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EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF *BACILLUS COAGULANS* MTCC 5856 AND ITS EFFECT ON GASTROINTESTINAL MOTILITY IN WISTAR RATS

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ABSTRACT

Probiotics are live microorganisms that confer a number of health benefits when consumed in adequate amounts. However, the beneficial effects of a probiotic are strain specific and hence each probiotic strain must be evaluated for their application. Thus, the present study was aimed to investigate the effect of *Bacillus coagulans* MTCC 5856 on castor oil induced diarrhoea and gastrointestinal (GI) motility using well established rodent models. Rats were divided into 6 different groups with six rats in each group i.e., 1) normal control (no treatment), 2) positive control (loperamide, 1 mg/kg), 3) negative control (maltodextrin) and 4, 5 and 6 treatment groups receiving orally 40, 80 and 160 × 10^6 cfu/kg body weights of *B. coagulans* MTCC 5856 spores respectively. Weight of faecal samples was recorded at 4, 8 and 12 h in castor oil induced diarrhoea. Furthermore, in an independent study, GI motility was assessed in fasting rats using charcoal meal as a marker. *B. coagulans* MTCC 5856 showed dose dependent anti- diarrhoeal activity, and the percentage inhibition of faecal weight was similar as loperamide group at a dose of 160 × 10^6 cfu/kg body weights in 4 h ($p>0.05$). However, the GI motility inhibition was not comparable with atropine sulphate (0.1 mg/kg body weight) which could be due to its intraperitoneal route of administration. *B. coagulans* MTCC 5856 elicited anti-diarrhoeal activity and inhibited the gastrointestinal motility in fasted rats. Therefore, *B. coagulans* MTCC 5856 could be a potential agent in the management of diarrhoea.

KEY WORDS: ***Bacillus coagulans*, diarrhoea, intestinal motility, probiotic.**

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INTRODUCTION

Lilly and Stillwell were the first to introduce the term "probiotics" in 1965[1]. Probiotics are defined as microbially derived factors that stimulate the growth of other organisms. The idea that probiotics have a beneficial effect on the host was first introduced by Roy Fuller in 1989[2]. Probiotics are live microbes that can be formulated into many different types of products, including foods, drugs and dietary supplements. A recent formal definition of probiotics was agreed by a working group of European scientists and is given as ''a live microbial feed supplement that is beneficial to health''. This emphasised the importance of definitive improvements in health as well as the possibility that probiotics could have effects systemic to the gut, e.g. vagina, skin, and mouth. The species *L. sporogenes* was originally isolated and described in 1933 by Horowitz-Wlassowa and Nowotelnow and subsequently reclassified as *Bacillus coagulans*. More recently, it has been evidenced that *L. sporogenes* shares the same characters of *B. coagulans*, and therefore it has been moved into *B. coagulans* group. According to the 8th edition of Bergey's Manual of Determinative Bacteriology, spore-bearing rods producing lactic acid, facultative or aerobic and catalase positive are to be classified within the genus Bacillus. Probiotics are marketed as health, or functional foods whereby they are ingested for their beneficial effects in the digestive tract and/or systemic areas like the liver, vagina or bloodstream. Although known since a long time, only in the last two decades probiotics have started to receive major attention from researchers, and several studies have been carried out on the effects of probiotic microorganisms, using different formulae and with numerous purposes of preventing or treating diseases [3, 4]. Most of the wide variety of novel probiotic products developed and marketed in European countries in the last decade mainly contain Lactobacilli, such as *L. acidophilus*, *L. casei*, *L. rhamnosus* for which several studies have evidenced some probiotic properties [5, 6]. Several products containing *B. coagulans* are now available in the market, promoting a large number of products. Most of them report the old nomenclature of *L. Sporogenes.* Probiotics are live microbial feed additions that improve human or animal health. Their activities are towards improving the composition of the gastrointestinal microbiota in a manner that reduces the risk of disorder. In some cases, probiotics are also used therapeutically. Traditionally, probiotics are incorporated in dairy products (yoghurts or fermented drinks) or in lyophilised form. Due to the stability and viability factors, heated products are not usually a target for probiotic use. This is because they are temperature sensitive. However, a spore-forming genus would have the ability to overcome this limitation. *L. sporogenes* is temperature resistant and it's indications cover all the usual range of probiotics, such as lactose intolerance, gastrointestinal infections, dyspepsia, hypercholesterolemia, non-specific vaginitis, urinary tract infections. An estimated 1.7 billion diarrhoeal cases occur every year, with about 760,000 children of 5 years and below in many developing countries, dying from the disease [7]. Diarrhoea is characterized by an increase in the frequency, fluidity and volume of faeces, much more than the normal for an individual [8]. Diarrhoea has been shown to be due to an imbalance between the secretory and absorptive processes in the intestines giving rise to increased frequency of bowel movements, watery stools and abdominal pain[9]. The consequences of diarrhoea are stark dehydration, bodily fluid and electrolyte loss[10]. With many of the available synthetic anti-diarrhoeal drugs associated with many unwanted side effects[11], the search for safe and effective anti-diarrhoeal agents continues. In this context, quite a lot of probiotics have been researched for potential anti-diarrhoeal properties and in the current study *B. coagulans* MTCC 5856 strain was evaluated for anti-diarrhoeal activity along with its effects on intestinal motility in Wistar rats.

MATERIALS AND METHODS

Bacillus coagulans MTCC 5856 strain has been deposited in the Microbial Type Culture Centre and Gene Bank (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India. *B. coagulans* MTCC 5856 samples used in the study were manufactured by Sami Labs Limited (Bangalore, India) by following a proprietary, in-house developed, good manufacturing process. Pure *B. coagulans* MTCC 5856 spores were spray–dried and standardized with food grade maltodextrin (Sanwa Starch Co. Ltd. Kashihara, Nara, Japan) to achieve the desired concentration of 16 × 10^9 cfu/gm, 8 × 10^9 cfu/gm and 4 × 10^9 cfu/g. One gram each of the above doses of *B. coagulans* MTCC 5856 was dissolved in 100 ml of sterile water, which had 160 × 10^6 cfu/ml, 80 × 10^6 cfu/ml and 40 × 10^6 cfu/ml, respectively.

Animals

Male Wistar rats were acclimatized for 5 days and were maintained under standard laboratory conditions (i.e. at room temperature of 24 ± 2°C and at a relative humidity of 45 to 65% in an approximate 12 h light/ dark cycle). During acclimatization, animals in groups of 3 were housed in polycarbonate cages with corn cob as bedding material. Each covered cage was fitted with a stainless steel grill having provision for holding pellet feed and a water bottle with stainless steel drinking nozzle. Irradiated rodent feed and autoclaved reverse osmosis water were fed to the animals except during fasting. In each cage, animals were identified with fur marking and cage label with study details. Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained for the conduct of the study (approval number is 37/38-15) and the studies were performed as per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines published in The Gazette of India, Dated: 15 December, 1998.

Chemicals and Reagents

Atropine sulphate (S.D Fine-Chem Limited, Mumbai), Loperamide (Micro Labs Limited, India), Castor oil (Ashwin Fine Chemicals and Pharmaceuticals, Mumbai), Charcoal meal (West-Coast Pharmaceuticals Works Limited, Ahmedabad, India)

and distilled water (served as the vehicle) were used in the study.

Experimental design for anti-diarrhoeal activity

A total of thirty six (36) male Wistar rats , and weighing between 150 to 200 grams from the breeding colony of the animal house of the Reliance Biosciences, Mumbai, India were used for the purpose of the study. The studies were conducted from 05 November to 25 November 2015. The animals were weighed before they were randomly divided into six groups of six (6) animals each for experiments on castor oil-induced diarrhoea . All three dosages of *B. coagulans* MTCC 5856 were dissolved in 100 ml of sterile water. 1 ml/kg body weight of all three dosages containing 40, 80 and 160 × 10^6 cfu/ml was administered orally to corresponding groups of animals. Loperamide at the dose of 1 mg/kg body weight was used as reference in this study and was administered at a constant volume of 1 ml/kg body weight orally to the positive control group. Castor oil was administered to all the animals in the groups at a dose of 1 ml/animal orally for the induction of diarrhoea [12]. Animals were fasted for 18 h (15:00 h to 09:00 h). Post fasting, animals were randomized into six groups (Table 1). Animals of group 1 were not treated and Group 2 animals were treated with 1 mg/kg loperamide. Group 3 animals were dosed placebo (maltodextrin) at 10 mg/kg. Groups 4, 5 and 6 were treated with different doses of *B. coagulans* MTCC 5856. Group 4 received the 40 × 10^6 cfu/Kg body weight, group 5 received the 80 × 10^6 cfu/kg body weight and group 6 received the 160 × 10^6 cfu/kg body weight. All the doses were administered orally. One hour after treatment, animals of groups 2 to 6 were administered 1 ml each, of castor oil orally to induce diarrhoea. During the study, all animals were housed individually in polycarbonate cages with fasting grills. The cage floor was layered with non-wetting paper. Animals were fasted during the study but were allowed access to water. Water consumption over the 12 h period was quantified. Animals were observed for defecation at 4 h, 8 h and 12 h post castor oil challenge. Weights of the faecal material were measured and water consumption over 12 h periods was also quantified. Microbial and chemical contaminant analysis of water and feed did not reveal the presence of any contamination which can affect the integrity of the study.

Effect of B. coagulans MTCC 5856 on gastrointestinal (GI) motility

A total of thirty (30) Wistar rats were fasted for 18 h and were randomly allotted to 5 groups of 6 rats each (Group 1 – 5) (Table 2). Charcoal meal was used as a diet marker. Animals were fasted for 18 h (17:00 h to 11:00 h). Animals of group 1 were administered 2% Gum acacia orally. Animals of group 2 were administered 1 mg/kg atropine sulphate intra-peritoneally. Group 3, 4 and 5 received *B. coagulans* MTCC 5856 at a dose of 40, 80 and 160 × 10^6 cfu/kg body weight, respectively. Four hours after dose administration to groups 1, 3, 4 and 5 orally and 2 h after dose administration to Group 2 animals intra-peritoneally, 3% deactivated charcoal in 2% aqueous Gum acacia was administered orally. As the current strain under study is a spore forming bacteria, a time gap of around 4 h was provided for the probiotic to germinate in the gut in groups 1, 3, 4 and 5. While the group 2 animals were given only 2 hours of time as it was administered intra-peritoneally. One hour after charcoal meal administration, animals were sacrificed by carbon dioxide asphyxiation. The distance covered by the charcoal meal in the intestine was measured from the pylorus to the caecum and was expressed as a percentage relative to control group [13].

Statistical analysis

Faecal weight and gastrointestinal motility were calculated (Table 1) for control and treated groups. Faecal weight of the treatment group animals were compared to negative control animals by ANOVA and that of positive control was compared with negative control by Student's *t*-test ($p \leq 0.05$). Similarly, gastrointestinal motility of treatment groups were compared with normal control by ANOVA and GI motility of positive control was compared with normal control by Student's *t*-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effect of B. coagulans MTCC 5856 on diarrhoea

At 4 h time point, the weight of faecal material expected was highest in the negative control. Loperamide treated group showed a significant decrease in weight at 4 h time point ($p<0.05$). There was a dose depended decrease in weight in the groups treated with the *B. coagulans* MTCC 5856. At a dose of 160 × 10^6 cfu/kg body weight, *B. coagulans* MTCC 5856 showed significant decrease in faecal weight ($p<0.05$) which was similar with loperamide treated group ($p>0.05$) . Percentage inhibition for loperamide treated group was 36% while that of the highest dose of *B. coagulans* MTCC 5856 was 33%. At time-points beyond 4 h similar trend was not observed. This could be due to the fact that the contents in the GI tract would be negligible owing to 31 h (18 h fasting and + 13 h of study) of fasting. Hence, the faecal output has decreased even for the negative control. While in the positive control, as it is a small molecule and has a short half-life, the gut contents could have got excreted by 4 h. This is the reason for higher faecal weight in the positive control group at 12 h time-point when compared with the negative control group. Water consumption at the end of 12 h did not show any changes between the treated and the control animals. To the best of our knowledge, this is the first study to report the anti- diarrhoeal activity of *B. coagulans* MTCC 5856 in rodent model. Castor oil usually results in the release of ricinoleic acid and this causes a change in the integrity of the fluid and electrolyte balance in the mucosa of the gastrointestinal tract [14]. Castor oil induced diarrhoea is an established model for a few years now [15]. It is evident that the administration of castor oil (1 ml) to Wistar rats facilitated the onset of diarrhoea, decreased the weight of wet stools (Table 3). Water consumption was found to be similar in all the groups (Table 4) indicating that none of the animal groups had abnormal water intake during the study period. Agents demonstrating anti-diarrhoeal properties had been evaluated by

their ability to delay diarrhoeal latency, decreased weight of diarrhoeal faeces as well as decreased number of faeces [16]. From the results of Castor oil induced diarrhoea, the diarrhoea was not found throughout the period of observation in loperamide group, indicating that loperamide elicited protection against diarrhoeal features associated with castor oil. Oral consumption of probiotics has been reported to be effective in the prevention and treatment of various forms of diarrhoea such as acute infectious diarrhoea, antibiotic-associated diarrhoea, and diarrhoea-predominant irritable bowel syndrome in clinical studies[17]. The mechanisms for these beneficial effects of probiotics could be proposed as reduction in luminal pH, inhibition of bacterial adherence, production of trophic factors such as spermine and spermidine, secretion of some antibacterial compounds, bacteriocins, to remove pathogenic bacteria, enhancement of intestinal epithelial barrier function growth and stimulation of mucin synthesis as well as secretion [18]. However, the mechanism of action at the molecular level is not fully understood yet.

Effect of B. coagulans MTCC 5856 on GI motility

Atropine sulphate blocks the muscarinic receptors on the smooth muscle cells of gut mucosa and thereby inhibits the gastrointestinal (GI) motility. Activated charcoal was deactivated before use with 2% aqueous gum acacia to obtain 3% deactivated charcoal. In the GI motility study using a deactivated charcoal meal as a marker, the highest dose of *B. coagulans* MTCC 5856 (160×10^6 cfu/kg body weight) exhibited almost similar effects on the GI motility to that of a standard drug, atropine sulphate (Figure 1). However, it did not reach statistical significance when compared with normal control. Moreover, a decreased trend in inhibition of GI motility has been seen with increased dose of *B. coagulans* MTCC 5856 and its highest dose of 160×10^6 cfu/kg body weight exhibited the maximum beneficial effect. Though, all probiotics are expected to have beneficial effects as per its definition, only few of them have demonstrated efficacy in diarrhoea. Bausserman and Michail reported a 6-week double blind randomized placebo controlled (DBPC) study investigating the efficacy of *Lactobacillus* GG in 50 children (aged 6–20) at the Children's Medical Center Pediatric Gastroenterology outpatient clinic in Dayton, Ohio[19]. Sinn D.H., et al, also reported a 4-week DBPC study of 40 subjects who were randomized to receive either placebo or *L. acidophilus* SDC 2012, 2013 at a dose of 2×10^9 cfu in an equal ratio. The study concluded that there was insufficient scientific evidence to support the use of *L. acidophilus* in the treatment of diarrhoea predominant IBS[20]. A meta-analysis on 82 randomized clinical trials addressed the prevention of antibiotic associated diarrhoea (AAD) with probiotic. The authors concluded that the pooled evidence suggests probiotics are associated with a reduction in AAD[21]. Another meta-analysis on 8 studies concluded that probiotics may be efficacious in reducing diarrhoea duration and stool frequency during a diarrhoea episode[22]. Thus additional research is needed to understand the effect of probiotics as adjunct therapy for diarrhoea among children [22]. Another study by E. J. Videlock and F. Cremonini in their meta-analysis on 34 studies confirmed earlier results supporting the preventive effects of probiotics in AAD [23]. Clinical efficacy of *B. coagulans* has been shown in treatment of diarrhoea, dysbiosis and as adjunct therapy for rheumatoid arthritis [24]. A recent study reported that the treatment with *Bacillus* probiotics during antibiotic therapy significantly decreased the incidence of AAD related to the use of antibiotics [25]. Our results presented here are in line with a clinical study on *Bacillus coagulans* MTCC 5856 strain wherein the probiotic strain exhibited significant ameliorative effects on diarrhoea predominant Irritable bowel syndrome (IBS) patients when compared with placebo (Majeed et al., US Patent application number 14536701).

Table 1
Animal groups for diarrhoea study

Group	Group Details	No. of animals
1	Normal control	6
2	Diarrhoea induced but treated with loperamide (1 mg/kg) – standard control	6
3	Diarrhoea induced but left untreated – Negative control	6
4	Diarrhoea induced but was treated with *B. coagulans* MTCC 5856 (40×10^6 cfu/kg body) (Dose 1)	6
5	Diarrhoea induced but was treated with *B. coagulans* MTCC 5856 (80×10^6 cfu/kg body) (Dose 2)	6
6	Diarrhoea induced but was treated with *B. coagulans* MTCC 5856 (160×10^6 cfu/kg body) (Dose 3)	6

Table 2
Animal groups for gastrointestinal motility test

Group	Group Details	No. of animals
1	Normal control – gum acacia + charcoal meal	6
2	Atropine (1 mg/kg) – standard control + charcoal meal	6
3	*B. coagulans* MTCC 5856 (40×10^6 cfu/kg body) (Dose 1) + charcoal meal	6
4	*B. coagulans* MTCC 5856 (80×10^6 cfu/kg body) (Dose 2) + charcoal meal	6
5	*B. coagulans* MTCC 5856 (160×10^6 cfu/kg body) (Dose 3) + charcoal meal	6

Table 3
Faecal weight and percentage inhibition for anti-* diarrhoeal *study on Wistar rats

Animal groups	Weight of stool (gm)			Percentage Inhibition		
	4 h	8 h	12 h	4 h	8 h	12 h
1	0.85 ± 0.70	0.59 ± 0.51	0.32 ± 0.12	NA	NA	NA
2	2.94 ± 1.54*	0.91 ± 0.72	1.04 ± 1.11	35.92	7.91	-38.14
3	4.59 ± 0.90	0.99 ± 0.40	0.75 ± 0.77	0	0	0
4	3.78 ± 0.92	0.57 ± 0.64	0.68 ± 0.58	17.54	42.09	9.76
5	3.62 ± 0.70	0.39 ± 0.46	0.59 ± 0.72	21.03	60.27	21.29
6	3.1 ± 1.48*	0.75 ± 0.42	0.66 ± 0.78	32.51	23.91	11.97

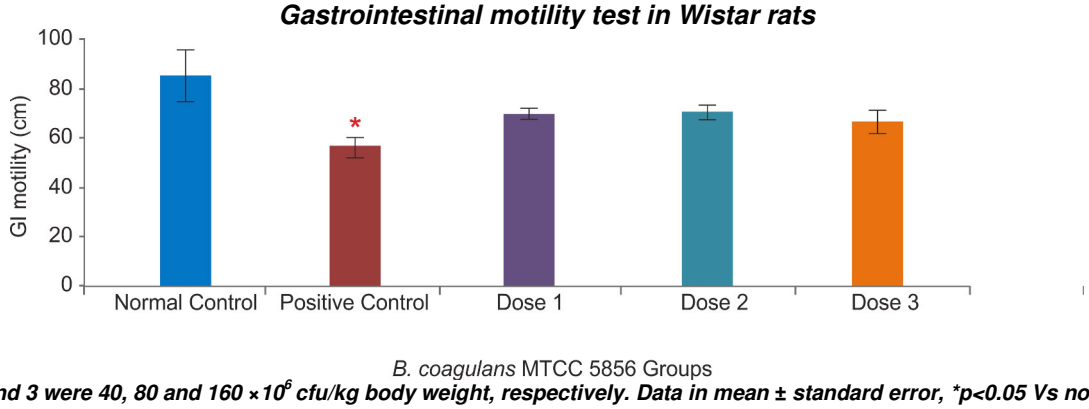
NA = Not Applicable, data in mean ± S.D.
**** Significantly lower than negative control (p<0.05)***

Table 4
Water consumption for anti-* diarrhoeal *study on Wistar rats

Animal groups	Dose	Water consumption (ml)
1	-	6.83 ± 2.79
2	1 mg/kg body weight	6.33 ± 1.75
3	-	6.50 ± 1.76
4	40 × 10^6 cfu/kg body weight	6.33 ± 1.86
5	80 × 10^6 cfu/kg body weight	6.17 ± 2.32
6	160 × 10^6 cfu/kg body weight	6.17 ± 2.04

mg = milligram, kg = kilogram, n = number of animals

Figure 1
Gastrointestinal motility test in Wistar rats



Dose 1, 2 and 3 were 40, 80 and 160 ×10^6 cfu/kg body weight, respectively. Data in mean ± standard error, *p<0.05 Vs normal control

CONCLUSION

In the efficacy study to assess the effect of *B. coagulans* MTCC 5856 in treatment of diarrhoea, it was found that *B. coagulans* MTCC 5856 treated animals showed decreased faecal output up to 4 h time point. Percentage inhibition of faecal output of the highest dose of *B. coagulans* MTCC 5856 (33%) was similar to positive control (36%). In the light of these findings, it can be concluded that *B. coagulans* MTCC 5856 was efficacious in the treatment of diarrhoea at a dose of 160 million cfu/kg body weight. The results of the study showed that the *B. coagulans* MTCC 5856 treated groups have a reduced gastrointestinal motility. Atropine sulphate group (positive control) decreased the GI motility by 34% while *B. coagulans* MTCC 5856 group at a dose of 160 × 10^6 cfu/kg body weight decreased GI motility by 22%. These experimental findings showed that *B. coagulans* MTCC 5856 possess significant anti- diarrhoeal activity and therefore it could be a potential source of anti-diarrhoeal agent in the future. However, the mechanism of action of *B. coagulans* MTCC 5856 against diarrhoea and GI motility needs to be further evaluated.

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