 control) Age: 18–75 years	4 Weeks	Increase in stool frequency (week 2 and week 4 [ $p < 0.0001$ ], week 3 [ $p = 0.016$ ]) Stool consistency and shape nearer normal parameters (week 3 [ $p = 0.0001$ ], week 4 [ $p < 0.0001$ ]) Reduction in frequency of abdominal symptoms (NS) Improvement of AGACHAN score ( $p < 0.0001$ )
Yang et al., 2008 <sup>12</sup>	<i>B. lactis</i> DN-173010, Yoghurt starters <i>S. thermophilus</i> and <i>L. bulgaricus</i>	RCT, parallel, placebo-controlled	<3 Defecations/week, hard stools, non-organic and habitual constipation	Women (63 treatment: 63 control) Age: 25–65 years	2 Weeks	Increase in stool frequency: week 1 ( $p < 0.01$ ) and week 2 ( $p < 0.01$ ) Improvement in defecation condition score (week 1 [ $p < 0.01$ ] and week 2 [ $p < 0.01$ ]) Improvement in stool consistency score after week 1 ( $p < 0.05$ ) and week 2 ( $p < 0.01$ )
(b)						
Bekkali et al., 2007 <sup>23</sup>	<i>B. bifidum</i> , <i>B. infantis</i> , <i>B. longum</i> , <i>L. casei</i> , <i>L. plantarum</i> and <i>L. rhamnosus</i>	Non-RCT, observational pilot study	Rome III	Children (20) Age: 4–16 years	4 Weeks	Increase in stool frequency in weeks 2 and 4 Decrease in number of faecal incontinence episodes Hard stools (NS) Abdominal pain decreased from 45% to 25% in week 2 and 20% at week 4.
Coccorullo et al., 2010 <sup>34</sup>	<i>L. reuteri</i>	RCT, parallel, placebo-controlled	Rome III	Infants (22 treatment: 22 control) Age: ≥6 months	8 Weeks	Higher stool frequency than placebo (week 2 [ $p = 0.042$ ], week 4 [ $p = 0.008$ ] and week 8 [ $p = 0.027$ ]) Improvement in stool consistency but NS. Insoluble crying episodes (NS)



Guerra et al., 2011 <sup>35</sup>	<i>B. longum</i>	RCT, crossover, yoghurt containing <i>B. longum</i> vs yoghurt	Rome III	Students (59) Age: 5–15 years	5 Weeks + 5 Weeks	Improvement in stool consistency in the probiotic group during the first part of the intervention ( $p = 0.03$ ). After crossing over, an NS inversion was observed. Overall for probiotic group compared to yoghurt only: Defecation frequency ( $p = 0.012$ ), defecation pain ( $p = 0.046$ ) stool consistency (NS) and abdominal pain ( $p = 0.015$ ) Increase in stool frequency (week 2 [ $p < 0.01$ ] and week 4 [ $p < 0.01$ ]) and week 4 [ $p = 0.03$ ]) Decrease in mean stool consistency score (week 2 [ $p = 0.07$ ] and week 4 [ $p = 0.03$ ]) Decrease in faecal incontinence (week 2 [ $p = 0.01$ ] and week 4 [ $p < 0.01$ ]), Pain during defecation decreased (week 2 [ $p = 0.10$ ] and week 4 [ $p = 0.08$ ]) Decrease in abdominal pain episodes (week 2 [ $p = 0.02$ ] and week 4 [ $p = 0.01$ ]) Stool frequency (NS) Stool consistency (NS) Fecal incontinence (NS) Pain during defecation (NS) Reduction in flatulence ( $p = 0.02$ )
Tabbers et al., 2011 <sup>18</sup>	<i>B. breve</i>	Non-RCT, observational pilot study	Rome III	Children (20) Age: 4–13 years	4 Weeks	
Tabbers et al., 2011 <sup>25</sup>	<i>B. lactis</i> DN-173010, Yoghurt starters <i>L. delbrueckii</i> ssp. <i>Bulgarius</i> CNCM I-1632 and I-1519, <i>S. thermophilus</i> CNCM I-1630 and <i>Lactococcus cremoris</i> CNCM I-1631	Multicenter RCT, parallel, placebo-controlled	Rome III	Children (74 treatment; 74 control) Age: 3–16 years	3 Weeks	

NS indicates not significant.

## 5. Conclusions

In conclusion, this randomized, placebo-controlled trial suggests that microbial cell preparation containing a mixture of *Lactobacillus* and *Bifidobacterium* is effective in increasing stool frequency and improving stool consistency. Furthermore, it could reduce the symptoms of straining and sensation of incomplete evacuation in adults with chronic functional constipation. Therefore, microbial cell preparation may be used as an alternative treatment for functional constipation.

## Conflict of interest

None of the authors had any personal or financial conflicts of interest.

## Funding

The study samples (microbial cell preparation and placebo) were provided by B-Crobes Marketing (M) Sdn Bhd. but they did not interfere with the study design, data collection, analysis, interpretation and the writing and submission of the manuscript for publication.

## Authors' contributions

SJ was involved in the design of the study, coordination of the research samples and recruitment as well as manuscript drafting. YNY recruited patients, was involved in the interpretation of data and drafted the manuscript. YR recruited patients and was involved in drafting the manuscript. RR was involved in the design of the study, statistical analysis and interpretation of data. KFC was involved in the design of the study, provided inputs on the interpretation of data and reviewed the manuscript. All authors have given approval of this version to be published.

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## Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.clnu.2013.03.004>.

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