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***Lactobacillus acidophilus* counts of human faeces after consumption of synbiotic dahi at different days (10^4 cfu/g)**

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Abstract

The recovery of *Lb. acidophilus* counts in human beings was studied by giving 100 g sample of dahi to each volunteer. When synbiotic dahi fed to human volunteers, *Lactobacillus acidophilus* strain V2 with prebiotics T1 (1 % Inulin) and T2 (1.5 % Inulin) have shown significantly increased trend in counts of *Lactobacillus acidophilus* as compared to other treatments. The maximum increment in count was observed for treatment T2V2 (1.5 % Inulin + strain V2). The count increased from 46.5 (zero day count) to 98.5 (seventh day count) i.e. net increase in count was 52 and for treatment T1V2 (1 % Inulin + strain V2) count increased from 12.5 (zero day count) to 55 (seventh day count) i.e. net increased in counts were 42.5. Prebiotic Inulin has shown the best results as compared to prebiotic raffinose.

Keywords: *Lactobacillus acidophilus*, faeces, synbiotic

Introduction

With the growing interest in self-care and integrative medicine coupled with our health-embracing baby boomer population, recognition of the link between diet and health has never been stronger. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods, is the small but rapidly expanding arena of probiotics – live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance. (Dave and Shah, 1997) [3] reported that several health benefits have been associated with the consumption of fermented foods, particularly dairy products. Certain of these health benefits are attributed to beneficial probiotic bacteria included in these fermented products. Attention, therefore, has been directed towards the inclusion of probiotic cultures in fermented products to promote health and nutritional benefits derived from the consumption of such products (Dave and Shah, 1997) [3]. The lactobacilli produce lactic acid during fermentation, which usually destroys or suppresses other less beneficial disease-causing bacteria (Chaitow and Trenev, 1990) [1]. Other claimed benefits of probiotic intake include prevention and treatment of infantile diarrhea, travelers' diarrhea, antibiotic induced diarrhea, colon cancer, constipation, hypercholesterolemia, lactose intolerance, vaginitis and intestinal infections (Franz *et al.*, 2003) [5].

On the other hand, prebiotics are non-digestible food ingredients that affect the host by selectively targeting the growth and / or activity of probiotic bacteria in the colon, and thus have the potential to improve health (Marchand and Vandenplas, 2000) [7]. Regular intake of prebiotic non-digestible complex carbohydrates (bifidogenic substances) can enhance the presence of probiotic bacteria, particularly bifidobacteria, in the gastro-intestinal tract (GIT) of the host. Subsequently, there is greater protection against infection by keeping the GIT relatively acidic. Potential benefits of prebiotic intake include reduction of cholesterol absorption, control of constipation, bioavailability of minerals and reduction in blood glucose levels when used to replace sucrose in diabetic diets (Niness, 1999) [8]. A functional food product is one that can positively influence one or more functions in the body, beyond its nutritional properties (Marchand and Vandenplas, 2000) [7].

A combination of prebiotics and probiotics results in a functional product referred to as a synbiotic product. Advantages of developed synbiotic products are due to their potential synergistic effects in the host consumer. The case in point is that, upon regular consumption of a synbiotic product, there is implantation and facilitation of growth of newly ingested probiotic bacteria, promotion of growth of existing probiotic bacteria as well as improvement in survival (Niness, 1999) [8].

A synbiotic is therefore, a combination of a probiotic and prebiotic in the same product. The advantages are that a commercial probiotic with known benefits can be used and the prebiotic aids the establishment of the organism in the complex gut environment (Kolida *et al.*, 2010) [6].

The production of fermented milk products is rapidly increasing in all major developing countries of the world. The fermented milk products like dahi, cultured butter milks, acidophilus milk, kefir, lassi etc. being manufactured in various countries. Fermented milks are known throughout the world for their taste, nutritive values and therapeutic properties. Dahi is one of the important Indian fermented milk product. The importance of dahi in a daily diet of Indian people has been well recognized from the vedic times. Its curative effect against gastro-intestinal disorder has been utilized by many civilizations (Sinha and Sinha, 2000) [12].

Material and methods

The composite samples of cow milk required for research were obtained from the herd maintained at the Research-Cum-Development Project on Cattle, Department of Animal Science and Dairy Science, Mahatma Phule Krishi Vidyapeeth, Rahuri. The prebiotic inulin and raffinose were purchase from Dodal Enterprizes, Aurangabad (M.S.).

The samples of synbiotic dahi were stored at refrigerated temperature for 7 days. The samples of stored synbiotic dahi were analyzed for their chemical and microbiological quality over a period of 7 days. The samples were analyzed for chemical and microbiological quality by standard analytical procedures. Experimental data was tabulated and analyzed with the help of Factorial Completely Randomized Design (FCRD) and Completely Randomized Design (CRD) method. The treatment combinations are as follows.

Treatment Details

T ₀ V ₁	:	Milk + <i>Lb. acidophilus</i> (strain 1) @ 1.5 %
T ₀ V ₂	:	Milk + <i>Lb. acidophilus</i> (strain 2) @ 1.5 %
T ₀ V ₃	:	Milk + <i>Lb. acidophilus</i> (strain 3) @ 1.5 %
T ₁ V ₁	:	Milk + 1 % Inulin + <i>Lb. acidophilus</i> (strain 1) @ 1.5 %
T ₁ V ₂	:	Milk + 1 % Inulin + <i>Lb. acidophilus</i> (strain 2) @ 1.5 %
T ₁ V ₃	:	Milk + 1 % Inulin + <i>Lb. acidophilus</i> (strain 3) @ 1.5 %
T ₂ V ₁	:	Milk + 1.5 % Inulin + <i>Lb. acidophilus</i> (strain 1) @ 1.5 %
T ₂ V ₂	:	Milk + 1.5 % Inulin + <i>Lb. acidophilus</i> (strain 2) @ 1.5 %
T ₂ V ₃	:	Milk + 1.5 % Inulin + <i>Lb. acidophilus</i> (strain 3) @ 1.5 %
T ₃ V ₁	:	Milk + 1 % Raffinose + <i>Lb. acidophilus</i> (strain 1) @ 1.5 %
T ₃ V ₂	:	Milk + 1 % Raffinose + <i>Lb. acidophilus</i> (strain 2) @ 1.5 %
T ₃ V ₃	:	Milk + 1 % Raffinose + <i>Lb. acidophilus</i> (strain 3) @ 1.5 %
T ₄ V ₁	:	Milk + 1.5 % Raffinose + <i>Lb. acidophilus</i> (strain 1) @ 1.5 %
T ₄ V ₂	:	Milk + 1.5 % Raffinose + <i>Lb. acidophilus</i> (strain 2) @ 1.5 %
T ₄ V ₃	:	Milk + 1.5 % Raffinose + <i>Lb. acidophilus</i> (strain 3) @ 1.5 %

Starter Culture, Its Maintenance and Propagation

The freeze dried probiotic pure cultures of *Lactobacillus acidophilus* were obtained from the National Dairy Research Institute, Karnal, Haryana and SMC College GAU, Anand. The cultures were maintained separately in sterilized reconstituted skim milk test tubes.

The sterilized skim milk test tubes were separately inoculated with these cultures and incubated at 37 °C for 8 hr and thereafter stored at refrigerated temperature. In order to keep these cultures active, they were propagated once in a week. (Shankar 1975) [11].

Preparation of Synbiotic Dahi

The synbiotic dahi was prepared by using the procedure prescribed by (De, 2008) [2] with some minor modifications. The flow diagram for preparation of synbiotic dahi is depicted in Fig. 1.

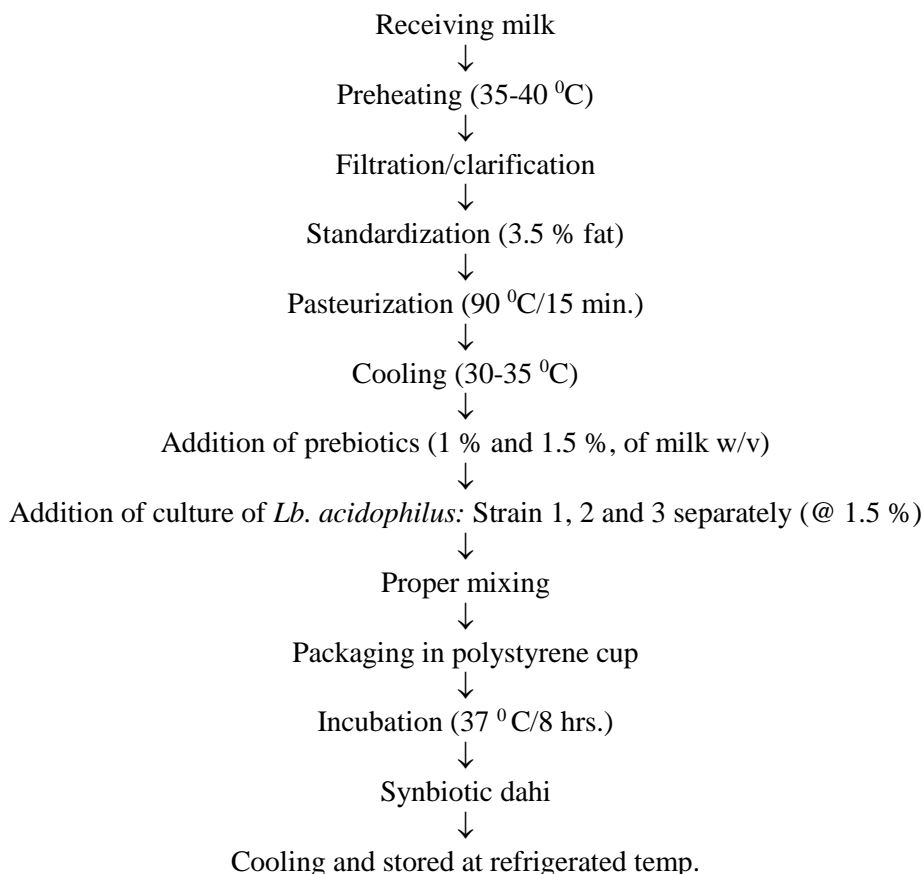


Fig 1: Preparation of synbiotic dahi

Result and discussion

The data regarding *Lactobacillus acidophilus* count of human faeces was presented in table 1.

Lactobacillus acidophilus counts of human faeces

The 100 gm samples of synbiotic dahi were given to the volunteers. The samples of faeces of volunteers were collected and analyzed for *Lactobacillus acidophilus* counts by using the MRS media. The results of *Lactobacillus acidophilus* counts of human faeces are presented in Table 1 and graphically presented in Fig. 2.

From Table 1, it is observed that, when the synbiotic dahi were fed to volunteers, there was increase in *Lactobacillus acidophilus* counts from their faeces as compared to control dahi. It was increased.

The synbiotic dahi of *Lactobacillus acidophilus* strain V₂ have shown the maximum *Lactobacillus acidophilus* counts for both prebiotics as compared to synbiotic dahi prepared from *Lactobacillus acidophilus* strain V₁ and V₃.

It was revealed that when synbiotic dahi fed to human volunteer's, *Lactobacillus acidophilus* strain V₂ with

prebiotics T₁ (1 % Inulin) and T₂ (1.5 % Inulin) have shown the significantly increased trend in of counts of *Lactobacillus acidophilus* as compared to other treatments. The maximum count increment was observed for treatment T₂V₂ (1.5 % Inulin + strain V₂). The counts increased from 46.5 (zero day count) to 98.5 (seventh day counts) i.e. net increased in count was 52 and from treatment. The T₁V₂ (1 % Inulin + strain V₂) count increased from 12.5 (zero day count) to 55 (seventh day count) i.e. net increased in counts were 42.5. Prebiotic Inulin has shown the best results as compared to prebiotic raffinose. (Saxelin, *et al.*, 1993) [10] found that, when fermented milk with the *Lactobacillus* GG as a probiotic organism was given at the rate of 1.2×10^{10} c.f.u./dose, there was a clear, statistically significant increase in mean faecal *Lactobacillus* GG. (Pandiyani, *et al* 2012) [9] found that faecal examination of human volunteers fed with symbiotic ice cream for an experimental period of 15 days carried out on 0, 7, 15 and 21 days post treatment showed significantly increased faecal *L. acidophilus* count and significantly ($P < 0.01$) reduced pH and coliform count.

Table 1: *Lactobacillus acidophilus* count of human faeces after consumption of synbiotic dahi at different days (10^4 cfu/g).

Treatment	Volunteers	Days							
		0	1	2	3	4	5	6	7
Strain V₁									
T ₀ V ₁	A	2	3	4	4	5	7	7	10
	B	3	5	4	6	7	8	11	14
	Mean	2.5	4	4	5	6	7.5	9	12
T ₁ V ₁	A	6	9	12	17	21	24	27	33
	B	8	11	16	19	23	26	29	37
	Mean	7	10	14	18	22	25	28	35
T ₂ V ₁	A	24	27	32	39	42	48	57	62
	B	26	29	36	38	44	50	51	54
	Mean	25	28	34	38.5	43	49	54	58
T ₃ V ₁	A	48	56	59	64	64	68	69	71
	B	47	52	53	56	59	61	64	68
	Mean	47.5	54	56	60	61.5	64.5	66.5	69.5
T ₄ V ₁	A	63	67	69	69	74	79	86	90
	B	60	63	69	72	76	79	81	84
	Mean	61.5	65	69	70.5	75	79	83.5	87
Strain V₂									
T ₀ V ₂	C	3	5	6	7	9	13	16	18
	D	2	4	5	8	9	13	11	14
	Mean	2.5	4.5	5.5	7.5	9	13	13.5	16
T ₁ V ₂	C	14	21	27	31	34	42	49	54
	D	11	17	26	29	33	39	44	56
	Mean	12.5	19	26.5	30	33.5	40.5	46.5	55
T ₂ V ₂	C	45	49	56	64	78	83	90	95
	D	48	57	67	69	77	88	96	102
	Mean	46.5	53	61.5	66.5	77.5	85.5	93	98.5
T ₃ V ₂	C	81	89	92	99	109	110	113	119
	D	93	93	91	97	102	111	117	122
	Mean	87	91	91.5	98	105.5	110.5	115	120.5
T ₄ V ₂	C	103	112	121	131	132	137	139	140
	D	113	121	129	133	139	144	149	156
	Mean	108	116.5	125	132	135.5	140.5	144	148
Strain V₃									
T ₀ V ₃	E	3	4	7	9	13	13	14	16
	F	2	5	8	8	9	10	12	14
	Mean	2.5	4.5	7.5	8.5	11	11.5	13	15
T ₁ V ₃	E	10	17	21	26	31	36	40	44
	F	8	13	20	23	31	33	37	41
	Mean	9	15	20.5	24.5	31	34.5	38.5	42.5
T ₂ V ₃	E	38	43	49	53	57	62	70	76
	F	36	40	47	50	56	61	69	74
	Mean	37	41.5	48	51.5	56.5	61.5	69.5	75

T ₃ V ₃	E	67	71	77	79	83	89	94	96
	F	64	63	64	68	73	80	87	92
	Mean	65.5	67	70.5	73.5	78	84.5	90.5	94
T ₄ V ₃	E	88	91	97	99	107	117	116	120
	F	81	87	90	97	99	103	109	112
	Mean	84.5	89	93.5	98	103	110	112.5	116

The growth, activity and retention of viability are specifically dependent on *Lactobacillus* strain. The addition of prebiotics to products containing probiotic bacteria such as *Lactobacillus* strains will have a significant effect, where unabsorbed prebiotics can be selectively utilized by them in the gut. Hence, a combination of a suitable *Lactobacillus* strain with a specific prebiotic would be a feasible approach in administering the beneficial bacteria *in vivo* (Desai, 2008) [4].

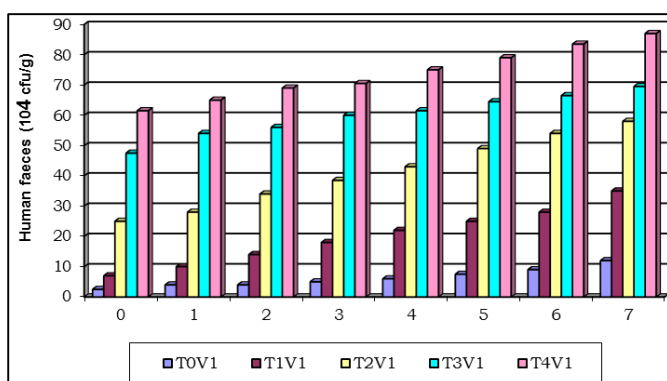
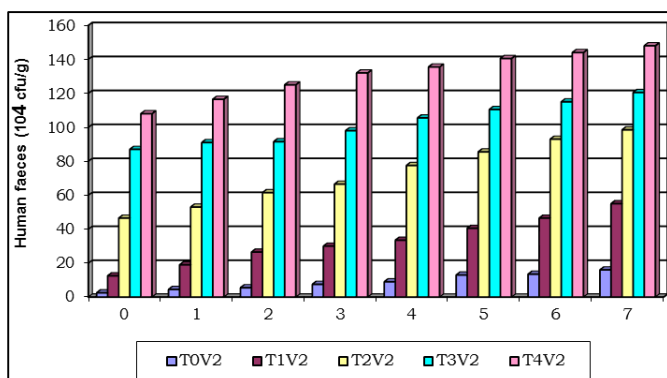
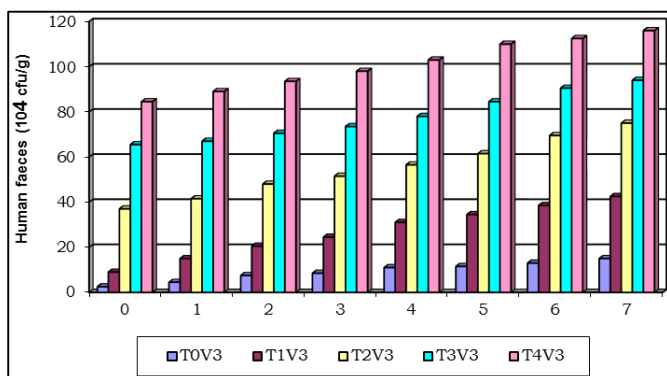
Strain V₁Strain V₂Strain V₃

Fig 2: *Lactobacillus acidophilus* count detection from human faeces (10^4 cfu/g).

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