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## Research Article

### PREBIOTIC POTENTIAL OF STARCH OF *TINOSPORA CORDIFOLIA*, A COMPONENT OF SOMAVIT®: AN IN VITRO STUDY ON GROWTH MODULATION OF BIFIDOBACTERIUM AND LACTOBACILLUS STRAINS

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#### Keywords:

*Bifidobacterium bifidum*; Gut microbiota; *Lactobacillus plantarum*; Prebiotic; Somavit®; Starch of *Tinospora cordifolia*

#### ABSTRACT

Prebiotic is a substrate that is selectively utilized by host microorganisms conferring a health benefit. The present study aimed to investigate the prebiotic potential of starch of *Tinospora cordifolia* (5% w/v) based on in vitro growth stimulation of *Lactobacillus plantarum* and *Bifidobacterium bifidum*, using individual strains and consortia. Bacterial cultures were incubated under anaerobic conditions with starch of *Tinospora cordifolia* (test) and with distilled water as the control. At various contact time points (0, 1, 48 and 96 hours), small aliquots were sampled. The organisms were plated on specific growth media under anaerobic conditions for *Bifidobacterium* and microaerophilic for *Lactobacillus*, and total viable count was determined by standard plate count. Starch of *Tinospora cordifolia* promoted growth of both bacterial strains, singly and in consortia. The mean log colony forming units (CFU)  $\pm$  standard deviation of *Lactobacillus* in test was significantly higher in comparison to control ( $8.09 \pm 0.07$  vs  $7.06 \pm 0.03$ ,  $p < 0.05$ ) at 48 hours incubation, thereafter the growth of *Lactobacillus* plateaued through 96 hours. CFUs for *Bifidobacterium* in test were  $8.52 \pm 0.28$  at 48 hours and  $8.74 \pm 0.14$  at 96 hours incubation, both significantly higher ( $p < 0.005$ ) when compared to control. *Bifidobacterium* in test exhibited 90-fold relative increase in growth when compared to control. *Lactobacillus* being more robust showed an immediate increase in growth, while *Bifidobacterium* demonstrated a delayed but sustained growth, which extended over a period of time. These findings suggest that Starch of *Tinospora cordifolia* may enhance the gastrointestinal health of the host through modulation of overall composition of gut microbiota.

#### INTRODUCTION

The human gastrointestinal tract harbors a diverse range of resident microorganisms, termed gut microbiota, which play a crucial role in health and disease of the host by maintaining immune and metabolic homeostasis, integrity of mucosal gut barrier and protecting against pathogens.<sup>1-3</sup> Antibiotics, pathogens, dietary variations, exercise, hygiene practices, stress, depression, smoking, vaccinations, or several environmental factors can

disrupt or alter the gut microbiome composition, thereby influencing the dominance of an organism under a particular circumstance.<sup>2,4-6</sup> Altered gut bacterial composition (dysbiosis) has been associated with the pathogenesis of a variety of human diseases ranging from luminal diseases such as inflammatory bowel disease (Crohn's disease and ulcerative colitis), metabolic diseases such as diabetes and obesity, allergic diseases, chronic

inflammatory diseases like rheumatoid arthritis and psoriasis to neurodevelopmental illness.<sup>7-10</sup> The beneficial effects of the gut microbial genera, particularly *Bacteroides*, *Eubacterium*, *Ruminococcus*, *Bifidobacterium* and *Lactobacillus*, have been noted and modulation of gut microflora for growth of these beneficial microorganisms offers a promising therapeutic strategy in illness borne from the gut dysbiosis or “leaky gut”.<sup>11</sup>

Antibiotics are generally a choice of therapy for the treatment for gastrointestinal infections, however, increased antibiotic resistance and antibiotic-associated alteration in native gut microbiota are major concerns.<sup>12, 13</sup> Probiotics are live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.<sup>14</sup> Probiotics are bacterial supplements recommended for a variety of clinical reasons, including stimulating the immune system, protecting against cardiovascular or metabolic diseases, enhancing post-infectious health, and improving bowel function. Probiotics are effective only if they are in their viable forms and resistant to gastric acid, bile, and pancreatic enzymes.<sup>15</sup>

On the contrary, the concept of prebiotics is based on the strategy to improve health by restoring or favorably modulating one's own gut microbiota composition. According to the current International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel, prebiotic is defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit.<sup>16</sup> Prebiotics are non-digestible, non-absorbable, and resistant to gastric acids and hydrolytic enzymes. They are fermented by gut microbiota and cause selective proliferation of the beneficial bacteria that already exist in the gut hence offering the benefits they inadvertently do. The effective combinations of antibiotic or probiotic with prebiotic supplement can produce sustainable changes in gut microbiota and can benefit the human host.

About 40-90% of gut microorganisms are difficult to culture in the in vitro settings,<sup>17</sup> hence most of the in vitro studies related to the effects of prebiotics on the gut microbiota have been focused on the stimulation of *Bifidobacterium* and *Lactobacillus*.<sup>18-20</sup> Prebiotics are typically complex carbohydrates or dietary fibers, containing fructooligosaccharides, inulin and galactooligosaccharides, which encourage growth of these beneficial gut bacteria. These bacteria express carbohydrate-active enzymes, which facilitate fermentation of complex carbohydrates to generate

short chain fatty acids (SCFAs) such as acetate, propionate and butyrate, which help in regulating immune system and inflammatory responses, and maintaining gut mucosal integrity.

*Tinospora cordifolia*, an herbaceous vine (family: Menispermaceae) indigenous to the tropical areas of India, Myanmar and Sri Lanka, is widely used in ethnomedicine to treat various ailments related to immune-related diseases. An arabinogalactan is a highly branched, high molecular weight, purified polysaccharide isolated from stems of *Tinospora cordifolia*. Arabinogalactan serves as a non-digestible substrate for beneficial gut habitant bacteria assisting in their repopulation and is thus considered to be responsible for immunomodulatory activity.<sup>21,22</sup> However, these ethnopharmacological uses are mainly with limited or without scientific evidence. A polyherbal formulation, commercially available as Somavit® (Millennium Herbal Care Ltd.) contains starch of *Tinospora cordifolia* as its key ingredient, and is recommended for its prebiotic activity. Thus, the present study aimed to investigate the prebiotic effect of starch of *Tinospora cordifolia*, based on the potential to stimulate the growth of *Lactobacillus plantarum* and *Bifidobacterium bifidum* in an in vitro setting.

## MATERIAL AND METHODS

### Sample preparation

Test samples were prepared by weighing 0.75 gm of starch of *Tinospora cordifolia* and dispensing in 15 mL of differential reinforced clostridial medium (DRCM) broth to achieve 5% w/v concentration in each test tube, while control samples were prepared in a similar manner by replacing starch of *Tinospora cordifolia* with 0.75 mL of deionized distilled water.

### Bacterial strains and growth conditions

Two standard test organisms, *Lactobacillus plantarum* (American Type Culture Collection [ATCC] 8014) and *Bifidobacterium bifidum* (ATCC 11863) were selected as representative resident gut microflora. The *Lactobacillus* and *Bifidobacterium* cultures were maintained in glycerol stocks at -80°C and subcultured on specified growth agar medium. *Bifidobacterium* strain was grown on DRCM agar under anaerobic conditions at 37°C±1°C for 5 days and *Lactobacillus* strain was grown on de Man, Rogosa and Sharpe (MRS) agar 30°C±1°C for 48 hours under microaerophilic conditions. Cell suspensions of *Lactobacillus* and *Bifidobacterium* cultures were prepared to have a cell density of 1.0×10<sup>5</sup> to 3.0×10<sup>5</sup> colony forming units (CFU)/mL.

### Quantitative determination of growth in bacterial count: prebiotic effect

Three systems of each, test and control (Table 1), (all in triplicates) were prepared to evaluate the effect of 5%w/v starch of *Tinospora cordifolia* (test) on the individual bacterial strains of *Bifidobacterium* and *Lactobacillus*, and in consortia of both these strains. Bacterial suspension was added to the respective system, homogeneously mixed and the viable counts of the test cultures were immediately determined which represented the 0 hour counts. Thereafter, all the systems were incubated under anaerobic conditions at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for up to 96 hours and sampled at the above defined contact times.

Growth promotion by starch of *Tinospora cordifolia* was evaluated by determining the bacterial population using 10-fold serial dilutions and standard plate count method. At all contact times, including the 0 hour sample, 1 mL of inoculated sample from both test and control systems were analyzed by carrying out 10-fold serial dilution up to  $10^{-8}$  and plating each dilution on specific growth agar medium using spread plate technique. All the plates were incubated at conditions specified for optimum growth of test strains used in the study (Table 1), and bacterial colonies were enumerated (Figure 1).

#### Statistical analyses

Descriptive results were presented as mean  $\pm$  standard deviation (SD). Paired t-test was applied to the data using Microsoft office excel, the p-values  $<0.05$  were considered as significant.

#### Results

Starch of *Tinospora cordifolia* (5%w/v) showed greater than 1 log difference in mean CFU/mL of *Lactobacillus plantarum* and *Bifidobacterium bifidum* following 48 hours incubation. The mean log colony count  $\pm$  standard deviation (SD) of *Lactobacillus* was  $8.09 \pm 0.07$  in test, which was significantly higher ( $p < 0.05$ ) when compared to control ( $7.06 \pm 0.03$ ) at 48 hours, thereafter the growth of *Lactobacillus* plateaued in both the systems through 96 hours. The mean log colony count of *Bifidobacterium* in test was  $8.52 \pm 0.28$  at 48 hours and  $8.74 \pm 0.14$  at 96 hours, which were significantly higher ( $p < 0.005$ ) when compared to control. Furthermore, *Bifidobacterium* continued to grow in contact with starch of *Tinospora cordifolia* even after 96 hours of incubation (Figure 2).

Relative difference in bacterial populations between the control and the test system was calculated to demonstrate the effect of starch of *Tinospora cordifolia* on the growth of test strains at

a given contact time. *Bifidobacterium bifidum* contact with starch of *Tinospora cordifolia*, at the contact time of 48 hour exhibited 90 percent relative increase in growth when compared with control, while in the consortium system, *Lactobacillus plantarum* exhibited better growth. The relative growth of *Lactobacillus plantarum* at the end of 48 hour contact time with starch of *Tinospora cordifolia* was found to be 70 percent as compared to 26 percent in its control system (Figure 3).

#### DISCUSSION

Probiotics and prebiotics are extensively studied to understand their influence and role on the gut microbiota.<sup>23</sup> Probiotics colonize the human intestine transiently which necessitates frequent dosing to maintain biologically significant numbers of probiotic strains. Also, there are variations across the gut microbiome of the host that would either resist or facilitate the colonization intended by the probiotic.<sup>24</sup> The clinical outcome of probiotic varies from one person to another since the number of viable bacteria populating or colonizing the intestine depends on several factors other than dose; including type of probiotic formulation, co-administration of food or milk, and the host's gastric pH, intestinal motility, adherence to intestinal epithelium and prior composition of gut microbiome. This was revealed in studies where the fecal recovery and biopsy microbiome analyses of the host were not equivalent after administration of probiotic.

In contrast, prebiotics are non-digestible, non-absorbable substrates that serve as food for existing gut microbiome towards a sustained healthy composition. Although probiotics are recommended by clinicians in a wide range of diseases, a polyherbal prebiotic like Somavit® might provide a host-centric delivery approach. In the present study, starch of *Tinospora cordifolia*, the principle ingredient of Somavit® was studied for its role as a prebiotic. Majority of studies have focused on pure oligosaccharides, including inulin, fructooligosaccharides, galactooligosaccharides, xylooligosaccharides, starch etc. for their prebiotic potential.<sup>20, 25</sup> The prebiotic potential of blueberry, pomegranate, almond skin, and red grape extracts on *Lactobacillus* and *Bifidobacterium* species have been assessed.<sup>26-29</sup> Furthermore, a study has reported the prebiotic effects of *Tulsi*, Ginger, and Black pepper using cultures of *Lactobacillus rhamnosus* GG and *Bifidobacterium infantis*.<sup>30</sup>

In the current study, the prebiotic potential of starch of *Tinospora cordifolia* was studied based on its influence on the growth of

*Bifidobacterium bifidum* and *Lactobacillus plantarum*, singly and their consortia. The use of consortia of *Lactobacillus* and *Bifidobacterium* for assessment of prebiotic potential supports the symbiotic behavior of these cultures. The entire contact study was conducted under anaerobic conditions to mimic the growth conditions in their natural ecosystem. The viable growth was assessed under anaerobic conditions for *Bifidobacterium* and microaerophilic conditions for *Lactobacillus*. Furthermore, since the Ayurvedic preparation tends to sediment to the base of the liquid medium, the entire study was conducted in a controlled liquid interface to facilitate uniform contact of the prebiotic. The study explored comprehensively the impact of starch of *Tinospora cordifolia* on the response of bacterial population studied individually and in consortium for the period of 1hour, 48hours and 96hours, which aligns with the time required for invitro multiplication and visualization of growth outcome by the chosen bacterial strains and the maximum time period that the prebiotic may remain in the gut.

This study inferred that the beneficial roles of both these organisms possibly proceed in a deferred manner; *Lactobacillus* being more robust exhibited a rapid growth in bacterial population, while *Bifidobacterium* displayed a delayed growth. Growth of *Lactobacillus* promotes enhancement of *Bifidobacterium*, allowing it to sustain for a longer duration in the human gut. The distinctive observation was the increase in count of *Bifidobacterium* even after 96 hours of contact time. These observations indicated the sustenance of these beneficial bacterial populations, which may help in reducing the dosing frequency of the prebiotic. These results suggest that the growth modulation of these bacterial populations with starch of *Tinospora cordifolia* could offer the desired benefits over an extended period of time.

#### CONCLUSION

In conclusion, starch of *Tinospora cordifolia*, a key component of Somavit® demonstrated a considerable growth stimulatory activity by increasing the population of beneficial gut microorganisms, *Bifidobacterium bifidum* and *Lactobacillus plantarum* in vitro and thus, confirmed a high prebiotic potential for this polyherbal formulation. Furthermore, a sustained growth modulation offered could probably reduce the dosing frequency. However, in order to substantiate the present in vitro data, further clinical studies to establish the prebiotic effect of starch of *Tinospora cordifolia* are warranted.

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**Author contributions:** Gandhi HI conceptualized and formulated Somavit SGC. Koli NM had primary role in study design along with Desai NJ and Samant SL. Desai NJ and Samant SL were involved in conceptualizing, executing, summarizing and inferencing the research work.

All authors met ICMJE criteria and all those who fulfilled those criteria are listed as authors. All authors had access to the study data and made the final decision about where to publish these data and approved submission to this journal.

**Conflict of Interest:** No potential conflict of interest was reported by the authors

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**Table 1: Summary of different bacterial systems, their growth conditions and growth characteristics**

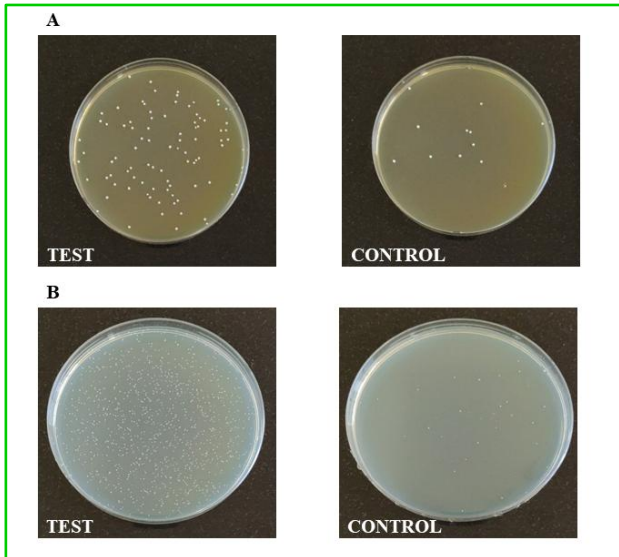
Set No	System	Growth condition	Colony characteristics
1	CONTROL- <i>Lactobacillus plantarum</i>	<i>Lactobacillus</i> MRS agar, 30°C ± 1°C, 48 hours, microaerophilic	
2	TEST- <i>Lactobacillus plantarum</i>	<i>Lactobacillus</i>	For <i>Lactobacillus plantarum</i> : Circular, convex, with entire edge smooth, and 1mm-2mm in size
3	CONTROL- <i>Bifidobacterium bifidum</i>	DRCM agar, 37°C ± 1°C, 5 days, anaerobically	
4	TEST- <i>Bifidobacterium bifidum</i>		
5 <sup>a</sup>	CONTROL-Consortium of <i>Lactobacillus plantarum</i> and <i>Bifidobacterium bifidum</i>	For <i>Lactobacillus plantarum</i> : MRS agar, 30°C ± 1°C, 48 hours, microaerophilic	For <i>Bifidobacterium bifidum</i> : Circular, flat, dull, and in different sizes
6 <sup>a</sup>	TEST-Consortium of <i>Lactobacillus plantarum</i> and <i>Bifidobacterium bifidum</i>	For <i>Bifidobacterium bifidum</i> : DRCM agar, 37°C ± 1°C, 5 days, anaerobically	

<sup>a</sup>Consortium systems were incubated in both the growth conditions separately for enumeration of *Lactobacillus* and *Bifidobacterium* colonies. TEST (containing 5% w/v starch of *Tinospora cordifolia*) and CONTROL (containing distilled water) systems were inoculated with cell suspensions of test strains of cell density of 1 x 10<sup>5</sup> to 3 x 10<sup>5</sup>. Abbreviations: MRS agar: de Man, Rogosa and Sharpe agar; DRCM: Differential reinforced clostridial medium.

### Figure legends

**Figure 1: Representative plates of growth of (A) *Lactobacillus plantarum* on MRS agar and (B) *Bifidobacterium bifidum* on DRCM agar at sampling point of 48 hour**

(A) observed in viable counts performed for Test and Control sets at 10-fold serial dilution of 1:100000 (1:10<sup>-5</sup>) after 48 hours of incubation under microaerophilic conditions; (B) observed in viable counts performed for Test and Control sets at 10-fold serial dilution of 1:10000 (1:10<sup>-4</sup>) after 5 days of incubation under anaerobic conditions. Abbreviations: MRS agar: de Man, Rogosa and Sharpe agar; DRCM: Differential reinforced clostridial medium



**Figure 2: Growth of bacterial strains (A) *Lactobacillus plantarum* (B) *Bifidobacterium bifidum*, expressed as average bacterial count in terms of Mean log<sub>10</sub> CFU/mL with increase in contact time with Test (5%w/v *Tinospora cordifolia*) and Control (5%w/v distilled water)**

Test: 5% w/v starch of *Tinospora cordifolia*; Control: 5% w/v Distilled water. Abbreviations: *B.bifidum*: *Bifidobacterium bifidum*; CFU: colony forming units; *L.plantarum*: *Lactobacillus plantarum*; SD: standard deviation

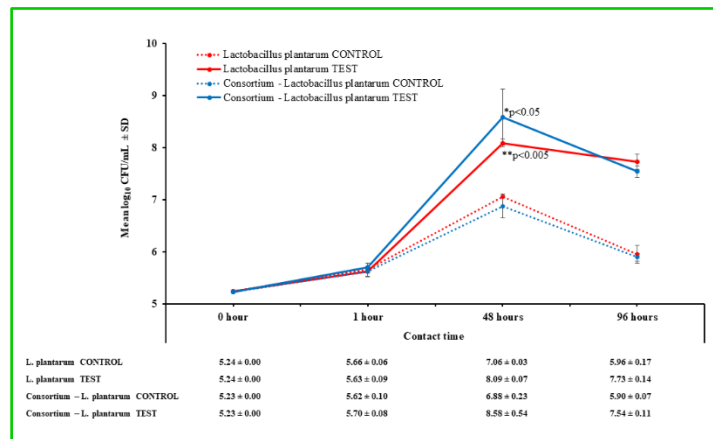


Figure 2A

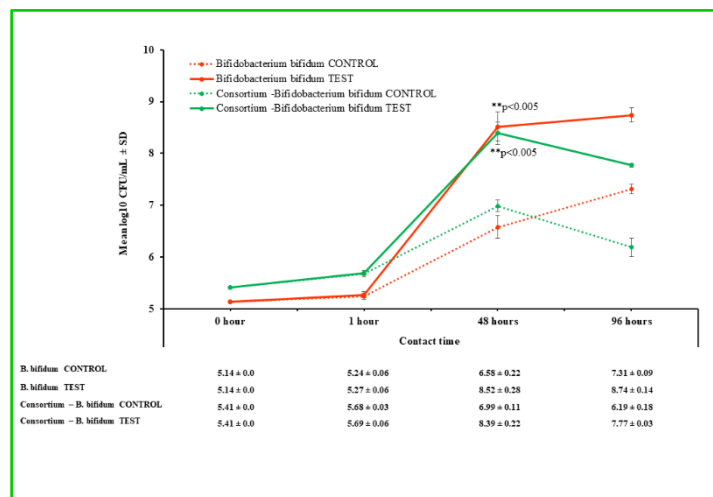
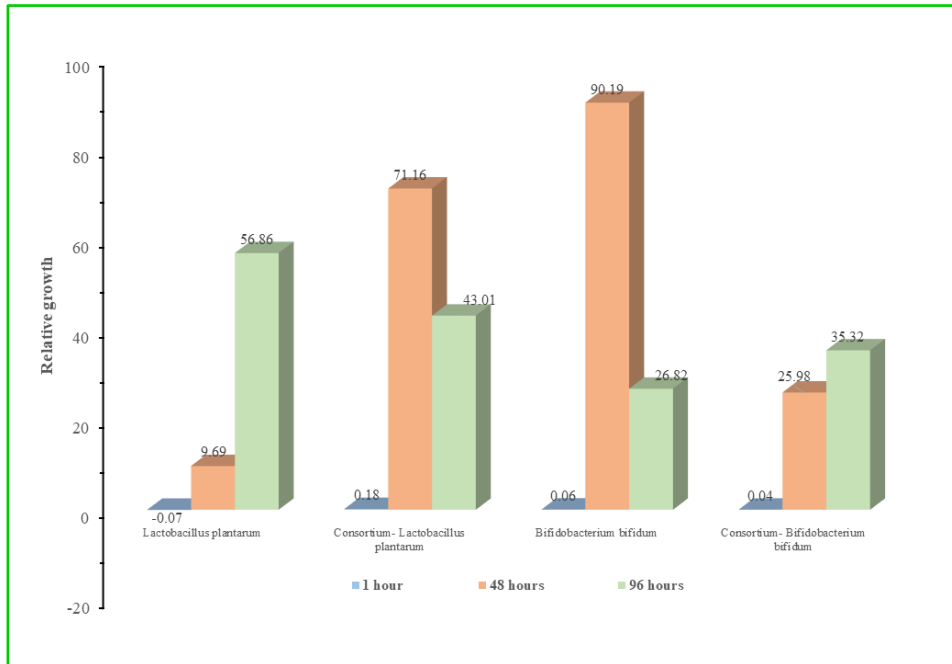


Figure 2B

**Figure 3: Relative percent increase in bacterial growth when in contact with the starch of *Tinospora cordifolia* as compared to control**



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