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A Double-Blind, Placebo-Controlled, Parallel Study Evaluating the Safety of *Bacillus coagulans* MTCC 5856 in Healthy Individuals

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Abstract

Objective: LactoSpore® containing probiotic strain *Bacillus coagulans* MTCC 5856 has been marketed as a dietary ingredient for nearly two decades. Clinical data on the safety and tolerance has not been evaluated at a dose of 2×10^9 cfu (spores)/day in healthy individuals. Thus, the primary objective of this study was to investigate the safety and tolerability of *B. coagulans* MTCC 5856 in healthy adults.

Study design: A total of 40 participants were randomized into one of two groups in a double-blind, randomized, placebo-controlled parallel study. One group of participants (n=20) were administered *B. coagulans* MTCC 5856 (600 mg tablet), containing 2×10^9 cfu (spores). The control group (n=20) was administered placebo tablets. Safety and tolerability of *B. coagulans* MTCC 5856 was assessed over 30 days by safety laboratory parameters (blood hematology and clinical chemistry parameters), anthropometric measures (weight, BMI, blood pressure and heart rate), adverse events, Bristol stool score, tolerability questionnaire and bowel habit diary.

Results: All laboratory parameters, anthropometric and vital sign measures remained within normal clinical range during the 30 day supplementation. Similar adverse events (AE's) were reported by participants in both the placebo and the *B. coagulans* MTCC 5856 group. The number of bowel movements and the Bristol stool scores were similar between the placebo group and *B. coagulans* MTCC 5856 group during the 30 days of supplementation. Participants also reported that *B. coagulans* MTCC 5856 tablets were tolerable and easy to swallow.

Conclusions: This study has verified that *B. coagulans* MTCC 5856 at a dose of 2×10^9 cfu (spores)/day was safe and tolerable in healthy participants when supplemented for 30 days.

Keywords: *Bacillus coagulans* MTCC 5856; Gut microbiota; LactoSpore®; Probiotic; Safety; Tolerability

Introduction

Intestinal microbiota plays a crucial role in several metabolic processes such as the regulation of intestinal epithelial proliferation, gut maturation, colonization, and resistance and modulation of the intestinal immune response [1-4]. The intestinal metabolome is composed of different species of beneficial and pathogenic bacteria. Beneficial bacterial species have several crucial functions in the intestine; restraining potentially pathogenic or harmful bacteria, activating immune responses, aiding proper digestion and absorption of food and acting as a barrier against harmful bacteria and toxins [5]. More than 500 species of indigenous bacteria colonize the colon and play a crucial role in human health and disease. In healthy individuals, the gut microbiota act as an important modulator of the immune system and serve as a source of non-inflammatory immune stimulation [6]. Modern lifestyle factors such as a poor diet, frequent travel and food or water contaminants in combination with increasing age may disturb the delicate balance of intestinal bacteria. Furthermore, medications such as broad spectrum antibiotics are commonly prescribed to curb infection which unfortunately kills beneficial bacteria disrupting the balance between the non-pathogenic and pathogenic species [7].

Probiotics are microorganisms that when administered in adequate amount provide health benefit to the host organism [8]. They induce health benefits by altering the intestinal microecology, producing antimicrobial compounds, and stimulating the body's immune response. Preparations of *Bacillus coagulans* has been found to contain a large number of viable lactic acid bacilli that retain their viability during storage prior to consumption as the spores are thermostable, survive in gastric secretions, reach and settle in the intestine producing sufficient lactic acid and other antagonistic substances inhibiting the growth of pathogenic bacteria. In vitro and in vivo studies on oral toxicity attest to the safety of *B. coagulans* [9,10]. *B. coagulans* is a probiotic, well known for its clinical efficacy in several human conditions [9-14]. *B. coagulans* based products are efficacious in adults with post-prandial intestinal discomfort, improving their quality of life. A potential for its application in adults with irritable bowel syndrome (IBS) have shown promising results in this arena [11,12,14]. Researchers have also reported on the relationship between *B. coagulans* and the immune system [15,16]. It is a well-established fact that the health benefits and the safety of a probiotic strain is strain specific, and not the species or genus-specific. This was clearly

indicated by the Joint Food and Agriculture Organization of the United Nations/World Health Organization and suggested to provide guidance to consumers or clinicians about the type and extent of safety assessments that have been conducted on the probiotic products [8]. Hence, it is a essential to verify the safety aspects of a probiotic strain.

LactoSpore® is a commercial proprietary probiotic preparation containing live spores of *Bacillus coagulans* MTCC 5856 (bearing internal reference number SBC37-01). It is a shelf stable, GRAS affirmed probiotic preparation which produces the beneficial L (+) form of lactic acid in the intestines and inhibits the growth of pathogenic bacteria [17]. Several preparations of *B. coagulans* in powder, tablet and capsule forms have been reported for the treatment of gastrointestinal disorders, vaginal infections, hypercholesterolemia, lactose intolerance, hepatic coma and as an adjuvant to antibiotic therapy in human clinical trials [17-20]. Spores of *B. coagulans* MTCC 5856 strain has the ability to withstand high temperature and reported to be stable during processing and storage of various functional foods [21]. It also reported that the *B. coagulans* MTCC 5856 did not alter either genetically or phenotypically and was found to be consistent over multiple years of commercial production [22]. Animal study revealed that *B. coagulans* MTCC 5856 elicited anti-diarrhoeal activity and inhibited the gastrointestinal motility in fasted rats [23]. However, clinical data on the safety and tolerability of *B. coagulans* MTCC 5856 has not been adequately established in a double-blinded, placebo-controlled study. Thus, the current double-blinded, placebo–controlled two arm study was aimed to evaluate the safety and tolerability of *B. coagulans* MTCC 5856 at a dose of 2×10^9 cfu (spores)/day in healthy adults over a 30 day supplementation period.

Methods and Materials

Product description

B. coagulans MTCC 5856 tablets (600 mg) contained 2×10^9 cfu (spores) (333.33 mg), microcrystalline cellulose, starch, sodium starch glycolate and magnesium stearate. Placebo tablets contained dibasic calcium phosphate, microcrystalline cellulose, starch, and magnesium stearate, No differences in color, taste, texture or packaging were detectable between the two products. Investigational product tablets were sealed in identically-appearing, high-density polyethylene bottles with desiccant.

Ethics and informed consent

The study was reviewed by the Natural Health Products Directorate (NHPD), Health Canada and a research ethics board. Notice of authorization was granted by the NHPD, Ottawa, Ontario (April 10, 2014) and unconditional approval was granted by the Institutional Review Board (IRB Services, Aurora, ON, Canada). This study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and its subsequent amendments. Informed consent was obtained from each subject at the screening visit (Visit 1) prior to any study-related activities being performed.

Study design

The study design is depicted in figure 1 and disposition of the study participant shown in figure 2. This randomized double-blind clinical study was conducted at a single site (Suite 1440, One London Place, 255 Queens Ave London, ON, Canada). The first subject was enrolled in June 2014 and the last subject completed the study in August 2014.

The sample size for this study was 40 subjects, with 20 subjects randomized to each of the two study arms in a double-blinded manner at a 1:1 ratio. In order to evaluate the primary objective, study assessments were conducted at baseline and day 30. The study consisted of a 30 day intervention period. Subjects met with the investigational team for screening, the baseline/randomization visit and at the end of the study (day 30).

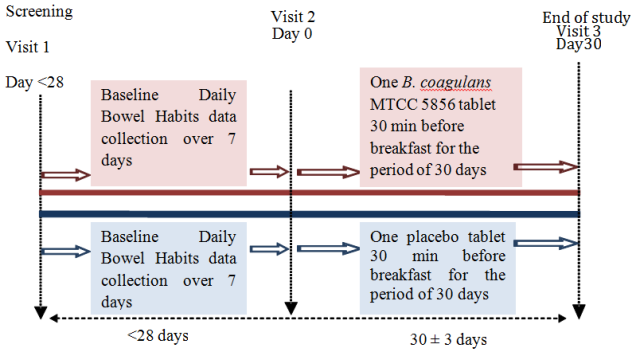

Figure 1: Study Design Diagram.

A description of Visits 1, 2 and 3 with study flow are provided in figure S1. No changes or amendments were made to the approved protocol after the trial commenced and no interim analysis was done during the study period. Independent investigator of the study monitored the progress of all clinical investigations that were conducted and ensured that the protocol is adhered in all aspects. This was a pilot safety study of 40 subjects and therefore the sample size calculation was not carried out. Each participant was assigned a 6-digit randomization code and the investigational products were dispensed by site personnel as per the randomization code list generated by an independent statistician. Double blinding to the investigational products was performed by an independent blinding of the dosing kits and therefore both clinical site staff and participants remained blinded to the treatment received throughout the study duration.

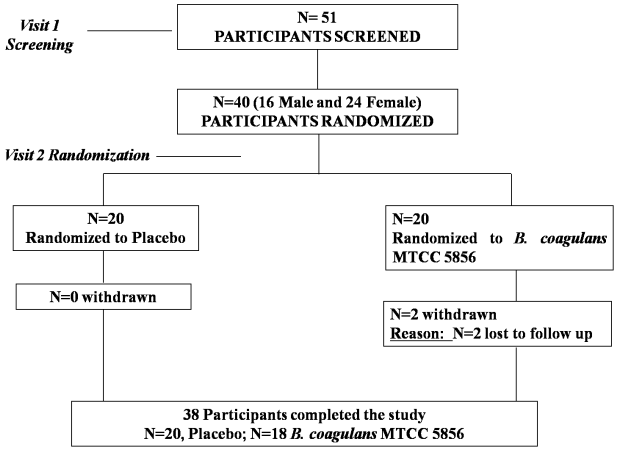

Figure 2: Disposition of study participants.

Selection of study population

A total of 40 healthy adult volunteers (16 males and 24 females) were randomized into two equal groups. All participants met the following eligibility criteria:

Inclusion criteria

1) Male or female ≥ 18 years of age. 2) Females not of child bearing potential, defined as females who had a hysterectomy or oophorectomy, bilateral tubal ligation or were post-menopausal (natural or surgically with >1 year since last menstruation) OR Female subjects of childbearing potential had to agree to use a medically approved method of birth control and have a negative urine pregnancy test result. Acceptable methods of birth control included: a) Hormonal contraceptives including oral contraceptives, hormone birth control patch (Ortho Evra), vaginal contraceptive ring (NuvaRing), injectable contraceptives (Depo-Provera, Lunelle), or hormone implant (Norplant System). b) Intrauterine devices. c) Vasectomy of partner (shown successful as per appropriate follow-up). d) Double barrier method (use of physical barrier by both partners). 3) Healthy as determined by laboratory results and medical history. 4) Normal BMI 18.5-29.9 kg/m^2. 5) Had given voluntary, written, informed consent to participate in the study.

Exclusion criteria

1) Women who were pregnant, breastfeeding, or planning to become pregnant during the course of the trial. 2) Subject had any clinically significant medical condition. 3) Subject required the use of prescribed medications (other than birth control). 4) Use of illicit drugs or history of drug or alcohol abuse within the past 5 years (or had been having more than 2 standard alcoholic drinks per day). 5) Participation in a clinical research trial within 30 days prior to randomization. 6) Clinically significant abnormal laboratory results at screening. 7) Allergy or sensitivity to test product ingredients. 8) Individuals who were cognitively impaired and/or who were unable to give informed consent. (9) Any other condition which in the Investigator's opinion may have adversely affected the subject's ability to complete the study or its measures or which may have posed significant risk to the subject if enrolled in the study.

Interventions

All subjects that met inclusion criteria were randomized into two groups. During the intervention period, one group received *B. coagulans* MTCC 5856 tablets containing 2 × 10^9 cfu (spores) while the other group received placebo tablets. The intervention period was 30 days in duration. Participants consumed 1 tablet daily 30 minutes before a meal. Participants were instructed to consume the tablet in the morning before breakfast.

Safety Outcomes

Adverse events and laboratory abnormalities

An adverse event (AE) was defined as any untoward medical occurrence in a clinical investigation subject who was administered an investigational product and which did not necessarily have a causal relationship with the treatment [24]. Pre-existing conditions which worsened during the study were to be reported as AEs. During the study, subjects recorded adverse effects in their diary. At each visit the subject was asked "Have you experienced any difficulties or problems since I saw you last?" Any AEs were documented in the study record and were classified according to the description, duration, intensity, frequency, and outcome. The Investigator assessed any AEs and decided causality. Intensity of AEs was graded on a three-point scale (mild, moderate, severe) and reported in detail in the study record.

Serious adverse events

A serious adverse event (SAE) was defined as any AE that resulted in death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability or incapacity and a congenital anomaly/birth defect in the offspring of a subject who received the study treatment. Important medical events that were not immediately life-threatening or resulted in death or hospitalization but may have jeopardized the subject or may have required intervention to prevent one of the outcomes listed above [24].

Laboratory test abnormalities

Laboratory blood tests [complete blood count (CBC), electrolytes, glucose, creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl aminopeptidase (GGT) and bilirubin] were conducted at screening, baseline/randomization and at the end of study visit (30 days). Laboratory blood tests were analyzed by LifeLabs (London, ON, Canada) by following standardized procedures.

Product tolerability

Participants ranked the *B. coagulans* MTCC 5856 tolerability on a scale of 0 to 10 (0 Representing “not tolerable at all” and 10 Representing “extremely tolerable”). Swallowing difficulty reported by participants by day over 30 days. Patients ranked the ease of swallowing on a scale of 0 to 10 (0 representing "difficult" and 10 representing "not at all difficult"). Participants ranked the performance of the *B. coagulans* MTCC 5856 in terms of ease of swallowing on a scale of 0 to 10 (0 representing “difficult” and 10 representing “not at all difficult”). Similarly, participants ranked the performance of the *B. coagulans* MTCC 5856 in terms of its effect on the stomach such as constipation, diarrhea and cramps on a scale of 0 to 10 (0 Representing “Many Stomach Problems” and 10 Representing “No Stomach Problems”).

Daily bowel habits

Participants were asked to maintain a daily bowel habits diary for the 7 days prior to randomization and for the duration of the study (30 days ± 3). Participants recorded the number of bowel movements per day as well as indicating if they experienced a feeling incomplete defecation, straining to start or stop defecation. Participants were provided with the Bristol Stool Scores (BSS) chart to be used to classify their stools [25]. The BSS is a validated score to classify human feces and is a useful tool to measure efficacy of probiotic formulations and their effects on a participants stool consistency. The BSS has 7 score types of classification. A score of 1-2 suggesting constipation, 3 and 4 being the ideal stool form and 5-7 suggesting diarrhea. The information reported by participants in this diary was used to assess if *B. coagulans* MTCC 5856 supplementation caused changes in the bowel habits or consistency during the supplementation.

Data quality assurance

The quality control procedures used in this study included cross-checking all data by research personnel against source document originals. Data entry and verification was executed according to KGK Synergize's Standard Operating Procedures (London, ON, Canada). All raw data and standard operating procedures used in this trial were maintained and archived where appropriate to satisfy regulatory requirements. Frequent monitoring was done during the study. All source documents were reviewed to ensure that all items were completed and that the data provided were accurate and obtained in the manner specified in the protocol.

Data Analysis

Assessment of normality

After the database was locked, but before it was unblinded, the analyzing statistician examined the distribution of all continuous variable endpoints. The Anderson-Darling test of normality was used to determine whether the variable was (1) sufficiently normally distributed to permit its use in parametric statistical tests (i.e. Student's t-test), or (2) sufficiently log-normally distributed to permit the logarithm of that variable to be used in parametric tests, or (3) so intractably non-normal, even with logarithmic transformation as to require non-parametric testing (i.e. Mann-Whitney tests).

Assessment of safety

Summary statistics for continuous variables were computed by treatment and visit as count, mean and standard deviation. Changes from baseline to subsequent time points were summarized the same way. Mean values and mean changes from baseline were compared between products by the unpaired Student t-test, or the non-parametric Mann-Whitney U test. Changes from baseline were tested for within group significance by the paired Student t-test or the Wilcoxon Signed-Ranks test. Summary statistics for discrete variables were tabulated at each time point, for each treatment group and for the combined groups as counts and percentages of group totals. For the assessment of safety laboratory parameters and anthropometric and vital sign measures, the change from baseline was defined as the difference between the value at day 30 and the value at the baseline visit. For the assessment of BSS and average number of bowel movements per day, the baseline was considered to be the average of the 7 day pre-baseline values.

Level of significance and statistical software

Probability (p) values ≤ 0.05 were considered statistically significant. All evaluations were carried out using the software package R 3.03. Data collection during the study and statistical analysis were performed by separate functional groups and a certified, independent statistician respectively.

Results

Forty participants were randomized to either placebo (n=20; 10 males and 10 females) or *B. coagulans* MTCC 5856 (n=20; 6 males and 14 females). The mean age for participants was 33.5 ± 13 years for placebo group and 33.5 ± 14.3 years for *B. coagulans* MTCC 5856 group and demographics were similar between *B. coagulans* MTCC 5856 group and placebo group (Table 1). The average BMI was 24.6 ± 2.7 kg/m^2 for the placebo group and 24.3 ± 3.5 Kg/m^2 for the *B. coagulans* MTCC 5856 group. Two females in the *B. coagulans* MTCC 5856 group withdrew from the study citing personal reasons for opting out of the study.

		Placebo	*B. coagulans* MTCC 5856	Total	P Value Δ
Gender					
	Female	10	14	24	0.333
	Male	10	6	16	
	Total	20	20	40	
Smokers					
	Ex-Smoker	16	18	34	0.797
	Non-Smoker	1	1	2	
	Smoker	3	1	4	
	Total	20	20	40	
Ethnicity					
	Hispanic	2	3	5	
	Non Hispanic	18	17	35	
	Total	20	20	40	
Races					
	Central American	0	1	1	0.95
	East Asian	1	1	2	
	South Asian	1	1	2	
	South American	0	1	1	
	South East Asian	0	1	1	
	Western European White	18	15	33	
	Total	20	20	40	
Alcohol use					
	None	5	3	8	0.452
	Occasionally	11	9	20	
	Weekly	4	8	12	
	Total	20	20	40	

Table 1: Demographics for all participants randomized into the Study. Δ Independence of treatment assessed by the Fisher's exact test. Probability values ≤ 0.05 are statistically significant.

Treatment compliance

The mean treatment compliance, measured as the number of dosage units taken by participants compared to the number expected to have been taken, was greater than 97% in both groups during the study. One participant had a compliance of 76.7%. Therefore, this participant's

data was only used for safety analysis and excluded from tolerability analysis.

Anthropometric and vital signs measures

There were no between group differences at Day 30 in BMI, weight, heart rate, and diastolic blood pressure (DBP) in participants supplemented with *B. coagulans* MTCC 5856 vs those on placebo (Table 2). There was a significant between group difference in systolic blood pressure (SBP) at Day 30 in subjects on placebo vs those on *B. coagulans* MTCC 5856 (p=0.006). This difference reflected an increase in the SBP in the placebo group while those on *B. coagulans* MTCC 5856 showed a decrease. However, the blood pressure in both groups remained within normal and acceptable clinical range at Day 30. Within groups, participants supplemented with *B. coagulans* MTCC 5856, showed no differences in weight, BMI, heart rate and systolic and diastolic blood pressure from baseline to Day 30. However, for participants in the placebo group, all of the anthropometric measures showed no statistically significant difference from baseline to Day 30 except for systolic blood pressure, which increased from baseline to Day 30 (p=0.023) but remained within a normal clinical range.

Parameters		Placebo (N=20)	*B. coagulans* MTCC 5856 (N=18)	P value Δ
Weight (Kg)				
	Baseline (Day 0)	72.2 ± 12.8	68.8 ± 13.6	0.598
	End of Study (Day 30)	71.8 ± 13.5	69.4 ± 14	0.604
	Change From Baseline to Day 30	p = 0.429	p = 0.390	0.968
BMI (kg/m^2)				
	Baseline (Day 0)	24.6 ± 2.7	24.5 ± 3	0.731
	End of Study (Day 30)	24.4 ± 2.9	24.3 ± 3.3	0.869
	Change From Baseline to Day 30	p = 0.381	p = 0.353	0.897
Heart Rate (BPM)				
	Baseline (Day 0)	68.3 ± 7.5	68.9 ± 8.4	0.839
	End of Study (Day 30)	65.9 ± 6.8	69.2 ± 8.6	0.193
	Change From Baseline to Day 30	p = 0.157	p = 0.486	0.159
Systolic Blood Pressure (mmHg)				
	Baseline (Day 0)	100.4 ± 10.3	104.0 ± 9.4	0.253
	End of Study (Day 30)	104.9 ± 10.4	100.0 ± 9.0	0.127
	Change From Baseline to Day 30	p = 0.023	p = 0.100	0.006
Diastolic Blood Pressure (mmHg)				
	Baseline (Day 0)	66.5 ± 7.6	68.4 ± 6.7	0.428
	End of Study (Day 30)	67.4 ± 7.6	69.0 ± 7.9	0.547
	Change From Baseline to Day 30	p = 0.590	p = 0.852	0.796

Table 2: Anthropometrics and Vital Signs for All Participants at Baseline and after 30 Days of Supplementation with *B. coagulans* MTCC 5856 or Placebo BPM, beats per minute; mmHg, millimeter of mercury; kg, kilograms; BMI, body mass index; kg/m^2, kilograms per square meter. Δ Between-group comparisons were made using the unpaired Student's t-test. Within-group comparisons were made using the paired Student's t-test. Probability (p) values ≤0.05 are statistically significant.

Laboratory parameters of safety

There were no differences in the laboratory parameters between participants in the *B. coagulans* MTCC 5856 group and those in the placebo after a 30 day supplementation with *B. coagulans* MTCC 5856 (Table S1). The baseline hemoglobin and hematocrit levels had statistically significant difference between participants supplemented with placebo and those supplemented with *B. coagulans* MTCC 5856 (p=0.017 and p=0.029 respectively). The placebo group consisted of more males (10 males and 10 females) in comparison to the *B. coagulans* MTCC 5856 group (6 males and 10 females). As hemoglobin and hematocrit levels tend to be higher in males compared to females, it is possible that the variation in gender between the two groups may be attributed to this difference. However, these differences did not carry through to Day 30 and values remained similar between groups after the supplementation period. Within groups, participants on placebo showed a decrease in mean corpuscular hemoglobin (p=0.027), and potassium concentration (p=0.017) and an increase in fasting glucose (p=0.015) and eGFR (p=0.022). The *B. coagulans* MTCC 5856 group showed a decrease from baseline in the red blood cell distribution width (RDW) after the 30 day supplementation (p=0.003). However all values remained within normal acceptable laboratory range. No other changes were seen between or within groups after the 30 day supplementation.

Adverse effect

A total of 9 AEs were reported in the placebo group by 8 participants. Six of these AEs were categorized as gastrointestinal with 1 AE (upset stomach) considered by the qualified investigator (QI) as "unlikely related" to the investigational product (IP) and 5 AEs

(nausea, upset stomach, bloating, borborygmus, abdominal cramps and stomach ache) considered by the QI to be "possibly" related to the investigational product. One AE was classified as a nervous system disorder; participant in the placebo group reported experiencing headache which was mild in severity and categorized as unlikely related to the IP by the QI. One AE was classified as infections and infestations; participant in the placebo group reported a cold, mild in severity and it was categorized as not related to the IP. Participant 031 in the placebo group accidently consumed gasoline during work which caused upset stomach and loose bowel movements, this AE was categorized as injury, poisoning and procedural complications and the AE classified as not related to IP. All AE's resolved without participants having to discontinue the study products.

In the *B. coagulans* MTCC 5856 group, 10 AEs were reported by 5 participants. Six of these were classified as gastrointestinal with 2 AEs (bloating and diarrhea) considered by the QI as "unlikely" related to the IP and 4 AEs (stomach pain, nausea, upset stomach and bloating) considered by the PI as "possibly" related to the IP. Two nervous system disorders (headache) were experienced by participants in the *B. coagulans* MTCC 5856 group. All nervous system disorder AEs were classified to be unlikely related to the IP. One AE experienced by a participant in the *B. coagulans* MTCC 5856 group was categorized as General Disorder and Administration site conditions. This AE was reported as fever and upset stomach and considered by the PI as unlikely related to the IP. One infection and infestation AEs was reported in the *B. coagulans* MTCC 5856 group (stomach flu) and was considered to be unlikely related to investigational product by the QI. All AEs resolved without participants having to discontinue the study products.

Tolerability

Participants ranked the IP tolerability, performance in term of ease of swallowing, and its effect on the stomach on a scale 0 to 10. The tolerability, swallowing difficulty and effect on the stomach was similar between placebo and *B. coagulans* MTCC 5856. Over the 30 days supplementation period, participants ranked the product to be tolerable with the mean tolerability of 9.02 ± 1.76 for placebo and 9.00 ± 1.96 for *B. coagulans* MTCC 5856 (0 represents "not tolerable at all" and 10 represents "extremely tolerable") (Figure 3). The participants also reported that both the placebo and *B. coagulans* MTCC 5856 were easy to swallow during the 30 days with the mean difficulty of 8.75 ± 1.66 for placebo and 8.82 ± 1.67 for *B. coagulans* MTCC 5856 (0 representing "difficult" and 10 representing "not at all difficult") (Figure 3). Both placebo and *B. coagulans* MTCC 5856 had minimal effect on the stomach such as constipation, diarrhea and cramps over the 30 days of supplementation. The effect of the product on the stomach was reported by participants as a mean of 8.94 ± 1.85 for placebo and a mean of 8.82 ± 2.24 for *B. coagulans* MTCC 5856 (0 representing "Many Stomach Problems" and 10 Representing "No Stomach Problems") over the 30 days of supplementation (Figure 3).

Bowel habits

There were no differences between groups in the daily number of bowel movements in either placebo or *B. coagulans* MTCC 5856 during the 7 days pre-dose period or the 30 day supplementation period (Table 3). The average number of bowel movements showed that there were no between group differences in this parameter for participants receiving either placebo or *B. coagulans* MTCC 5856 the 30 days supplementation (Table 3).

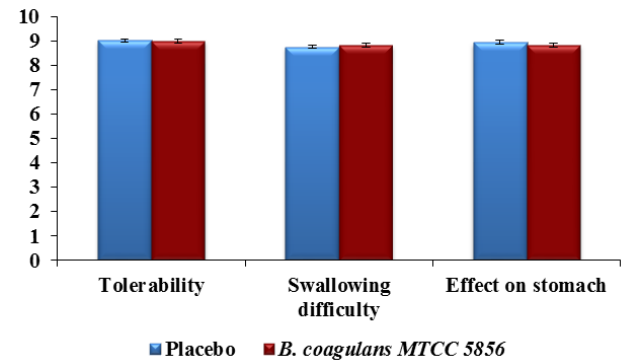

Figure 3: Average of daily product effect on the tolerability, swallowing difficulty and Stomach reported by participants over 30 days. The error bars represent the standard error of the means.

Week of Study	Placebo	*B. coagulans* MTCC 5856	P value Δ
	(N=20)	(N=18)	
Baseline (Day 0)	1.32 ± 0.42	1.19 ± 0.51	0.416
End of Study (Day 30)	1.14 ± 0.45	1.19 ± 0.75	0.842
Change From Day 0 to Day 30	p=0.123	p=0.864	0.509

Table 3: The average number of daily bowel movements of all participants during the 30 Day supplementation with either *B. coagulans* MTCC 5856 or Placebo. Δ Between-group comparisons were made using the unpaired Student's t-test. Within-group comparisons were made using the paired Student's t-test.

During the 30 days of supplementation, there were no between group differences in the number of participants who experienced at least one bowel movement that required straining to start defecation (Table 4) or to stop defecation (Table 5) in the placebo vs *B. coagulans* MTCC 5856 group.

Week of Study	Straining	Placebo	*B. coagulans* MTCC 5856	P value Δ
		(N=20)	(N=18)	
Baseline (Day 0)	No	12	11	1
	Yes	7	9	
End of Study (Day 30)	No	7	14	0.187
	Yes	4	2	

Table 4: Number of participants who experienced at least one bowel movement that required straining to start defecation, during the 30 day supplementation with *B. coagulans* MTCC 5856 or Placebo. Δ Between-group comparisons were made using the Fisher's exact test.

Similarly, there were no differences between subjects who experienced at least one "Feeling of Incomplete Defecation", during the

30 Day supplementation with either *B. coagulans* MTCC 5856 or Placebo (Table 6).

Week of Study	Straining	Placebo	*B. coagulans* MTCC 5856	P value Δ
		(N=20)	(N=18)	
Baseline (Day 0)	No	17	15	0.407
	Yes	2	5	
End of Study (Day 30)	No	11	15	1
	Yes	0	1	

Table 5: Number of participants who experienced at least one bowel movement that required straining to stop defecation, during the 30 Day supplementation with either *B. coagulans* MTCC 5856 or Placebo. Δ Between-group comparisons were made using the Fisher's exact test.

Week of Study	Incomplete Defecation	Placebo	*B. coagulans* MTCC 5856	P value Δ
		(N=20)	(N=18)	
Baseline (Day 0)	No	13	8	0.111
	Yes	6	12	
End of Study (Day 30)	No	11	16	-
	Yes	0	0	

Table 6: Number of subjects who experienced at least one "Feeling of Incomplete Defecation", in a given week, during the 30 Day supplementation with either *B. coagulans* MTCC 5856 or Placebo. Δ Between-group comparisons were made using the Fisher's exact test.

Bristol stool score

The Bristol Stool form scale provides illustrations of the seven stool types, which can be used to help fill out the **stool diary** [25]. It remains in use as a research tool to evaluate the effectiveness of treatments for various conditions of the bowel, as well as a clinical communication aid. Over the 30 day supplementation period, *B. coagulans* MTCC 5856 did not show any adverse effects on the consistency of the faeces of participants. There were no between group differences in the daily BSS for participants in the placebo vs *B. coagulans* MTCC 5856 during the pre-dose week (baseline) (data not shown). The average BSS were not different between the placebo and the *B. coagulans* MTCC 5856 group (Table 7).

Week of Study	Placebo	*B. coagulans* MTCC 5856	P value Δ
	(N=20)	(N=18)	
Baseline (Day 0)	3.76 ± 0.63	3.91 ± 0.86	0.608
End of Study (Day 30)	3.61 ± 0.62	4.40 ± 0.87	0.275
Change from Baseline to Day 30	p=0.336	p=0.021	0.022

Table 7: The average BSS for all participants during the 30 Day supplementation with either *B. coagulans* MTCC 5856 or Placebo. Δ Between-group comparisons were made using the unpaired Student's t-test. Within-group comparisons were made using the paired Student's t-test.

Discussion and Conclusions

Oral administration of *B. coagulans* was found to be safe in several subchronic, chronic and reproductive toxicity animal studies which did not reveal any adverse effects at a dose of 6.88×10^{10} cfu (spores)/kg body weight [9,20,26]. In another animal study, *B. coagulans* containing 5×10^9 cfu (spores)/g was administered by gavage to male mice at dose levels of 1, 3 or 5 g/kg body weight and no deaths occurred, nor was there any abnormality such as diarrhea. Hence, the results of the study suggested that the LD50 of a powder containing *B. coagulans* was greater than 5 g/kg body weight [9,26]. From the animal studies, the current double-blinded, placebo-controlled two arm study was conducted to evaluate the safety and tolerability of *B. coagulans* MTCC 5856 at a dose of 2×10^9 cfu (spores)/day in healthy adults over 30 days supplementation period. The treatment compliance was 99% for the *B. coagulans* MTCC 5856 group and 97% for the placebo group. There were no statistically significant differences in the laboratory parameters between participants in the *B. coagulans* MTCC 5856 group and those in the placebo after a 30 day supplementation with *B. coagulans* MTCC 5856. The baseline hemoglobin and hematocrit levels were statistically significantly higher in participants randomized to the placebo group compared to those randomized to the *B. coagulans* MTCC 5856 group. However, this difference did not translate into a statistically significant difference between groups after the 30 day supplementation. Within groups, participants supplemented with *B. coagulans* MTCC 5856, showed no differences in weight, BMI, heart rate and systolic and diastolic blood pressure from baseline to Day 30. Further, there were no significant differences in the anthropometric measurements between *B. coagulans* MTCC 5856 and placebo groups. To the best of our knowledge, this is the first study demonstrating clinical safety (blood hematology, clinical chemistry parameters and anthropometric measures) of *B. coagulans* MTCC 5856 probiotic at a dose of 2×10^9 cfu (spores)/day in healthy adults over 30 days supplementation period. However, there are many other strains of *Bacillus coagulans* consumed worldwide and well-studied for their clinical efficacy and safety [10,27-32].

Adverse events were rigorously monitored in the study in order to document all events that occurred during the 30 day supplementation with *B. coagulans* MTCC 5856. Special emphasis was directed to any gastrointestinal related symptoms such as vomiting, diarrhea and abdominal pain. Participants receiving *Bacillus coagulans* MTCC 5856 did not report any adverse events such as vomiting and diarrhea. Participants on placebo reported two adverse events classified as abdominal pain and those on *B. coagulans* MTCC 5856 reported one adverse event classified as abdominal pain. Therefore, abdominal pain was not limited to the *B. coagulans* MTCC 5856 group. *B. coagulans* MTCC 5856 and placebo tablets were reported by the participants to be tolerable, easy to swallow and had minimal effects on the stomach during the 30 days of supplementation. The number of bowel movements was similar between the placebo group and *B. coagulans*

MTCC 5856 group during the baseline week and during the 30 days of supplementation. BSS were also similar between placebo and *B. coagulans* MTCC 5856 during the 30 day supplementation period. The results from the current study are in the agreement with the published literature [10,27-32]. For the first time, we report a detailed safety and tolerability of *B. coagulans* MTCC 5856 at a dose of 2×10^9 cfu (spores)/day in healthy individuals during the 30 days of supplementation. However, in another study, 36 subjects were randomized into two groups who received either *B. coagulans* MTCC 5856 or placebo. *B. coagulans* MTCC 5856 treatment revealed a significant change/decrease in the clinical symptoms such as bloating, vomiting, diarrhea, abdominal pain and stool frequency towards end of the study [33].

Ugba, a fermented African OIlbean seeds (*Pentaclethra macrophylla*, Benth) is a popular protein-rich solid, flavorful alkaline food in the Ibo ethnic group of Nigeria. *B. coagulans* was reported to be major organism responsible for the fermentation of Ugba [34]. This validates the traditional safe use of *B. coagulans*. Several clinical studies on the safety aspects of *B. coagulans* have been reported. However, it is essential to evaluate the safety of every probiotic strains which is intended for human consumption [8]. Additionally, *B. coagulans* has been granted Qualified Presumption of Safety (QPS) status since 2008 by the European Food Safety Authority [35] and the Japanese Ministry of Health and Welfare has also approved *B. coagulans* for improvement in symptoms caused by abnormalities in the intestinal flora or in dysbiosis [17, 20]. In addition to the above approved uses, USFDA had also issued a "no questions" letter to the GRAS notices on the use of *B. coagulans* spores preparations to be used at a maximum level of approximately 2×10^9 cfu/serving in several food categories.

In conclusion, this study has verified that *B. coagulans* MTCC 5856 at a dose of 2×10^9 cfu (spores)/day in healthy individuals was safe and tolerable in healthy participants supplemented for 30 days. *B. coagulans* MTCC 5856 was also easy to swallow, tolerable and had no statistically significant effects on the stomach during the 30 days of supplementation. The results of the study suggested that oral administration of *B. coagulans* MTCC 5856 at a dose level of 2×10^9 cfu (spores)/day for 30 days was safe and well tolerated in healthy subjects.

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LactoSpore® stable probiotic is a registered logo (U.S Trademark Registration No.4068336) of Sabinsa Corporation, 20 Lake Drive, East Windsor, NJ, USA 08520.

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