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RAPID COMMUNICATION

Effect of special Hungarian probiotic kefir on faecal microflora

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Abstract

AIM: To investigate the effect of a four-week consumption of a special Hungarian probiotic agent (Biofir®) on the faecal microflora in human healthy subjects.

METHODS: The effect of Biofir® with $10^6/cm^3$ initial germs on the faecal microflora was studied in 120 healthy volunteers (71 females, 49 males). The traditional Russian type kefir was used as control. The various germ groups and pH values were determined in wk 2, 4 and 6.

RESULTS: The number of all microbes increased during the 4-week probiotic treatment. The number of microbes increased 4.3-fold in the control group and 6.8-fold in Biofir-treated group. The probiotic kefir caused multiplication of the probiotic flora, meanwhile the undesired bacteria multiplied in the control group. No significant change of pH values of the faeces was found in both groups.

CONCLUSION: The Hungarian probiotic kefir (Biofir®) is capable of promoting multiplication of probiotic bacterial flora in the large bowel.

Key words: Hungarian probiotic agent (Biofir); Faecal microflora; Large bowel

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INTRODUCTION

Human intestinal flora contains as many as 10^{14} bacteria classified into 400-500 species, which are ten times higher than all the cells in the human body. Some bacteria of the intestinal flora such as Clostridia, Proteus and Pseudomonas aeruginosa can be harmful, while others like bifidobacteria and Lactobacilli belonging to the so-called probiotic strains are favorable for the organism. The microflora in the large intestine plays an important part in the life of the host organism[1, 2]. Its composition may change several times during our life. However it can still be regarded as nearly constant. Non-pathogenic, pathogenic and potentially pathogenic microorganisms living in a state of equilibrium determined by their own ecosystem within the large intestine take part in the local immunological and metabolic processes as well as in those affecting the organism as a whole[3, 4].

Some strains produce metabolites, such as short chain fatty acids and bactericins, which are of antibacterial effects. As a consequence of the relationship between cells of the mucous membrane and the microflora, the expression of certain mucosal genes may change, the cytokin release may increase, the proliferation of mucous membrane may change and produce a significant effect on the intestine-associated lymphoid tissue which is the largest immune organ of the organism containing 80% of cells producing antibodies[5-9]. Certain strains of bacteria are capable of improving the barrier function of the mucous membrane and increase the differentiation of B cells as well as IgA secretion[4, 6].

The increasinfg data raise the idea of enriching foods with probiotics, prebiotics and symbiotic, a mixture of these two. Probiotics which are beneficial non-pathogenic bacteria live in the intestinal canal and play a role in the preservation of health[10, 11]. The majority of prebiotics are oligosaccharides, i.e. the indigestible constituents of our plant food, which promote the multiplication, growth and efficacy of the strains of probiotic bacteria in the large intestine. Nowadays several strains of probiotic bacteria are known, but their utilization is restricted by the fact that an effective probiotic is supposed to proceed along the acidy pH of the stomach and is able to resist the digestion of bile and pancreatic juice and finally sticks to the surface of some cells in the intestinal wall. The lifespan of the stuck probiotic is short, ranging from a few days to a few weeks and it usually lasts for a short period of time following its regular intake. Colonizing probiotics compete with other microrganisms for nutrients and appropriate binding sites. Only probiotics capable of colonizing even if only temporarily, can exert the required immunological effect[1, 7, 8].

Table 1 Composition of kefirs

Components	Kefir: control product	Biofir
Fat content	3.5%	3.5%
Prebiotic content	-	0.4%
Number of microbes x 10^6 Cfu		
Lactobacilli	0.15	1.48
Streptococci	165.00	227.50
Yeasts	0.40	0.18

The *in vitro* efficacy of the well-known probiotic strains has already been proved in clinical practice [12]. The most widely used strains are those taken from sour dairy products and the intestinal system. The most frequently studied species include various species of Lactobacilli, Streptococcus, thermophilus, bifidobacteria, Saccharomyces boulardii, but under certain conditions other strains in the intestinal microflora, such as *Escherichia coli* (*E. coli*) can also be used as probiotics[13, 14]. In order to become suitable for producing health improving foods, namely functional foods, individual probiotic strains need to meet certain requirements[15, 16]. The following criteria are listed for effective probiotic bacteria[17, 18]: maintenance of the biotic potential, good taste and flavor following fermentation, mild acidity in the course of storage, preservation of the capability of colonizing in the course of food technology and storage, high degree of stability during storage, stability in the course of freeze-drying or other drying procedures, accurate and reliable determination of the strain, and the dose-dependent effects

Biofir® is a traditional, so-called Russian type of kefir based on probiotic lactic acid bacteria culture composed of termophilic strains (producing exopolysacharides) developed by the Hungarian Dairy Research Institute. The present study was undertaken to investigate the effect of a four-week-long consumption of Biofir® on the faecal microflora.

MATERIALS AND METHODS

Patients

One hundred and twenty healthy volunteers (71 females and 49 males) were included in the study following the permission of the Ethical Committee. Their age ranged from 18 to 59 years. The basic selection criterion was that no antibiotics were taken by the subjects 2 mo prior to the investigation.

Composition of kefirs (Table 1)

Kefir containg 3.5% of fat was made by stirring the frozen kefir culture. The initial number of cells was $10^6/cm^3$. Biofir® containg 3.5% of fat was a mixture of curd kefir made by stirring the symbiolact-1 culture. The ratio of ingredients was 1:1. The initial number of cells was $10^6/cm^3$.

Prior to the investigation, blood, urine and faece samples were taken from the subjects for routine laboratory analysis and faecal microflora examination. The investigations lasted for 6 wk, while the subjects followed standard diet which was free of sour dairy products and other probiotic foods. The diet was controlled regularly by the dietitian. Then the control group (60 persons) consumed 0.5 L of Russian type kefir daily for four week, while those included in the study group (60 persons) consumed a daily amount of 0.5 L of probiotic kefir.

Table 2 Germ groups and cultures used in study

Germ groups	Cultures
All germs	Plate count skim milk agar
Streptococci	M-17-agar according to Terzaghi
Lactobacilli	MRS-agar De Man, according to Rogosa and Sharpe
Bifidobacteria	MGLP modified Garche agar
Bacteroids	Anaerob blood agar base according to CDC
Coliforms	Violet red bile (VRB) agar
Escherichia coli	Lauryl sulfate broth
	Eosin methylene-blue lactose sucrose (EMB) agar
Enterobacteria	Violet red bile dextrose (VRBD) agar according to Mossel
-Lactose- positive	Hektoen enteric agar
Lactose-negative	
Enterococci	Citrate azide tween carbonate (CATC) agar base
Anaerob spora	Reinforced clostridial agar
	Anaerobic agar according to Brewer
Yeasts and moulds	Yeast extract glucose chloramphenicol (YGC) agar

Faece samples were taken in the 2nd, 4th and 6th weeks. The screening type of blood sample analysis was repeated in the 4th wk. Faece samples were stored in sterile containers at 4 °C and sent to the Hungarian Dairy Research Institute in 4-6 h, where determination of the various germ groups was carried out in internationally accepted cultures. In addition to the analysis of the microflora, the pH value of the faeces was also determined (Table 2).

Statistical analysis

Evaluation of the data included calculation of the changes in percentage. Determination of the differences with regard to the self-control was carried out using one sample T probe, while two-sample T probes were used for the comparison of the two groups.

RESULTS

The essential question in the course of analyzing the faecal microflora was how the consumption of each type of kefir influenced the number of primary probiotic Streptococci, Lactobacilli and bifidobacteria within the total number of germs.

The effect of the tested dairy products on the essential faecal microflora during the 4-wk clinical investigation is listed in Table 3.

The results indicated that the number of all microbes increased 4.3-fold in the control group consuming the traditional Russian type of kefir and 6.8-fold in the group consuming the probiotic Biofir®, respectively after 4 wk. A very important difference was found between the two groups. The rate of probiotic microbes decreased from 8.9% to 2.7% in the control group by the end of the 4th wk and increased from 12.7% to 72% in the target group. Within the total number of the probiotic germs, the greatest increase (59.7-fold) resulting from the consumption of Biofir® could be observed in bifidobacteria, a lower increase (6.8 fold) was found in Streptococci, while the index

Table 3 Effect of consumption of tested dairy products on the essential microflora in 4-wk clinical investigation

Dairy product consumed	Number of subjects	Germ group	Unit	Germ values of faece samples		
				wk 0	wk 2	wk 4
Traditional Russian type of kefir	15	Total germ number (Aerobe + anaerobe)	10^6/g	10 585 ± 8350	7876 ± 4272	45 125 ± 19463
			Index	1.0	0.7	4.3
			10^6/g	220 ± 105	374 ± 179	285 ± 123
			Index	1.0	1.7	1.3
		-Streptococcus	10^6/g	226 ± 97	37 ± 12	164 ± 84
		-Lactobacillus	Index	1.0	0.2	0.7
		-Bifidobacterium	10^6/g	500 ± 205	197 ± 94	759 ± 223
			Index	1.0	0.4	1.5
			10^6/g	946	608	1208
			Index	1.0	0.4	1.5
		Probiotics	Ratio%	8.9	7.7	2.7
			10^6/g	4360 ± 2958	38 278 ± 13260	29 785 ± 12945
			Index	1.0	8.8	6.8
			10^6/g	134 ± 83	593 ± 94	930 ± 154
Probiotic Biofir® (Experimental)	57	Total germ number	Index	1.0	4.4	6.9
			10^6/g	78 ± 15	81 ± 23	96 ± 44
		-Streptococcus	Index	1.0	1.0	1.2
		-Lactobacillus	10^6/g	342 ± 132	3723 ± 528	20 414 ± 1564
		-Bifidobacterium	Index	1.0	10.9	59.7
			10^6/g	554	4397	21440
		Probiotics	Index	1.0	7.9	38.7
			Ratio%	12.7	11.5	72.0

of Lactobacilli showed no change. No significant change was found in faece pH values in both groups.

The participants followed the prescribed diet but consumed no probiotic food for another two weeks following the 4-wk period of consumption. Microflora analysis of the faeces was repeated in the 6th wk. The results indicated that both the total number of germs and the number of probiotic germs returned to the initial values in both groups.

DISCUSSION

Various types of medical treatment may damage the probiotic flora in human organ. Besides, similar damage can result from the lack of appropriate nutrients in the normal flora. The findings of several controlled studies in the field have proved that various probiotic strains are capable of shortening the duration of gastroenteritis induced by rotavirus. The beneficial effects of probiotics on chronic inflammatory intestinal conditions have been reported. These effects may be attributed to the proteolytic activity of the intestinal flora, which contributes to the breaking up of enteral antigens, reduces secretion of inflammation mediators, helps the normal activity of the intestinal mucosal barrier, normalizes intestinal permeability and increases the production of mucosal IgA[19-21]. The production of specific IgE, which prevents the allergic reactions, is inhibited by the healthy intestinal microflora[19, 22].

A well-known probiotic effect is that some bacteria produce beta galactozidase, which makes their use desirable and beneficial in the case of lactose intolerance. The decreased activity of faecal enzymes and the low levels of faecal toxins are due to the healthy intestinal flora, which is an important factor for the prevention of large intestine cancer.

Prebiotics play an important part in the maintenance and regeneration of healthy intestinal flora as they are components of several plant foods and oligosaccharides with beneficial effect on the host organisms by selectively increasing the multiplication of probiotic bacteria and their activity in the large intestine.

One of the probiotic strains of bacteria used in the present study is capable of producing mucus. The mucus produced by it is a polysaccharide, which serves as a prebiotic agent for other probiotic strains. Besides, it increases the preservability of food products containing it and prevents their acidification.

The present study proved that Biofir®, a probiotic kefir, was capable of promoting the multiplication of the probiotic flora in the large intestine but the traditional Russian type kefir could not. As a result of the consumption of Biofir® most probiotic microbes grew significantly but the consumption of traditional kefir did not change the number (index) of these microbes.

In the present study, the initial heterogeneous color and the consistency of faecal samples were totally homogenuous after four weeks of Biofir® consumption, probably due to the advantageous bio-physiological processes caused by the multiplication and ultimate predominance of the useful probiotic microbes.

In conclusion, Biofir® meets all the criteria of functional foods and can be used in the prevention and treatment of various acute and chronic inflammatory conditions and nutritional allergies. Further investigations should be

undertaken to determine the effect of probiotic strains on various pathological conditions.

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