Banana flour as a substrate for the development of probiotic health foods

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Abstract
A staple based food mixture if developed from the commonly used foods in a community and then fermented with probiotic organism will have a better profile of nutrients, acceptability and therapeutic value. The present study was undertaken to develop a banana based probiotic fermented food mixture involving *Lactobacillus acidophilus*. The total viable count of *L. acidophilus* in the products was maximized while variables like substrate concentration, quantity of the inoculum, time of incubation, pH and temperature were kept at acceptable levels. Each developed food mixture (25g) was mixed with 150 ml water and stirred to obtain uniform slurry, the pH adjusted to 4.5 and autoclaved at 121°C (1.5 kg cm^{-2}) for 15 mts, cooled, inoculated with 300µl(119×10^6 cfu ml^{-1}) liquid culture of *L. acidophilus* (24 hour old culture) and incubated at 37°C for 24 hours. After fermentation it was freeze dried. The results revealed that banana flour is a good substrate for developing a probiotic food with a total viable count of *L. acidophilus* ranging from 9.13 to 9.45 log cfu g^{-1}. The viable count of *L. acidophilus* in the developed probiotic food mixtures was within the recommended level of probiotic organism to assure health benefits.

Keywords: Banana, Probiotic, *Lactobacillus acidophilus*, Optimisation.

Introduction

A variety of foods and their components are emerging as factors capable of modifying growth, development, performance and disease resistance. Such discoveries have influenced perceptions about appropriate nutrition. The term functional food is coined out of the benefits from food that go beyond those attributes to essential nutrients. Within the concept of functional foods we can identify foods known as 'probiotics'. Probiotics are viable microbial food supplements which beneficially influence the health of the host.

Probiotic bacteria break down hydrocarbons which mean the food is split into its most basic elements. This allows almost total absorption through the digestive system. In this way probiotics dramatically increase overall nutrition and enhance rapid cellular growth and development. Probiotics also produce many important enzymes and increase the availability of vitamins and nutrients, especially vitamin B, vitamin K, lactase, fatty acids and calcium (Khaterpaul, 2005).

Prebiotics are often referred to as co factors of probiotics and can be defined as non digestible food ingredients that beneficially affect the host. Tannis (2011) stated that raw green bananas offer a form of prebiotic referred to as resistant starch which enables probiotic bacteria to survive the acidic environment of the stomach so that they can reach the small and large intestine. The present study was undertaken with the objective to optimise the conditions for the development of a probiotic food using banana flour as a substrate, involving the bacteria *L. acidophilus*.

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Materials and Methods

Collection of raw materials and preparation of food mixtures

Raw banana (Nendran *Musa* AAB) was purchased from the local market. This was peeled, washed, sliced, dried and powdered to a flour of 40 mesh particle size. This banana flour was used as a source of starch in all food mixtures. The foods selected for developing the probiotically fermented food mixtures were defatted soya flour (as a source of protein), ripe mango, papaya and tomato and these foods were purchased from the local market.

Pure cultures of *L. acidophilus* (MTCC 447) used for the fermentation of food mixtures was obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

Six food mixtures were prepared after initial standardisation and the treatments are listed in Table1 .

1. *Optimisation of variables for the fermentation of the food mixtures with L. acidophilus*

Using *L. acidophilus* for fermentation, total viable count in the products was maximized while variables like substrate concentration, quantity of the inoculum, time of incubation, pH and temperature were kept at acceptable levels.

Table 1. Selected food combinations in each treatment

Food mixtures (Treatments)	Combinations (percent)
T_1	B-70, DS-20, M-10
T_2	B-60, DS-20, P-20
T_3	B-60, DS-20, T-20
T_4	B-60, DS-20, M-10, P-10
T_5	B-60, DS-20, M-10, T-10
T_6	B-70, DS-,20, P-5, T-5

B- Banana, DS- Defatted soya flour, M- Mango, T-Tomato, P-Papaya

1. Optimisation of substrate concentration

From each food combination 25g, 50g and 75g were weighed, and a slurry made by mixing with 150 ml of water in conical flasks. This was then autoclaved at 121°C for 15 mts, cooled and inoculated with 100 µl(107×10^6 cfu ml^{-1}) of 24 hour old culture of *L. acidophilus*. The flasks with samples were incubated at 37°C for 24 hours and then freeze dried and enumerated for viable counts of *L. acidophilus* using MRS medium. One gram of the mixture was weighed and transferred to a tube containing 9 ml sterile distilled water (dilution: 10^{-1}). This was then serially diluted upto 10^{-7}. The samples were enumerated for microbial count by pour plate method using MRS agar and the results expressed as cfu g^{-1}.

2. *Optimisation of pH*

The best substrate concentration (with maximum viable count of *L. acidophilus*) was taken and slurries were prepared with 150 ml water and the pH was adjusted to 3.5, 4.5, 5.5 and 6.5 using citric acid (20 per cent). These were then autoclaved at 121°C for 15mts, cooled and inoculated with 100µl (107×10^6 cfu ml^{-1}) of 24 hour old culture of *L. acidophilus*, incubated at 37°C for 24 hours, freeze dried and enumerated for viable counts of *L. acidophilus.*

3. *Optimisation of temperature for fermentation*

Each food combination with best substrate concentration was taken and slurries were prepared with 150 ml water and adjusted to the selected optimum pH, autoclaved at 121°C for 15mts, cooled and inoculated with 100µl(107×10^6 cfu ml^{-1}) of 24 hour old culture of *L. acidophilus* and incubated at varying temperatures of 37°C, 41°C and 45°C for 24 hours, freeze dried and enumerated for viable counts of *L. acidophilus.*

4. *Optimisation of time of incubation*

Each food combination with best substrate concentration was taken and slurries were prepared with 150 ml water and adjusted to the optimum pH. The slurries were autoclaved, cooled and inoculated with 100µl(107×10^6 cfu ml^{-1}) of 24 hour old culture of *L. acidophilus*, incubated at the optimum temperature for varying periods of 18 hours, 24 hours and 30 hours, freeze dried and enumerated for viable counts of *L. acidophilus.*

5. *Optimisation of inoculum concentration for fermentation*

Each food combination with best substrate concentration was taken and slurries were prepared with 150 ml water and adjusted to the optimum pH. The slurries were autoclaved at 121°C for 15 mts, cooled and inoculated with varying inoculum concentration of 100µl (107×10^6 cfu ml^{-1}), 200µl (116×10^6 cfu ml^{-1}) and 300µl (119×10^6 cfu ml^{-1}) and kept for incubation at the optimum temperature for the optimum period of fermentation. After fermentation the samples were freeze dried and were enumerated for viable counts of *L. acidophilus.*

Development of fermented food mixtures

After optimising the variables for fermentation, each treatment of food mixture was fermented under optimum conditions. Each developed food mixture (25g) was mixed with 150 ml water and stirred to obtain uniform slurry. The pH was adjusted to 4.5 and autoclaved at 121°C (1.5 kg cm^{-2}) for 15 mts. After cooling this was inoculated with 300µl(119×10^6 cfu ml^{-1}) liquid culture of *L. acidophilus* (24 hour old culture) and incubated at 37°C for 24 hours and freeze dried and packed in metalised polyester polyethylene laminated pouches and stored for a period of six months under ambient conditions.

Microbial enumeration and viable count of L. acidophilus in the food mixtures during storage

The total microbial counts in the food mixtures were enumerated by serial dilution and plate count method as described by Agarwal and Hasija (1986). 10 g of powdered sample was added to 90 ml sterile water and agitated for 20 minutes. One ml of this solution was transferred to a test tube containing 9 ml of sterile water to get 10^{-2} dilution and similarly 10^{-3}, 10^{-4}, 10^{-5} and10^{-6} dilutions were also prepared.

Enumeration of total microbial count was carried out using nutrient agar media for bacteria, Potato dextrose agar media for fungus and Sabouraud's dextrose agar media for yeast. The dilution used for bacteria was 10^{-7} whereas for fungus and yeast 10^{-3} dilutions were used. Viable counts of *L.acidophilus* in food mixtures were enumerated as said above.

Results and discussion

The foods selected for developing the probiotically fermented food mixtures were banana flour, defatted soya flour, ripe mango, papaya and tomato.

Products combining *Lactobacillus acidophilus* (as probiotic) and certain fruits such as banana (as prebiotic) that might provide functional benefits (as synbiotic) have been suggested and studied by Prajapathi et al. (1987). Banana possesses high contents of sugars mainly sucrose, glucose and fructose and is suitable for microbial fermentation (Vega et al., 1988).

Studies by Saarela et al. (2002) indicated that soya is a good substrate for probiotic bacteria. Rani and Khetarpaul (1999) developed a probiotic fermented food mixture RSMT containing freshly ground rice, defatted soya flour, skimmed milk powder and fresh tomato pulp (2:1:1:1 w/w) with good acceptability. Studies conducted by Babu et al. (1992) also proved tomato and papaya pulp as good substrates for *Lactobacillus acidophilus*. In another similar study

by Sindhu and Khetarpaul (2004), barley flour, milk co-precipitate, green gram paste and tomato pulp (2:1:1:1 w/w) were used for *Lactobacillus acidophilus* fermentation, and the food mixtures were found to be organoleptically acceptable to human palate and maintained adequate cell viability.

Optimisation of variables for fermentation of food mixtures

1. *Optimisation of substrate concentration*

Three different quantities of each food mixtures (25, 50 and 75g) were taken for optimising the substrate concentration. After fermentation, the freeze dried samples were enumerated for viable counts of *L. acidophilus*.

Table 2. Viable count [x 10^7 cfu g^{-1}] of *L. acidophilus* in food mixtures with different substrate concentrations

Treatments (Food mixtures)	Quantity of substrate (g)		
	25	50	75
	Viable count		
T_1	53	30	TFTC
T_2	39	TFTC	0
T_3	71	32	TFTC
T_4	48	TFTC	TFTC
T_5	65	58	TFTC
T_6	47	27	TFTC

All values are means of 3 independent enumerations

TFTC- Too few to count

10^7dilutions of all the treatments were compared, each with three substrate concentration. In all the treatments, substrate concentration of 25g showed maximum viable count of *L. acidophilus* ranging from 39 to 71 x 10^7 cfu g^{-1} (Table 2). When expressed in log cfu g^{-1}, the viable count of treatments with a substrate concentration of 25g ranged between 8.59 to 8.85 log cfu g^{-1}.

2. *Optimisation of pH*

Twenty five gram of each food mixture was taken for further standardisation procedures. pH of the substrate was adjusted to 3.5, 4.5, 5.5 and 6.5 using citric acid (20 percent). The substrates with pH 3.5 got hydrolysed to a thin watery consistency after fermentation so it was not possible for freeze drying, and hence eliminated. Other samples were freeze dried and viable counts were enumerated by pour plate method.

10^7dilutions of all the treatments were compared, each with different pH levels. In all the treatments, a pH of 4.5 showed maximum viable count of *L. acidophilus* ranging from 40 to 99 x 10^7 cfu g^{-1} (Table 3). When expressed in log cfu g^{-1}, the viable count of the treatments with a substrate concentration of 25g at pH 4.5 ranged between 8.60 to 8.99 log cfu g^{-1}.

Table 3. Viable count of *L. acidophilus* [x 10^7 cfu g^{-1}] in food mixtures with different pH levels

Treatments	pH		
	4.5	5.5	6.5
T_1	63	37	TFTC
T_2	40	TFTC	0
T_3	99	43	TFTC
T_4	51	TFTC	0
T_5	65	61	TFTC
T_6	53	27	TFTC

All values are means of 3 independent enumerations.

TFTC- Too few to count

3. *Optimisation of temperature for fermentation*

Twenty five g of each food mixture was taken and pH was adjusted to 4.5. After fermentation, incubation was done at 37°C, 41°C and 45°C. The freeze dried samples were enumerated for total viable count of *L. acidophilus.*

10^7dilutions of all the treatments were compared, and each incubated at different temperatures. In all the treatments, a temperature of 37°C showed maximum viable count of *L. acidophilus* ranging from 37 to 97 x 10^7 cfu g^{-1} (Table 4). When expressed in log cfu g^{-1}, the viable count of treatments with a

Table 4. Viable count of *L. acidophilus* [x 10^7 cfu g^{-1}] in food mixtures with different temperatures for incubation

Treatments	Temperature (°C)		
	37	41	45
	Viable count		
T_1	65	32	0
T_2	41	35	0
T_3	97	32	0
T_4	37	26	0
T_5	67	37	TFTC
T_6	57	31	0

All values are means of 3 independent enumerations.
TFTC- Too few to count

substrate concentration of 25g with pH 4.5 incubated at temperature 37 °C ranged between 8.57 to 8.99 log cfu g^{-1}.

4. *Optimisation of time of incubation*

Twenty five gram of each food mixture after adjusting the pH at 4.5 was fermented at 37°C for three different periods, i.e., 18 hours, 24 hours and 30 hours. The freeze dried samples were enumerated for total viable count.

Table 5. Viable count of *L. acidophilus* [x 10^7 cfu g^{-1}] in food mixtures with different time of incubation**.**

Treatments	TIME (h)		
	18	24	30
	Viable count		
T_1	32	63	27
T_2	30	40	TFTC
T_3	34	99	21
T_4	26	40	TFTC
T_5	30	66	TFTC
T_6	31	54	TFTC

All values are means of 3 independent enumerations
TFTC- Too few to count

10^7dilutions of all the treatments were compared, each incubated for different intervals of time. In all the treatments, 24 hour of incubation showed maximum viable count of *L. acidophilus* ranging from 40 to 99 x 10^7 cfu g^{-1} (Table 5). When expressed in log cfu g^{-1}, the viable count of treatments with a substrate concentration of 25g at pH 4.5 incubated at 37°C for 24 hours ranged between 8.60 to 9.00 log cfu g^{-1}.

5. Optimisation of inoculum concentration for fermentation

Twenty five gram of each food mixture after adjusting the pH at 4.5 was inoculated with three different concentration of inoculum (*L. acidophilus*) i.e. 100µl (107x10^6 cfu ml^{-1}), 200µl (116x10^6 cfu ml^{-1}) and 300µl (119 x10^6 cfu ml^{-1}) and was kept for incubation at 37°C for 24 hours, freeze dried and enumerated for the total viable count.

Total viable count was highest with 300 µl of the inoculum in all the treatments which ranged from 139 to 282 x 10^7 cfu g^{-1} (Table 6)

Thus for all the treatments, fermentation with 25g of the substrate at pH 4.5, inoculated with 300µl (119 x10^6 cfu ml^{-1}) and incubated at 37°C for 24 hours gave the maximum total viable count of *L.*

Table 6. Viable count of *L. acidophilus* [x 10^7 cfu g^{-1}] in food mixtures with different concentrations of inoculum.

Treatments	Inoculum(µl)		
	100	200	300
	Viable count		
T_1	67	85	147
T_2	42	72	139
T_3	95	137	282
T_4	45	69	137
T_5	65	104	210
T_6	51	50	205

All values are means of 3 independent enumerations
TFTC- Too few to count

Table 7. Total bacterial count in fermented food mixtures on storage [x 10^7 cfu g^{-1}]

Food mixture	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	149[a]	144[b]	135[c]	126[d]	115[e]	108[f]	98[g]
T_2	140[a]	133[b]	126[c]	114[d]	109[e]	96[f]	92[g]
T_3	283[a]	273[b]	257[c]	223[d]	207[e]	189[f]	135[g]
T_4	139[a]	126[b]	114[c]	98[d]	91[e]	81[f]	74[g]
T_5	211[a]	200[b]	189[c]	173[d]	159[e]	144[f]	123[g]
T_6	206[a]	192[b]	177[c]	166[d]	147[e]	128[f]	110[g]

Values are means of three independent enumerations
DMRT row wise comparison
Values with different superscripts differ significantly at 5 % level

Table 8. Fungal count in fermented food mixtures on storage [x 10^3 cfu g^{-1}]

Food mixture	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	Nil	Nil	1.0	1.3	1.8	2.0	2.1
T_2	1.0	1.0	1.3	1.5	2.0	2.1	2.3
T_3	Nil	1.0	1.0	1.3	1.5	2.0	2.1
T_4	Nil	1.0	1.0	1.3	1.8	2.0	2.1
T_5	1.0	1.5	1.6	1.8	2.0	2.1	2.3
T_6	Nil	Nil	1.0	1.5	2.0	2.1	2.3

Values are means of three independent enumerations

Table 9.Viable count of *L. acidophilus* in fermented food mixtures on storage [x 10^7 cfu g^{-1}]

Food mixture	Storage period in months						
	Initial	1	2	3	4	5	6
T1	147[a]	142[b]	132[c]	123[d]	112[e]	105[f]	95[g]
	(9.16)	(9.15)	(9.12)	(9.08)	(9.04)	(9.02)	(8.97)
T2	139[a]	132[b]	124[c]	112[d]	106[e]	93[f]	89[g]
	(9.14)	(9.12)	(9.09)	(9.05)	(9.03)	(8.96)	(8.94)
T3	282[a]	271[b]	255[c]	221[d]	205[e]	187[f]	133[g]
	(9.45)	(9.43)	(9.40)	(9.34)	(9.31)	(9.27)	(9.12)
T4	137[a]	124[b]	111[c]	95[d]	88[e]	77[f]	70[g]
	(9.13)	(9.09)	(9.04)	(8.97)	(8.94)	(8.86)	(8.84)
T5	210[a]	198[b]	187[c]	171[d]	156[e]	141[f]	120[g]
	(9.32)	(9.29)	(9.27)	(9.23)	(9.19)	(9.14)	(9.08)
T6	205a	191[b]	175[c]	164[d]	144[e]	125[f]	106[g]
	(9.31)	(9.28)	(9.21)	(9.21)	(9.16)	(9.09)	(9.03)

Figures in parenthesis are log cfu g^{-1}
Values having different super scripts differ significantly at 5% level
DMRT row wise comparison

acidophilus MTCC 447 ranging from 9.14 to 9.45 log cfu g^{-1} and this total viable count is above the desired level of probiotic organism (4.7 to 8.9 log cfu g^{-1}) in probiotic foods as recommended by Shah et al. (1995).

Earlier, similar optimization studies were conducted by Santos and Soccol (2003) in the development of a probiotic beverage using cassava flour and strains of *Lactobacillus casei* and *Lactobacillus acidophilus*. The optimised parameters were temperature of incubation of 35°C, fermentation time of 16 hours, cassava flour concentration of 20 per cent and inoculum rate of 4 per cent for *Lactobacillus casei* and 4 per cent for *Lactobacillus acidophilus*.

Total Microbial population and viable count of L. acidophilus in food mixtures on storage

Initially, total bacterial population (Table 7) varied from 139 to 283 x 10^7 cfu g^{-1}. Maximum bacterial count was observed in T_3 and the minimum in T_4. There was a significant reduction in the total bacterial count in FFM during storage. After six months, total bacterial count was significantly reduced and varied from 74 to 135 x 10^7 cfu g^{-1}. Maximum total bacterial population was in T_3 and minimum in T_2.

Initially, fungal population (Table 8) was nil in T_1, T_3, T_4 and T_6 and a fungal count of 1 x 10^3 cfu g^{-1} was observed in T_2 and T_5. There was an increase in the fungal count in FFM during storage. After six months, fungal count increased, and varied from 2.1 to 2.3 x 10^3 cfu g^{-1}. This low bacterial and fungal count may have been be due to the antagonistic properties processed by *L. acidophilus* bacteria present in the food mixtures

There were no traces of yeast and insect infestation in fermented food mixtures throughout the storage period.

Viable count of L. acidophilus in fermented food mixtures on storage

Initially, viable counts of *L. acidophilus* varied from 137 (T_4) to 282 (T_3) x 10^7 cfu g^{-1}. After six months of storage, viable count significantly reduced which varied from 70 (T_4) to 133 (T_3) x 10^7 cfu g^{-1} (Table 9). Even though there was a reduction in viable count, the viable count after six months of storage was within the desired level of probiotic organism as recommended by Shah et al.(1995). Products sold with any health claims should meet the criterion of a minimum 10^6 cfu ml^{-1} probiotic bacteria at the expiry date, because the minimum therapeutic dose per day is suggested to be 10^8–10^9 cells (Kurmann and Rasic, 1991).

One of the requirements for microorganisms to be used as dietary adjuncts is the need to retain viability and activity in the food vehicle before consumption. The main factors for the loss of viability of probiotic organisms have been attributed to the decrease in pH of the medium and accumulation of organic acids as a result of growth and fermentation (Hood and Zottola, 1998; Shah and Jelen, 1990).

A common principle is that the higher the initial cell concentration, the longer the shelf life of the products (Costa et al., 2002). In the present study the initial viable count of *L. acidophilus* was very high in the food mixtures so that they retained high viable counts even after 6 months of storage.

Application of banana as a medium for lactobacillus fermentation has also been studied by Aegerter and Dunlap (1980) and De Porres et al. (1985).Banana is said to possess high contents of sugars, mainly sucrose, glucose and fructose which are suitable for microbial fermentation. Bananas are an exceptionally rich source of fructooligosaccharides (FOS), a group of compounds which have been shown to exhibit beneficial health effects by stimulating the growth of lactic acid bacteria in the colon by suppressing putrefactive pathogens (Crittenden and Playne, 1996).

Hence, it can be concluded that banana is a good substrate for the growth of *L acidophilus* and it is possible to produce probiotic foods with acceptable properties.

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