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Original Research Article

In vitro screening of indigenous plant materials for prebiotic potential

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A B S T R A C T

Prebiotics, typically the non-digestible carbohydrates, render physiological benefits to improve gut health. Present study was planned to screen 29 indigenous plant materials for their prebiotic potential. Standard fructooligosaccharide (FOS) was used as the positive control. Growth response of 9 lactic, 2 *Streptococci* and 2 marketed probiotic consortia A & B (*Probiotic acidophilus*, Nutrition Now, USA and Total Probiotics, Nutri-West, USA) on plant materials was studied. Consortium A, *L. casei* and *S. thermophilus*, *S. lactis*, *L. plantarum*, *L. acidophilus*, *L. rhamnosus* showed growth above 60% relative to FOS for all experimental materials. Bael, papaya, pomegranate, ber, tamarind, fig, guava, grapes, gum, apple, spinach and okra were identified first time for promising *in vitro* prebiotic potential with more than 60% growth of probiotics relative to FOS. Three formulations F1, F2 and F3 were confirmed as most promising prebiotic formulations through batch fermentation using human fecal slurry inoculum, with increased butyrate levels and increased growth of probiotics.

Introduction

The human gastrointestinal (GI) tract, principally the colon, is an intense site of microbial activity (Gibson, 2004). Nutritionists and clinicians have recognized that consumption of food ingredients (carbohydrates) that have a selective metabolism in the lower gut can have a major effect on the gut microbiota activities and in turn can influence the health of the host. In recent years, increasing attention has been focussed on the possible beneficial effects of prebiotics. Prebiotics have been proved for their effect in increasing absorption and bioavailability of calcium, magnesium, zinc and iron (Miyazato *et al.*, 2010). Prebiotics are also studied for immune stimulation (Peppelenbosch and Ferreira, 2009) property and in colonic cancer prevention (Wollowski *et al.*, 2001). In addition, a prebiotic may inhibit the growth of pathogens for overall beneficial health (Roberfroid, 2001).

Majority of the reported studies are on chicory, inulin, Jerusalem artichoke,

asparagus, salsify, leeks or garlic and onions, are of western origin (Yang *et al.*, 2012) and there is a need to tap the potential of locally available edible plant materials for the prebiotic activity. The literature suggests that majority of studies have focussed on pure oligosaccharides for their prebiotic potential. However, if the fruits and vegetables naturally enriched in oligosaccharides are used then other functional properties might be of benefit by using whole fruit approach. Moreover, fruits and vegetables have historically been considered as rich sources of some essential dietary micronutrients and fibres. Recently, they have been recognized as important sources for a wide array of phytochemicals that individually, or in combination, may benefit health (Rechkemmer, 2001) conferring the status of "functional foods."

Fruits and vegetables provide an assortment of protective nutrients, many of which provide antioxidant or disease-fighting effects. Evidence indicates that for the effect of fruit and vegetable consumption on health from the whole fruit may be more than the sum of the parts; individual components appear to act synergistically where the influence of at least some of these is additive (Yahia, 2009). Fruits and vegetables can be easily supplemented and provided through diet as prebiotic foods. Indian fruits and vegetables need to be given attention for development of cheaper prebiotic functional food supplement. The view taken in the *in vitro* fermentation study using human fecal inoculum is that, whilst it is not feasible to fully characterize the changes occurring in the colonic microflora, it is possible to monitor the populations of selected species believed to be indicative of the state of health of the colon. In light of these facts, present study was planned to assess prebiotic potential of various indigenous plant materials through *in vitro* batch fermentation. Therefore, this study was aimed to assess indigenous plant materials for their prebiotic potential. The objectives were

1) To assess indigenous plant materials for their prebiotic potential
2) To study probiotic potential and growth response of 9 lactic, 2 *Streptococci* cultures and their consortia and find their synbiotic combinations
3) To study the effect of drying of plant materials on prebiotic activity
4) To study batch fermentation of formulations using human fecal flora.

Materials and Methods

Pepsin, pancreatin, bile salts, sodium bicarbonate, MRS broth and its ingredients were purchased from HiMedia laboratories. The standard 11 cultures (Table 1) were purchased from NCIM (National Collection of Industrial Micro-organisms), Pune and two marketed consortia A and B were purchased from USA. Fructooligosaccharide (FOS) (NutraFlora, GNC, USA) was over the counter marketed formulation. Three glass vessels (500 ml) with three neck inlets and one outlet were fabricated procured from glass fabricator. Peristaltic pump, autoclavable pH electrodes (Innovative Analyticals Ltd, Pune) were used for development of chemostat model.

Composition of MRS medium, without glucose, was (g/L): Peptone (10), Agar (10), Beef extract (8), Sodium acetate 3(H_2O) (5), Yeast extract (4), K_2HPO_4 (2), Tri ammonium citrate (2), $MgSO_4.7H_2O$ (0.2), $MnSO_4.4H_2O$ (0.05), Sorbiton monooleate (1ml), pH - 6.2 ± 0.2.

For batch fermentation of three formulations, feces of healthy infant were obtained. Anaerobic dilution medium (pH

6.5) replacing glucose, serum bottles, sterile syringes were used for experiment. All media were obtained from HiMedia. Nutrient agar was used for total aerobes, whereas Wilkins-Chalgren agar was used for total anaerobes. Eosin methylene blue agar was used for *E. coli*, tryptose sulfite cycloserine agar for *Clostridia*, bifidobacteria broth for total *Bifidobacteria*, mannitol salt agar for *Staphylococci* and de Man, Rogosa, Sharpe agar for total *Lactobacilli*. 1:1 O- Phosphoric acid was used for acidification of samples for VFA analysis.

Characterization of probiotic nature of NCIM cultures

All the 11 organisms were initially assessed for their probiotic properties using physiological approach of GI digestion treatment.

pH and bile salt tolerance and resistance to digestive enzymes

Bacteria were grown in MRS broth (HiMedia) at 37^{o}C in duplicate. Exponentially growing cells were harvested by centrifugation at 12000 x g for 10 min, washed twice with sterile saline (0.85% NaCl, W/V pH 7.0) and resuspended in saline. Sterile saline of pH 2.0 and pH 7.6 were prepared. Pepsin (0.1g/ml) in 0.1M HCl was added to saline of pH 2.0. Bile pancreatin (2.5g bile salts + 0.4 g pancreatin in 100 ml of 0.1 M $NaHCO_3$) was added in saline of pH 7.6.

Individual pellet of organism was then inoculated in the saline of pH 2.0 and pH 7.6, respectively. Tubes were incubated for 2h. After 2h, pellets were harvested by centrifugation. Samples of 0h and 2h were taken and analyzed for enumeration of the organisms using total viable count.

Antimicrobial activity

Antimicrobial activity was tested using agar well diffusion method. Cell free supernatant was used to analyse the antimicrobial activity against common pathogens like *E. coli*, *S. aureus* and *S. typhi*. These three cultures were obtained through Bharati Medical College, Katraj, Pune, India.

Collection and processing of materials

Indigenous plant materials from local market of Pune were chosen depending on their hydrocolloidal and mucilaginous properties (Table 2). Materials (M1-M31) were grouped as fruits, vegetables and others which included roots, plant exudates like gums and flowers etc. These were dried in oven and stored as fine powder at -20^{o}C. Moisture contents of all materials were determined simultaneously till constant weight to ensure complete drying.

In vitro GI digestion treatment for enrichment of prebiotic content

All the materials were given GI digestion treatment, where other components like proteins and sugars are removed due to the action of pepsin and pancreatin (mixture of amylase, lipase and protease). Free sugars were removed by continuous ethanol washings.

Simulated GI digestion protocol was followed according to modified method of Agte *et al.* (1995). Dried, powdered material (5%) was added to distilled water and 2 ml pepsin (pH 2.0) and kept in shaker incubator for 2h at 37^{o}C. After peptic digestion, 10 ml bile- pancreatin solution (pH 7.6) was added, incubated for 2h, solution was filtered and residue was collected, dried and used as undigested residue of material. These powders were treated with ethanol till

the sample was completely free of sugar. This undigested residue was dried and used for assessment of prebiotic potential.

Assessment of in vitro prebiotic potential

Prebiotic potential was assessed for each of the sample in fresh, non treated dried and undigested dried states. The assay was done as per the protocol reported in our previous study (Agte *et al.,* 2010). Glucose from MRS medium was replaced by 20 mg of respective residue of sample material as the only source of carbohydrate. FOS and MRS medium containing respective samples were individually inoculated with 100 µl of 24h old individual culture of 0.4 optical density (O.D.) and were incubated under partially anaerobic conditions at 37^{o}C. O.D. at 600 nm was recorded at 0, 24 and 48h of inoculation as a measure of growth using spectrophotometer (Spectronic 21, Bausch and Lomb, UK). Growth of individual organisms in MRS containing FOS alone was taken as positive control and compared with the growth in presence of samples. Growth response for all the probiotics was measured in triplicate for all the individual materials and expressed as % FOS.

Formulation development of shortlisted plant materials

The materials shortlisted from screening study were used for formulation development. The materials were mixed in various proportions for development of twelve formulations. These formulations were tested for *in vitro* prebiotic potential using above mentioned protocol where glucose was replaced with the respective formulations (0.4% W/V). Growth performance of three promising organisms such as consortium A (Probiotic acidophilus, USA), L10 and L8 was tested in presence of twelve formulations.

Effect of prebiotics on relative growth of probiotics using batch culture

In vitro fermentation of the three formulations (F1, F2 and F3) was studied using human fecal inoculums in single stage chemostat model. The used method was similar to that of Lehmann *et al.* (2002) with the modification of using anaerobic dilution medium according to Ranade and Gadre (1988) for preparing the fecal slurry. Considering the end use of prebiotic formula for infants, feces of healthy infant were taken for the experiment. Fresh feces of healthy infants (3) were taken and mixed with pre-reduced anaerobic dilution medium (pH 6.5) while gassed with nitrogen to obtain a 5% fecal suspension. The serum bottles were prepared with anaerobic dilution medium under N_2 flushing, sealed and autoclaved at 15 psi for 20 min. The bottles were kept ready for serial dilution of fecal slurry with sterile anaerobic dilution medium.

For the fermentation, sample formulation at concentration of 10mg/ml was inoculated with 5% fecal slurry using sterile syringe and incubated at 37°C in a shaking water bath (180 strokes/ min). Samples were taken at 0h and 24h for enumeration of microorganisms and for volatile fatty acid (VFA) analysis samples were taken at 0, 3, 6, 21, 24, and 48h.

Plates of *Lactobacilli* were incubated anaerobically at 37°C for 48 h in anaerobic jars. Plates for the enumeration of total aerobes, *E. coli* and *Staphylococci* were incubated at 37°C for 48 h in controlled aerobic incubators (Liong and Shah, 2006). For VFA analysis, samples were acidified with 1:1 o-phosphoric acid and analysis was done using gas chromatography.

Statistical analysis

All the observations were studied in triplicates and data were summarized as mean values and standard deviations. One-way and two-way ANOVA, critical differences, confidence interval, % coefficient of variation (CV), student t test and paired t test were applied to the data using Microsoft Office Excel 2007. The p values $p<0.05$ were considered as significant.

Results and Discussion

Prebiotics are short-chain carbohydrates that alter the composition or metabolism of the gut microbiota in a beneficial manner and help to improve health. Since prebiotics also imply carbohydrate residues that escape digestion, 29 experimental plant materials were given to a simulated GI digestion treatment and were assessed for *in vitro* prebiotic potential. Growth of probiotics using experimental plant materials as the only source of carbohydrate was considered as the first biomarker for determination of prebiotic potential.

Probiotic properties of lactic and *Streptococci* cultures

All the 11 organisms were initially assessed for their probiotic properties based on the criteria like tolerance to low pH and bile salt, resistance to digestive enzymes and antimicrobial activity *E. coli*, *S. aureus*, and *S. typhi* (Table 1 and Fig. 1). Among 11 cultures, *L. acidophilus* 2660 followed by *L. bulgaricus*, *L. plantarum*, *L. helveticus* and *L. acidophilus* 2902 showed good pH and pepsin tolerance as compared to other organisms. *L. delbrueckii* subsp *delbrueckii* showed best tolerance to bile salt and pancreatin treatment. *L. acidophilus* 2660, *L. helveticus* followed by *L. plantarum*, *L. bulgaricus* and *L. fermentum* also showed good tolerance to bile and pancreatin treatment.

S. thermophilus and *S. lactis* showed best antimicrobial activity against all three pathogens like *E. coli*, *S. aureus* and *S. typhi. L. casei var. rhamnosus* and *L. fermentum* also showed antimicrobial activity against *S. typhi. L. acidophilus* 2660 followed by *L. bulgaricus, L. helveticus, L. delbrueckii* subsp *delbrueckii*, *L. plantarum, L. fermentum, L. acidophilus* 2902 and *L. casei var. rhamnosus* showed good antimicrobial activity against *S. aureus. L. helveticus* followed by *L. plantarum, L. bulgaricus, L. fermentum, L. delbrueckii subsp delbrueckii*, *L. acidophilus* 2660, *L. casei var. rhamnosus* and *L. acidophilus* 2902 showed good antimicrobial activity against *E. coli*.

Hence, the criteria of tolerance to pH, bile salt and digestive enzymes along with antimicrobial activity satisfy probiotic nature of the lactic and *Streptococci* cultures under study.

Standardisation of prebiotic activity protocol

FOS was used as standard prebiotic source and growth of all the experimental materials was expressed as average % growth with respect to FOS. It was felt essential to assess the variability of the growth for FOS during various sets of experiment. One way ANOVA indicated no significant differences between various sets of FOS (N=10) suggesting repeatability of results of FOS and the assay conditions ($F=0.86$, $p>0.1$). Secondly, % CV was observed to be 21 % in all the sets of FOS. Considering the mean ± 2S.D. for FOS and the confidence interval (56%-143%), a growth corresponding to 60% of FOS was considered as cut off for

promising prebiotic response for screening the plant materials.

Prebiotic potential of fruits (undigested dry)

Between 13 different fruits, when expressed as % FOS, a wide range of 25–119% of growth was observed. One way ANOVA showed significant differences ($p<0.001$) between the fruits. It was interesting to find that, amongst 13 fruits, 11 (M8 (pomegranate), M7 (papaya), M24, M1, M28, M5, M2, M9, M3, M25, M4) showed promising prebiotic potential (Fig. 2).

Prebiotic potential of vegetables (undigested dry)

In case of vegetables (5), prebiotic potential range was found to be lower than that for fruits (43–66%). One way ANOVA for vegetables showed significant differences ($p<0.0001$) in the growth potential as compared to FOS. Only M12 and M6 showed promising growth with respect to FOS (Fig. 2).

Prebiotic potential of other materials (undigested dry)

Amongst other materials (10), range of growth response was found to be 55–78%. Materials M29, M16, M23, M27, M18 and M17 showed more than 60% prebiotic potential (Fig. 2), with respect to FOS. Additionally, one way ANOVA indicated significant differences ($p<0.0001$) in the growth response of these 'other materials' as compared to FOS.

Average growth of probiotic cultures for synbiosis

The growth responses of individual cultures showed considerable variability. When the entire data was examined for the growth of particular probiotics, average growth of 13 probiotics showed a range from 40–109%. However, consortium A, *L. casei* (L8), *S. thermophilus* (L10), *S. lactis* (L11), *L. plantarum* (L4), *L. acidophilus* (L1) and *L. rhamnosus* (L5) showed promising growth i.e. above 60% for all experimental materials (Fig. 3). One way ANOVA showed no significant difference between 6 organisms indicating that they were equally promising for use in further experiments. Also, materials M8 and M7 showed higher growth response as compared to FOS to all the organisms (Fig. 2) suggesting that combinations of M8 and M7 with these 6 organisms might be beneficial as synbiotics.

Effect of drying on prebiotic potential

The prebiotic assay was done by the same protocol by using fresh and non treated dry samples of same materials (n=12) representing same amount of dry matter. Figure 4 indicates increase in the prebiotic potential in dry M1 (10%), M8 (35%) and M13 (8%) than fresh whereas materials like M2 (5%), M3 (7%), M4 (47%), M5 (20%), M6 (17%), M9 (11%), M10 (31%), M11 (10%) and M12 (6%) showed decrease in the prebiotic potential of dry as compared to fresh material. But, paired t test assuming unequal variances showed no significant difference in prebiotic potential between fresh and dry materials. The results overall, suggested that there was no significant difference in prebiotic potential due to drying.

Formulation development of shortlisted plant materials

ANOVA (two-factor without replication) showed significant differences between organisms and between materials ($F = 11.41$, 17.44, $p< 0.001$) for % growth response

with respect to FOS. Consortium A, L10 and L8 showed promising probiotic potential for all experimental materials. M7, M8, M13, M14 and M28 exhibited growth response higher than FOS. Finally, pooled statistical analysis was done for ranking and critical difference was calculated. The materials with first 6 ranks were selected for formulation development.

In vitro prebiotic potential of twelve formulations

Formulations I, L and K showed promising prebiotic potential (Fig. 5). ANOVA (two-factor with replication) showed significant differences between organisms and between formulations ($F = 23.31, 80.46$, $p < 0.001$) for % growth response with respect to FOS. These promising formulations were designated randomly as F1, F2 and F3.

In vitro fermentation of formulations with human fecal flora

Changes in human fecal bacterial populations present in the batch cultures are summarized in Table 3. All the formulations F1 ($p=0.01$), F2 ($p=0.0005$) and F3 ($p=0.001$) showed significant increase in the total lactobacilli count after fermentation up to 24h. The *E.coli* population after fermentation with F1 ($p=0.04$), F2 ($p=0.01$) and F3 ($p=0.003$) showed significant decrease after 24h. Similarly, significant decreases were seen in total aerobes numbers with F1 ($p=0.0001$), F2 ($p=0.0001$) and F3 ($p=0.0001$). Total staphylococci were found to be significantly decreased after fermentation with F1 ($p=0.01$) and F2 ($p=0.04$) while F3 showed non-significant reduction after 24h.

Results of VFA analysis (ppm) indicated that, n butyric acid was formed only at 48h and F1 followed by F2 showed highest production. F1 (862.69±3.54) followed by F2 (771.08±13.47, $p<0.01$) showed significant increase in n-butyrate levels as compared to F3 (292.14±9.36, $p<0.001$). Levels of acetic acid also showed similar trend as F1 (3037±6.02) followed by F2 (2173±20.4, $p<0.01$) showed significant increase compared to F3 (1963±2.0, $p<0.001$). In case of i-butyrate, F1 (1531±16.2) followed by F3 (1438±3.8, $p<0.001$) showed significant increase as compared to F2 (1270±4.6, $p<0.01$). Increased levels of VFA indicated the fermentation of the respective formulations and hence utilization of the substrate. As butyrate is involved in immune functions, increased levels will be used as marker to identify the effectiveness of formulations in immunological disorders.

A prebiotic is 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefit upon host well-being and health' (Gibson *et al.*, 2004). According to Licht *et al.* (2012), consumption of prebiotics is now considered as a prevention mechanism for reducing diet related complications. Thus, prebiotics are also getting further attention for research in identification of new plant materials, their health beneficial effects and use for product development.

Though, inulin and FOS are available in market, their cost is not affordable to the common man. While majority of fruits and vegetables reported for prebiotic activity are of western origin, Indian plant materials are underexploited for this particular property. As a regular dietary source, it makes sense to search for prebiotics potential from fruits or vegetables and the promising ones can be supplemented through diet. Prebiotics based on locally available plant sources will also be affordable. This was the mindset behind

taking up the present work.

Manderson *et al.* (2005) and Mandalari *et al.* (2007) have used the approach of extraction of the oligosaccharides. However, the physiological approach of *in vitro* GI digestion treatment was used in the present study for simulation of the human system. During *in vitro* GI digestion treatment, enzymes digest the sugars and the end products released are free of digestible ingredients. These end products are not available to the probiotics. Thus the residue enriched with the non digestible oligosaccharides is provided to probiotics as the only source of carbohydrate. This approach being physiological is felt to give better estimate of the probiotic growth by utilisation of specific prebiotics. This aspect of *in vitro* GI digestion also satisfies the first criteria of non digestibility of the prebiotic materials. Ting and DeCosta (2009) supports our result that standard lactobacilli cultures showed resistance to acidic conditions. All the lactic, streptococci cultures under study have shown antimicrobial activity against *E. coli* and *S. aureus*, whereas, L5, L9, L10 and L11 only showed antimicrobial activity against *S. typhi*. According to criteria summarized by Dunne *et al.* (2001), the cultures under study have shown resistance to gastric acidity, bile toxicity, resistance to digestive enzymes and antimicrobial activity against common gut pathogens. Hence these results are supported by Dunne *et al.* (2001), satisfying the criteria of probiotic.

Table.1 Study of probiotic property of study organisms (tolerance of pH and digestive enzymes)

Organisms	Code	NCIM No.	Properties studied	
			Tolerance to gastric conditions (pH 2.0* & Pepsin*)	Tolerance to intestinal conditions (bile salts* & Pancreatin*)
L. acidophilus	L1	2902	++	+
L. delbruckeii	L2	2025	+	++++
L. helveticus	L3	2733	++	+++
L. plantarum	L4	2084	++	++
L. rhamnosus	L5	2364	+	+
L. acidophilus	L6	2660	+++	+++
L. bulgaricus	L7	2056	++	++
L. casei	L8	2651		
L. fermentum	L9	2165	+	++
S. thermophilus	L10	2904	+	+
S. lactis	L11	2114	+	+
Consortium A	CA	Probiotic acidophilus, Nutrition Now, Inc. USA		
Consortium B	CB	Total probiotics, Nutri-west, Douglas		

*Pancreatin (Mixture of amylase, lipases and proteases);
%TVC/ml : +: 0-25, ++:26-50, +++: 51-75, ++++: 76-100

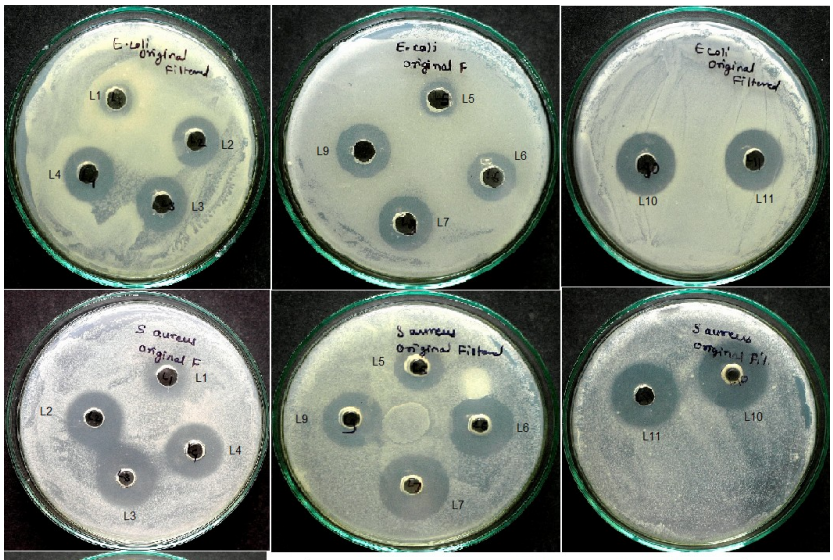
Table.2 Plant materials used in this study

Code	Common Name	Scientific Name	Family
M1	Ber	*Zizipus jujuba Lam.*	*Rhamnaceae*
M2	Fig (Anjir)	*Ficus carica Linn.*	*Moraceae*
M3	Gooseberry	*Emblica officinalis Gertan.*	*Euphorbiaceae*
M4	Guava	*Psidum guajava Linn.*	*Myrtaceae*
M5	Grapes	*Vitis venifera Linn.*	*Vitaceae*
M6	Okra	*Hibiscus esculantus Linn.*	*Malvaceae*
M7	Papaya	*Carica papaya Linn.*	*Caricaceae*
M8	Pomegranate	*Punica granatum Linn.*	*Punicaceae*
M9	Red tamarind	*Tamarindus indica Linn.*	*Leguminosae*
M10	Mint	*Mentha spicata Linn.*	*Labiatae*
M11	Safflower leaves	*Carthamus tinctorius Linn.*	*Compositae*
M12	Spinach	*Spinacia oleracea Linn.*	*Chenopodiaceae*
M13	Custard apple	*Annona squamosa Linn.*	*Annonaceae*
M14	Chikoo	*Achras sapota Linn.*	*Sapotaceae*
M15	Arvi (Colocasia)	*Arum curvatum Roxb.*	*Araceae*
M16	Sweet potato	*Ipomoea batatas Lam.*	*Convolvulaceae*
M17	Yam (Suran)	*Amorphophalus companulatus Roxb.*	*Araceae*
M18	Bhatkanda(edible tuber)	*Manihot esculanta Crantz.*	*Euphorbiaceae*
M19	Purple Yam	*Dioscorea bulbifera Linn.*	*Dioscoreaceae*
M22	Drumstick Gum	*Moringa olifera Linn.*	*Moringaceae*
M23	Kathilya gum	*Acacia senegal Linn.*	*Fabaceae*
M24	Bael fruit	*Aegel marmelos Corr.*	*Rutaceae*
M25	Cluster fig	*Ficus racimosa Linn.*	*Moraceae*
M26	Ivy Gourd	*Coccinia indica W. & A.*	*Cucurbitaceae*
M27	Water-Chestnut	*Trapa bispinosa Roxb.*	*Onagraceae*
M28	Apple fruit	*Malus sylvestris Mill.*	*Rosaceae*
M29	Idlimbu (Citron)	*Citrus aurantium*	*Rutaceae*
M31	Mahua flowers	*Madhuca latifolia Linn.*	*Sapotaceae*

Table.3 Changes in human fecal bacterial populations present in the batch cultures

Formulations	Time	Log10 CFU/ml			
		E. coli	Staphylococci	Lactobacilli	Total aerobes
F1	0h	9.195±0.10	8.624±0.06	8.975±0.01	10.311±0.03
	24h	9.012±0.02*	8.476±0.03**	9.159±0.08*	9.851±0.01***
F2	0h	9.163±0.04	8.608±0.08	9.067±0.04	10.238±0.04
	24h	9.024±0.04**	8.422±0.08*	9.282±0.01***	9.851±0.03***
F3	0h	9.183±0.06	8.591±0.1	9.024±0.02	10.276±0.04
	24h	8.931±0.05*	8.387±0.08ns	9.225±0.04**	9.851±0.02***
*: p<0.01, **: p<0.001, ***: p<0.0001, ns: non-significant					

Fig. 1 Antimicrobial activity of experimental cultures



	Zone of cell free supernatant (cm)		
	E. coli	*S. aureus*	*S. typhi*
L1	0.5	0.8	No zone
L2	1.0	1.3	No zone
L3	1.2	1.5	No zone
L4	1.2	1.3	No zone
L5	0.6	0.8	0.8
L6	1.0	1.7	No zone
L7	1.1	1.7	No zone
L9	1.1	1.2	1.3
L10	1.4	1.8	1.3
L11	1.4	1.7	1.4

Fig. 2 Relative growth response of plant materials to probiotics (mean ± S.D. Average of growth response by materials for 13 organisms)

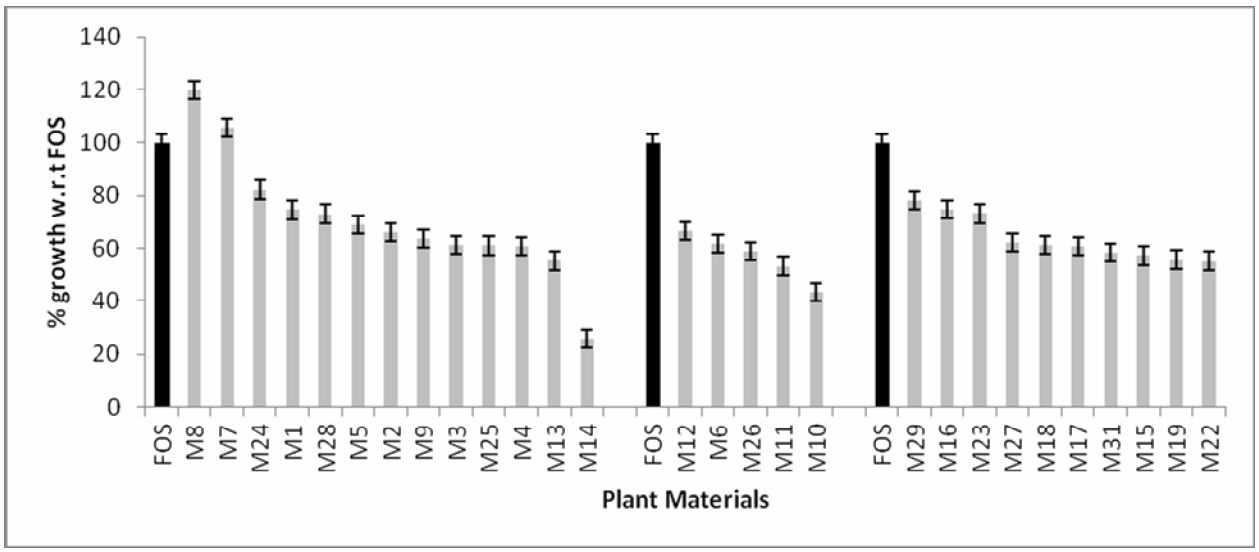


Fig. 3 Growth of probiotics to plant materials with respect to FOS (mean ± S.D. Average of growth response by 29 materials)

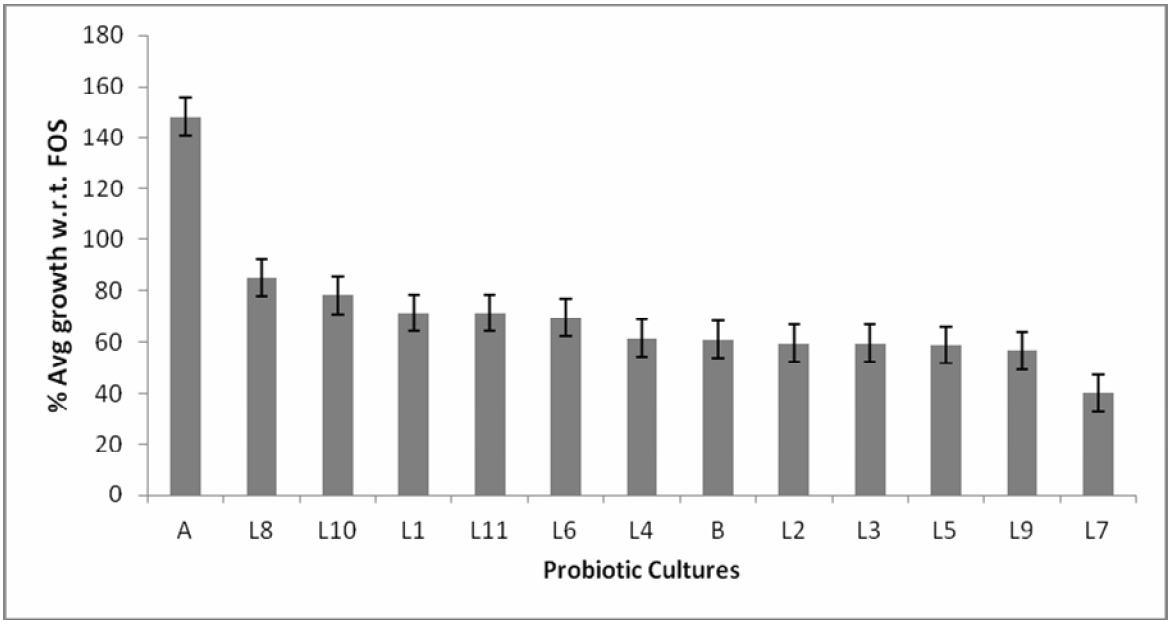


Fig.4 Prebiotic potential of dry vs. fresh material

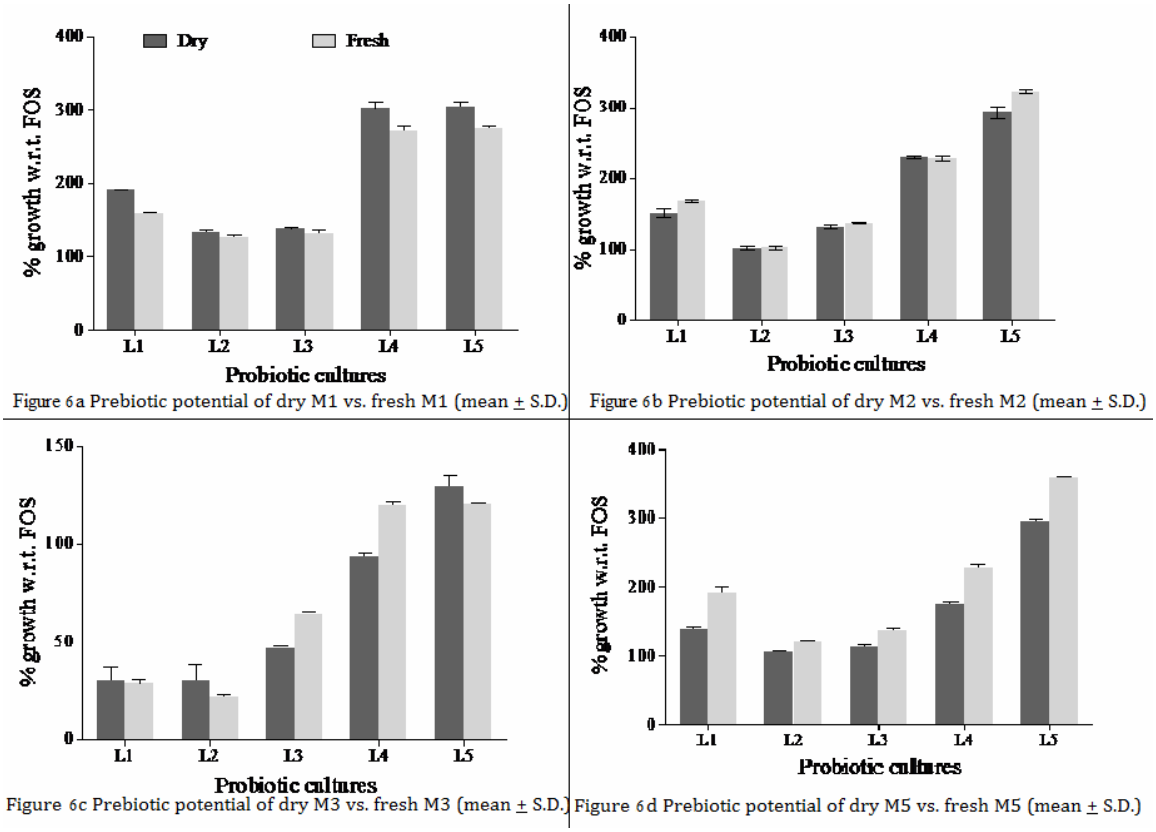


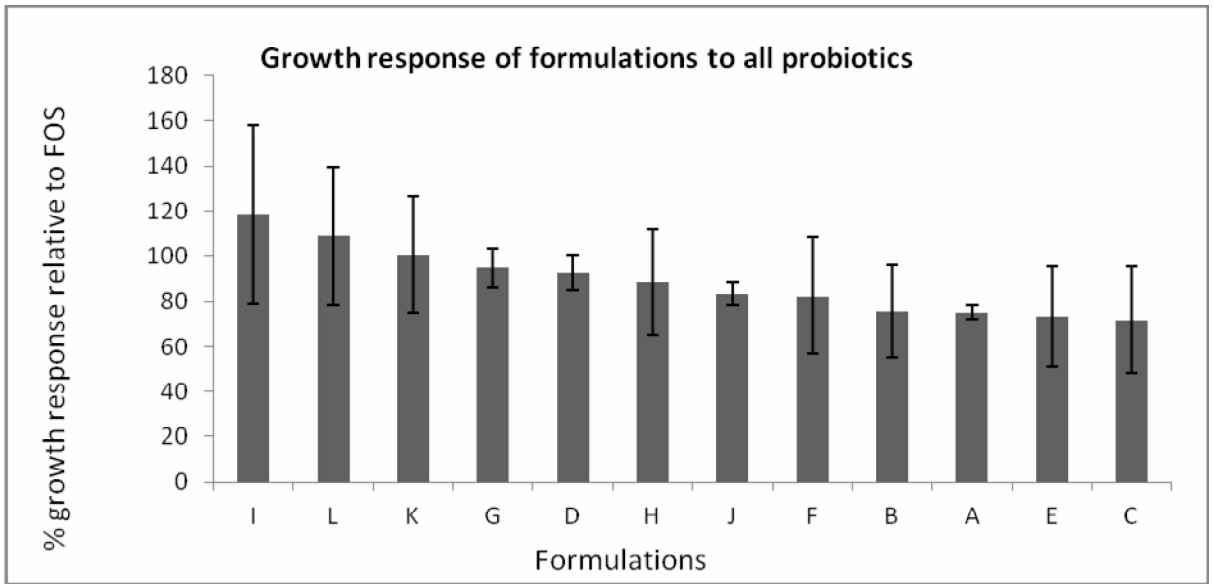
Figure 6a Prebiotic potential of dry M1 vs. fresh M1 (mean ± S.D.)

Figure 6b Prebiotic potential of dry M2 vs. fresh M2 (mean ± S.D.)

Figure 6c Prebiotic potential of dry M3 vs. fresh M3 (mean ± S.D.)

Figure 6d Prebiotic potential of dry M5 vs. fresh M5 (mean ± S.D.)

Fig. 5 *In vitro* prebiotic potential of formulations (mean ± S.D.)



Present study incorporated the use of consortia of various lactic and *Bifidobacterium* cultures for assessment of prebiotic potential of plant materials. This factor supports the synbiotic behaviour of these cultures and simulates the physiological conditions more closely. In present study, *in vitro* fermentation using various experimental plant materials has shown the significant increase in the growth of *lactobacillus* and the consortia having *Bifidobacteria* and *lactobacillus* significantly but to a variable extent. This indicated selective fermentability of the experimental materials where the second criteria (Roberfroid, 2001) for prebiotic is satisfied. It is more likely that these effects will require the introduction of a consortium of strains, such as those observed in our studies (e.g. *Lactobacilli* and *Bifidobacteria*). This result is supported by Dunne *et al.* (2001). Results of Liong and Shah (2006) also support our study where *L. casei* was found to have highest growth response for variety of the experimental plant materials. Previous report of Modler *et al.* (2005) supports finding of present study that there was no significant difference in the prebiotic activity due to drying and storage at -20^{o}C, indicating maintenance of prebiotic activity of the materials.

The results of the present study for changes in the microflora are in agreement with Manderson *et al.* (2005) and Mandalari *et al.* (2007). Manderson *et al.* (2005) reported that orange peel POS showed increase in *Lactobacilli* population later in the fermentation than did FOS, suggesting that the orange peel POS produced a more sustained prebiotic fermentation. Present study suggests that the fermentation of formulation F1 showed significant reduction for *E. coli*, *Staphylococci* and total aerobes. The changes in the number of *Lactobacilli* showed significant increase. Similarly formulations F2 and F3 found effective in terms of increasing the *Lactobacilli* number significantly. Simultaneous decrease for all other groups was also found significant for both the formulations. Finally, increase in butyrate levels suggests important role of formulations in the immunological disorders. Formulation F1 and F2 followed by F3 showed significant increase in the n-butyrate levels after 48 h fermentation.

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