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EFFECT OF A CYANOBACTERIAL BIOMASS ENRICHED WITH TRACE ELEMENTS ON THERMOPHILIC DAIRY STARTER CULTURES

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1 INTRODUCTION AND AIM

Fermented dairy products, which are manufactured with starter cultures, are often referred to as the queens of dairy products. If they are consumed on a regular basis, a wide range of beneficial effects may be expected, in humans. The latest statistics show that the average Hungarian consumer purchases about 50-60% less fermented dairy product than his/her Western European counterpart. It is worth recording, however, that sales of fermented dairy products are growing steadily in Hungary, despite the fact that there has been a decline in the purchasing power of people in the past few years, which is well reflected in the overall consumption of milk and dairy products.

The consumption of fermented dairy products shows an upward tendency worldwide, not only in Hungary. This phenomenon can be accounted for by the fact that people have been aware of the high nutritional value and excellent sensory properties of these products. Dairy companies endeavor to maintain and further stimulate these favorable tendencies by improving quality continuously, widening the assortment of products and packaging goods in an increasingly practical and attractive way.

The beneficial properties of fermented dairy products, which are largely due to the presence of starter bacteria in high counts and the change in the composition of milk, can be summarized as follows.

- The characteristic micro-organisms in the product secrete lactic acid and sometimes also acetic acid, which may show specific toxicity against certain species of yeasts or bacteria or, by lowering the pH of the intestinal contents, inhibit the growth of putrefactive organisms in general.
- The starter culture bacteria of human origin can colonize the human digestive tract, thereby replacing the protective effect that was seriously depleted by treatment with antibiotics against pathogens.
- The bifidogenic growth-promoting factors in the product help bifidobacteria and lactobacilli colonize the intestinal tract.
- Owing to proteolytic activity and precipitation of casein, both protein digestion and absorption improve as compared with milk.
- Digestion and absorption of milk fat and resorption rates of calcium, phosphorous and iron also improve.
- By lowering the cholesterol level of blood, they play a key role in the prevention of coronary heart diseases.
- Fermentation substantially improves the nutritional properties (e.g. non-protein nitrogen content, free amino nitrogen content, specific protein utilization, “half-period” of vitamin C etc.) of milk.
- They act as stimulants of the immune system.
- Owing to the β-D-galactosidase activity of certain micro-organisms, these products can be consumed by the majority of people who suffer from lactose maldigestion because the lactose content of milk is decomposed and changed into organic acids.
- Certain starter cultures may possess antitumorigenic properties.

- The fermented dairy products may be superior to milk as regards vitamin content.
- Because of the low pH of the final product, the supplements of beneficial physiological effect (mineral complex compounds, vitamins etc.) become highly stable.
- The ingestion of fermented dairy products contributes to an increased secretion of saliva, bile salts and gastric juices.
- A large variety of fermented dairy products can be manufactured with respect to taste and flavor if the available species of thermophilic starter bacteria are combined with one another and various additives are also employed.
- By total or partial ultrafiltration of the milk, addition of milk protein concentrates and standardization of the fat content, dietary products and roborants of almost any composition can be manufactured.
- Extremely long shelf-life and stability can be ensured by heat treatment or freeze-drying (lyophilization) of the final product, in accordance with market demands.

The importance of probiotic (therapeutic) micro-organisms, including *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, has recently come into prominence in several countries which have highly developed dairy production. The probiotic properties of the above-mentioned starter culture bacteria include prevention of traveler's diarrhea, reduction of diarrhea and rotavirus infection in infants, prevention of constipation in elderly people, contribution to a faster recolonization of the intestinal microflora after administration of antibiotics, improvement in lactose intolerance, reduction of cholesterol level in the blood, stimulation of the immune system and improvement in defense against cancer. There is a wide range of fermented dairy products on the market all over the world. Table 1 shows the major types of fermented milks manufactured with starter cultures containing micro-organisms that are indigenous to the intestinal tract.

Table 1 Fermented milk products manufactured with starter cultures containing micro-organisms indigenous to the human intestinal tract

Starter Culture Bacteria (SCB) in the Product		Product or Trade Name
SCB of Intestinal Origin	Other SCB	
Lactobacillus acidophilus	—	Acidophilus Milk
	Lactobacillus bulgaricus + *Streptococcus thermophilus*	Acidophilus Yogurt
		ACO Yogurt
Bifidobacterium bifidum	—	Bifidus Milk
	Streptococcus thermophilus	Bifighurt®
	Lactococcus lactis subsp. *lactis* biovar. *taette*	Bioghurt®
	Lactobacillus bulgaricus + *Streptococcus thermophilus*	Bifidus Yogurt
Bifidobacterium bifidum + *Lactobacillus acidophilus*	—	Fermented AB Milk
		Cultura®
	Streptococcus thermophilus	Fermented ABT Milk
		Biogarde®
	Lactobacillus bulgaricus + *Streptococcus thermophilus*	Acidophilus Bifidus Yogurt

Only few of the above fermented milks are available in Hungary. It is most desirable that consumers should include them in their diet because these products possess specific health benefits. They are expected to become increasingly available and gain widespread acceptance in the long term if the purchasing power of consumers increases. In several of Europe's highly developed countries (e.g. Denmark, France, the Netherlands, Germany etc.) the market of probiotic products is characterized by an annual growth of 15% or more and, in some cases, this is despite a 70% price premium over regular yogurts. However, high quality and attractive packaging do not ensure success automatically. In any market, popular brands are built through heavy investment in marketing communications. As for the probiotic fermented dairy products, advertisements should be based on a scientifically established and documented health claim.

Even the current consumption level of fermented dairy products could easily contribute to consumers' ingesting fewer artificially produced vitamin and trace element preparations and less medicine if fermented dairy products were enriched with vitamins, proteins, essential fatty acids and trace elements of natural origin. A simple way of attaining this goal is the use of *Spirulina platensis* biomass enriched with trace elements for the manufacture of fermented milk products. The presence of bioactive substances in this cyanobacterial biomass is of great importance because it further improves the high nutritional value of fermented milks.

Productivity is one of the main concerns of dairy technologists and scientists. According to German and Japanese authors, acid development by and growth rate of certain lactic acid bacteria can be stimulated by addition of extracts of green algae.

This work aims to find out whether stimulation of single- and multiple-strain type thermophilic dairy starter cultures can be brought about by a cyanobacterial biomass and identify the substances responsible for the effects observed.

The effect of 3 g l^{-1} *Spirulina platensis* biomass enriched with trace elements (iodine, zinc, selenium) on the rate of acid development by and growth rate of pure and synchronized mixed cultures of *Streptococcus salivarius* subsp. *thermophilus* CH-1, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2, *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 was evaluated in a model milk medium. The components of the cyanobacterial biomass responsible for the stimulation caused were also identified in laboratory simulations wherein trace elements (iodine, zinc, selenium), vitamins (B-complex, C, A, E) and nitrogenous compounds (peptone, adenine, hypoxanthine) were tested.

This Dissertation meets a long-felt want since reports on similar experiments carried out with this cyanobacterium species, these dairy starter cultures and such a large variety of bioactive substances were not to be found in the literature.

Thereafter storage experiments were conducted to reveal the changes in the characteristic and undesirable microbial flora of cyanobacterial and control yogurts produced according to regular technology of manufacture. The cyanobacterial yogurt was richer in vitamins, trace elements and further bioactive substances than the "normal" fermented milk products and thus it possessed functional properties.

The experiments required milk in large quantities. About 100 l of UHT milk was purchased and deep-frozen for the researches done in a model milk medium and for the preliminary experiments. As to the products manufactured for the storage experiments, 50 l of pasteurized market milk was used as raw material.

2 MATERIALS AND METHODS

2.1 Researches done in a model milk medium

2.1.1 Raw material

UHT milk free of inhibitory substances, tested by means of Delvotest® SP MINI, was used. It was deep-frozen (in Ultra Low Freezer U 41085, New Brunswick Scientific) and stored at -75°C so that all the experiments would be done with raw material from the same lot of production and thus the results could be compared. It contained 28 g l^{-1} fat, 34 g l^{-1} protein, 47 g l^{-1} lactose and 7 g l^{-1} ash. Although having been treated at an ultra high temperature, milk was heated to 90°C and held for 5 min before being cooled to inoculation temperature so that the levels of thermal denaturation of whey proteins would be increased. The experiments were carried out in the Institute of Food Science at Pannon University of Agricultural Sciences, Faculty of Agricultural Sciences at Mosonmagyaróvár.

2.1.2 Starter culture strains

Four freeze-dried strains, manufactured by Chr. Hansen A/S, were kindly supplied by the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár. The strains were subcultured twice at 37°C for 12-72 h before being employed for the experiments. The single strains and the combinations of strains used for the fermentation experiments are listed in Table 2.

Table 2 Single strains and combinations of strains employed in the fermentation experiments

Starter Culture Bacteria (SCB)		Name of product manufactured with the given starter culture
SCB of Intestinal Origin	Other SCB	
—	*Strep. thermophilus*	—
	Lact. bulgaricus	—
	Strep. thermophilus + *Lact. bulgaricus*	Yogurt
Lact. acidophilus	—	Acidophilus Milk
	Strep. thermophilus	—
	Strep. thermophilus + *Lact. bulgaricus**	Acidophilus Yogurt
		ACO Yogurt
Bifid. bifidum	—	Bifidus Milk
	Strep. thermophilus	Bifighurt®
Lact. acidophilus + *Bifid. bifidum*	—	Fermented AB Milk (Cultura®)
	Strep. thermophilus	Biogarde®
		Fermented ABT Milk

Strep. thermophilus, *Streptococcus salivarius* subsp. *thermophilus* CH-1; *Lact. bulgaricus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* CH-2; *Lact. acidophilus*, *Lactobacillus acidophilus* La-5; *Bifid. bifidum*,

Bifidobacterium bifidum Bb-12; *, Since *Lact. bulgaricus* is added to such products in order to ensure more pronounced acid development, no experiment was performed with this combination

2.1.3 Spirulina platensis biomass

The *Spir. platensis* biomass, which is a licensed food supplement in Germany, was obtained from the Institute of Cereal Processing Inc. (Institut für Getreideverarbeitung GmbH) at Bergholz-Rehbrücke. The composition of this product is shown in Table 4.

Table 3 Composition of the *Spirulina platensis* biomass

Component	Quantity (in 1 kg of biomass)
Original component	
Dry matter	941 g
Protein	576 g
Total lipids	111 g
Ash	114 g
Zn	515 mg
Cu	7 mg
Cd	<0.01 mg
Pb	0.05 mg
Enriched component	
KI	0.131 g
$ZnCl_2$	2.052 g
$Na_2SeO_3 \cdot 5H_2O$	0.333 g

Since the product was added to the milk at a concentration of 3 g l^{-1}, 0.393 mg l^{-1} KI, 6.156 mg l^{-1} $ZnCl_2$ and 0.999 mg l^{-1} $Na_2SeO_3 \cdot 5H_2O$ were used when the individual effect of trace elements on acid development by the starter culture strains was tested.

The idea of employing the cyanobacterial (CBA) biomass at a concentration of 3 g l^{-1} was borrowed from a previous work of ours wherein the effective and economic concentration of CBA biomass resulting in good sensory properties was determined.

2.1.4 Further components tested

In addition to iodine, zinc and selenium, the following vitamins and nitrogenous substances were tested separately. Vitamin B_1 (0.5 mg l^{-1}), vitamin B_2 (2.0 mg l^{-1}), nicotinic acid (1.0 mg l^{-1}), pantothenic acid (4.0 mg l^{-1}), vitamin B_6 (0.6 mg l^{-1}), vitamin B_{12} (5.0 µg l^{-1}), vitamin C (50 mg l^{-1}), vitamin A (1.0 mg l^{-1}), vitamin E (3000

IU l^{-1}), peptone (1.0 g l^{-1}), adenine (2.0 mg l^{-1}), hypoxanthine (3.5 mg l^{-1}). The effect of peptone + adenine and that of peptone + hypoxanthine were also investigated. All these substances, including iodine, zinc and selenium, were purchased from Merck Kft.

2.1.5 Experimental conditions

The heat-treated and cooled milk was portioned out into 250 ml Erlenmeyer flasks and supplemented with the substrate(s) to be tested (CBA biomass or its components). As for single strains, the rate of inoculation was 1% (v/v) except for *Bifid. bifidum* (6%, v/v). *Strep. thermophilus* and *Lact. bulgaricus* were incubated at 42.5°C whereas *Lact. acidophilus* and *Bifid. bifidum* at 37.5°C. As regards combinations of strains, preliminary experiments had been conducted so that the levels of inoculation giving approximately the same counts of colony forming units (cfu) of starter culture organisms at the end of the fermentation process could be determined. The rate of inoculation was thus between 0.1% (v/v) and 6.0% (v/v) with respect to the single strains. The mixed culture of *Strep. thermophilus* and *Lact. bulgaricus* was incubated at 42.5°C whereas the combinations of strains containing *Lact. acidophilus* and/or *Bifid. bifidum* at 37.5°C. Yeast extract (0.25 g l^{-1} from Merck Kft.) was added to the samples containing *Bifid. bifidum* so that the special nutritional requirements of this species would be satisfied. Acid production was determined by hourly pH measurements and growth was checked by enumeration of viable cells. All the experimental results are means of 3 replicates (n=3).

2.1.6 pH measurement

The pH of samples was measured at room temperature with a HANNA 8521 pH meter and combined glass electrode.

2.1.7 Enumeration of micro-organisms

The circumstances of the enumeration of micro-organisms are outlined in Table 4.

Table 4 Determination of viable cell counts of mixed culture components

	Mixed Culture Component	Culture Medium	Incubation		
			Conditions	Time (h)	Temp. (°C)
1	*Strep. thermophilus*	M 17 Agar	Aerobic	48	37
	Lact. bulgaricus	MRS Agar*	Anaerobic	72	37
2	*Strep. thermophilus*	M 17 Agar	Aerobic	48	37
	Lact. acidophilus	MRS Agar	Anaerobic	72	37
3	*Strep. thermophilus*	M 17 Agar	Aerobic	48	37
	Bifid. bifidum	MRS Agar	Anaerobic	72	37
4	*Lact. acidophilus*	MRS Agar	Aerobic	72	37
	Bifid. bifidum	MRS+NNL† Agar	Anaerobic	72	37
5	*Strep. thermophilus*	Lee's Agar	Aerobic	120	37

Lact. acidophilus	MRS-Maltose Agar	Anaerobic	72	37
Bifid. bifidum	MRS+NNLP‡ Agar	Anaerobic	72	37

*, Acidified to pH 5.4; †, Nalidixic acid + Neomycin sulfate + Lithium chloride; ‡, Nalidixic acid + Neomycin sulfate + Lithium chloride + Paromomycin sulfate

The pour-plate method was used in all cases. Oxoid anaerobic jars were employed, atmospheric oxygen being absorbed by means of ANAERO*GEN*^{TM} AN 25 paper sachets (from Oxoid Kft.). Components of the NNL and NNLP solutions were purchased from Sigma-Aldrich Kft. and all the other components of the culture media from Merck Kft.

2.2 Manufacture of a model product and storage experiments

The cyanobacterial and control yogurts needed for the storage experiments were manufactured in the pilot plant of the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár.

The composition of the raw material (i.e. pasteurized market milk) was identical with that of the UHT milk outlined in subchapter 2.1.1. Homogenization resulted in an average diameter of fat globules of <0.6 μm, without cluster formation. Heat treatment took 5 min at 90°C. FYE-43 yogurt starter culture (kindly supplied by the Hungarian Dairy Research Institute Inc. at Mosonmagyaróvár) was employed at a concentration of 3% (v/v). Incubation took 2.5 h at 42°C.

In the case of cyanobacterial yogurt the *Spir. platensis* biomass was added to the product when pH dropped to 5.2, at a temperature of 25°C; thereafter the normal technology of yogurt manufacture was followed. Both the cyanobacterial and the control samples were filled into 40 retail containers apiece, which were then sealed with aluminum foil. After a day's pre-cooling, half of the samples was placed into a cooling and heating thermostat at 15°C while the rest of them was stored in a refrigerator at 4°C. Three (n=3) retail containers apiece were opened after 0, 3, 6, 9, 12 and 15 days of storage in the case of cyanobacterial and control yogurt samples stored at 15°C and after 0, 7, 14, 21, 28 and 35 days of storage in the case of cyanobacterial and control samples stored at 4°C. The viable cell counts of the following micro-organisms and microbial groups were determined according to the standard methods of the International Dairy Federation: total microbial count, counts of *Strep. thermophilus*, *Lact. bulgaricus*, yeasts and molds, total enterococci and coliform organisms (Table 5).

Table 5 Enumeration of micro-organisms during the storage experiments

Micro-organism (Group)	Method	Culture Medium*	Incubation	
			Time (h)	Temp. (°C)
Strep. thermophilus	Pour-plate	M 17 Agar	48	37
Lact. bulgaricus	Pour-plate	MRS Agar†	72‡	37
Total Microbial Count	Addition∇	—	—	—
Yeasts and Molds	Pour-plate	YGC Agar	96	25
Coliform Organisms	MPN	BRILA Broth	24-48	37
Enterococci	Pour-plate	KEA Agar♦	72	37

*, All culture media were purchased from Merck Kft.; †, Acidified to pH 5.4; ‡, Incubated anaerobically; ∇, *Strep. thermophilus* count + *Lact. bulgaricus* count (calculated values); ♦, Kanamycin Esculin Azide Agar.

Along with the microbiological investigations, the pH of samples was measured so that data concerning the degree of post-acidification would also be gained.

2.3 Mathematical-statistical evaluation

The pH values of experimental samples and those of the corresponding controls were tested for significance of difference by means of the MicroCal Origin 3.0 program package. The terms “significantly (different)” or “significant (difference)” etc. mean that controls and test samples were significantly different at the P=0.05 level.

The average growth rate of the individual starter culture strains, during a given interval of time, was calculated by the formula $\mu_{i(y;x)} = (\log N_x - \log N_y) / (x-y) \cdot \log 2$, where $\mu_{i(y;x)}$ is the average growth rate of strain “i” during time interval y–x; N_x is the average viable cell count of strain “i” at time x; N_y is the average viable cell count of strain “i” at time y; x-y is the length of the interval of time studied, expressed in hour.

3 RESULTS

3.1 Effect of the *Spirulina platensis* biomass and that of its active components on acid development by single-strain type thermophilic dairy starter cultures in a model milk medium

The addition of cyanobacterial biomass resulted in increasing the rate of acid development by all four starter culture strains significantly, although to varying degrees.

The *Spir. platensis* biomass enhanced acid development by *Strep. thermophilus* significantly during hours 2-5 of the fermentation process. This increase in acid production was partly due to the presence of trace elements and to a greater extent to that of nitrogenous compounds (peptone, adenine, hypoxanthine). The addition of vitamins C, A and E, in which cyanobacteria are abundant, also resulted in a considerable drop in pH.

The cyanobacterial biomass stimulated acid production of *Lact. bulgaricus* to a greater extent than that of *Strep. thermophilus*. The increase in the rate of acid development during the main phase of fermentation could be attributed to the additive effect of nitrogenous substances (peptone, adenine, hypoxanthine). The acid tolerance of this moderate acid-producing strain was substantially improved by the cyanobacterial biomass, which could be partly accounted for by the stimulating effect of vitamin C on fermentation activity during the stationary phase of fermentation. The addition of inorganic selenium resulted in a small, although significant decrease in the rate of acid development.

Acid production of *Lact. acidophilus* was also stimulated significantly by the *Spir. platensis* biomass. Nitrogenous compounds (mainly peptone) and vitamin C were found to be the most stimulatory of all the individual substrates tested whereas the inhibitory effect of selenium and that of vitamin E were clearly visible too; i.e. among the antioxidants preventing membrane lipid peroxidation by free radicals, only vitamin C proved to stimulate acid production of *Lact. acidophilus* while the rest of them (vitamins A, E and selenium) retarded it to some extent. The B-complex vitamins reduced the rate of acid production as well.

Most of the substrates tested had similar effects on *Bifid. bifidum* and on *Lact. acidophilus*, although the two species differ widely in some of their major characteristics, including their metabolic system. However, adenine and hypoxanthine had an adverse effect or no effect at all on acid production of *Bifid. bifidum*. The influence of adenine employed in combination with peptone proved to be stimulatory on acid production, which was in contrast to what had been experienced when adenine being tested alone. Peptone was the only substance to stimulate acid development by *Bifid. bifidum* significantly but this stimulation did not account for the one caused by the cyanobacterial biomass. The *Spir. platensis* biomass must have also contained other effective components than peptone.

3.2 Effect of the *Spirulina platensis* biomass on acid production and growth of combinations of thermophilic dairy starter culture strains in a model milk medium

The effect of the cyanobacterial biomass on the combinations of strains could only partly be accounted for by what had been experienced with the single strains because the combinations of strains formed more complex systems than the individual strains.

Acid development by the mixed culture of *Strep. thermophilus* (0.5%, v/v) and *Lact. bulgaricus* (0.5%, v/v) was over 4 times higher in the cyanobacterial samples than in controls during the first 3 h of the fermentation process. This was due to the stimulatory effect produced by the cyanobacterial biomass on the growth of *Lact. bulgaricus*. As a result of this, the increase in average viable cell count of *Lact. bulgaricus* was 0.67 log cycle in the cyanobacterial samples and only 0.40 in the controls during the period mentioned. However, the *Spir. platensis* biomass had no influence on the growth of *Strep. thermophilus* in the course of this model experiment.

As for the combination of *Strep. thermophilus* (0.1%, v/v) and *Lact. acidophilus* (1.0%, v/v), the results showed an increase in the rate of acid production only 6-10 h after the start of fermentation, which was 2-2.5 times greater in the cyanobacterial samples than in controls. This could be accounted for by the significantly higher average growth rate and average viable cell count values of both *Strep. thermophilus* and *Lact. acidophilus* in the cyanobacterial samples than in controls during the first 10 h of fermentation.

The cyanobacterial biomass had no influence at all either on fermentation activity or on growth of the mixed culture of *Strep. thermophilus* (0.1%, v/v) and *Bifid. bifidum* (6.0%, v/v).

As regards acid development, the mixed culture of *Lact. acidophilus* (1.0%, v/v) and *Bifid. bifidum* (6.0%, v/v) gave results similar to those produced by the combination of *Strep. thermophilus* and *Lact. acidophilus* in the case of cyanobacterial and control samples alike. The *Spir. platensis* biomass had a stimulatory effect on the growth of *Lact. acidophilus* in both mixed cultures. It stimulated the acid production of this combination of strains by increasing the average growth rate and average viable cell count values of both *Lact. acidophilus* and *Bifid. bifidum* during the first 10 h of fermentation.

The average differences in pH between cyanobacterial and control samples inoculated with the mixed culture of *Strep. thermophilus* (0.1%, v/v), *Lact. acidophilus* (1.0%, v/v) and *Bifid. bifidum* (6.0%, v/v) were minimal. Although the acid-producing activity of *Bifid. bifidum* was not so pronounced as that of the other two starter culture organisms, its growth results were significantly better in the cyanobacterial samples than in controls. The growth of *Lact. acidophilus*, unlike that of *Strep. thermophilus*, was also stimulated significantly by the *Spir. platensis* biomass.

The results clearly indicate that the cyanobacterial biomass did not always have the same effect on the same strain in various mixed cultures. This is well illustrated by the example of *Strep. thermophilus*. As regards the mixed culture of *Strep. thermophilus* and *Lact. bulgaricus*, the *Spir. platensis* biomass had a significant stimulatory effect on acid development but it did not influence the growth of *Strep. thermohilus*. In the case of the combination of *Strep. thermophilus* and *Lact. acidophilus*, the cyanobacterial biomass stimulated both acid production and the growth rate of *Strep. thermophilus*. As for the mixed culture of *Strep. thermophilus* and *Bifid. bifidum*, neither acid production nor the growth of *Strep. thermophilus* was affected by the *Spir. platensis* biomass.

As can be seen from the results, it was the mixed cultures containing *Lact. bulgaricus* or *Lact. acidophilus* that the cyanobacterial biomass had the most stimulatory effect on. The growth rate of these two strains was accelerated in each case and *Bifid. bifidum* was also stimulated whenever it was combined with a rod-shaped starter organism (i.e. *Lact. acidophilus*). As to the mixed culture of *Strep. thermophilus*, *Lact. acidophilus* and

Bifid. bifidum, the growth of *Bifid. bifidum* was found to be significantly faster in the cyanobacterial samples than in controls. Since such an effect was not observed in the case of the combination of *Strep. thermophilus* and *Bifid. bifidum*, it is supposed that the growth rate of *Bifid. bifidum* was accelerated by certain metabolic products of *Lact. acidophilus* and/or by some specific nutrients found in the *Spir. platensis* biomass.

On the whole, the cyanobacterial biomass stimulated the rod-shaped starter bacteria to a greater extent than the coccus-shaped ones, but its effect on cocci was largely dependent on what kind of rod the cocci were combined with. In other words, the effect of the *Spir. platensis* biomass on mixed cultures also depended on the interaction between/among strains composing the mixed cultures.

3.3 Microbiological and physical-chemical changes in cyanobacterial and control yogurts during storage

Characteristic viable cell counts of over 10^8 cfu g^{-1} were found both in control and cyanobacterial yogurts, regardless of storage temperature. However, the viable cell counts were significantly higher in the cyanobacterial samples than in controls at 4 °C.

The storage temperature of 15°C resulted in considerable post-acidification in control and cyanobacterial yogurts alike while the pH of all the samples stored at 4°C remained above 4.0 during the entire storage period.

The count of yeasts and molds, both in the cyanobacterial and control samples, rose to a level of 10^1 cfu g^{-1} by the 6^{th} day and to a level of 10^5 cfu g^{-1} by the 15^{th} day of the storage period at 15°C; whereas the cyanobacterial yogurt, after one month of storage at 4°C, had a significantly lower count of yeasts and molds than the control yogurt. These results suggest that substance(s) possessing fungistatic properties can be found in the *Spir. platensis* biomass.

None of the samples contained enterococci or coliform organisms at either temperature during the storage period.

All things considered, the properly stored (cooled) cyanobacterial yogurt was found to have a shelf-life of one month, despite the fact that it had been produced according to regular technology of manufacture.

4 NEW FINDINGS

- The *Spir. platensis* biomass, which is rich in essential amino acids, trace elements, unsaturated fatty acids and vitamins, has a beneficial effect on the nutritional value of cow's milk.

- The cyanobacterial biomass significantly increases the rate of acid development by and growth rate of certain thermophilic dairy starter cultures (i.e. *Strep. thermophilus*, *Lact. bulgaricus*, *Lact. acidophilus*, *Bifid. bifidum*).

- In the case of combinations of strains, the *Spir. platensis* biomass stimulates the rod-shaped starter bacteria to a greater extent than the coccus-shaped ones, but its effect on cocci is dependent on what kind of rod the cocci are combined with. In other words, the effect of the cyanobacterial biomass on mixed cultures also depends on the interaction between/among strains composing the mixed cultures.

- The stimulating effect of the *Spir. platensis* biomass on thermophilic dairy starter organisms is largely due to nitrogenous substances (i.e. free amino acids, hypoxanthine, adenine).

- In certain instances, the vitamin (B-complex, C, A, E) and trace element (iodine, zinc, selenium) contents of the cyanobacterial biomass may have a stimulatory or retardative effect on the above-mentioned starter bacteria. However, this is of slight importance from a practical point of view.

- The *Spir. platensis* biomass inhibits the growth of yeasts and molds contaminating the fermented dairy products and it maintains the count of characteristic micro-organisms at a high level provided that the product is stored at a low temperature.

5 SUGGESTIONS (USE OF NEW FINDINGS FOR THEORETICAL AND PRACTICAL PURPOSES)

The use of *Spir. platensis* biomass for the manufacture of fermented milk products can be recommended for various reasons.

Owing to its composition, the cyanobacterial biomass increases the trace element and vitamin contents and improves the fatty acid composition of cow's milk. However, these beneficial effects are largely dependent on the employed concentration of the biomass. The effective and economic concentration resulting in good sensory properties has been found to be 3 g l^{-1}.

The abundance of bioactive components in the *Spir. platensis* biomass is of paramount importance from a nutritional point of view because the cyanobacterial biomass thus creates a new opportunity for the manufacture of functional dairy products.

The vitamins and trace elements are also beneficial components of the *Spir. platensis* biomass in that they have either slight or no effect on acid development by the starter cultures, that is to say the presence of these bioactive substances does not affect adversely the cyanobacterial biomass.

The significant stimulatory effect of the *Spir. platensis* biomass on acid production (and growth) of thermophilic dairy starter cultures is of practical importance because thus shorter time is needed for the manufacture of the same amount of fermented milk and consequently productivity will improve. Besides, a rapid rate of acid production prevents the growth of undesirable micro-organisms and is also essential for texture and flavor. Stimulation of growth and acid production is extremely important in the case of *Bifid. bifidum* because this species grows, and produces acetic and lactic acids, very slowly in milk. Considering this, the fact that *Bifid. bifidum* Bb-12 is stimulated by the *Spir. platensis* biomass to a very high degree can be regarded as one of the major findings of this work.

The discovery that, in mixed cultures, the cyanobacterial biomass stimulates the rod-shaped starter organisms to a greater extent than the coccus-shaped ones offers a new way of ensuring the optimal ratio of cocci to rods in the finished product.

By inhibiting the growth of yeasts and molds which contaminate the fermented dairy products and maintaining the count of characteristic micro-organisms at a high level during storage, the *Spir. platensis* biomass extends the shelf-life of products stored at a low temperature (i.e. 4^{o}C).

When the question of using the cyanobacterial biomass for the manufacture of fermented milk products is considered, the economic aspect of the issue must also be looked at. The current market price of the *Spir. platensis* biomass is 38-40 DM/kg. If roughly 3 g of biomass was employed for the making of 1 kg of finished product, the cost of production would increase by 0.12 DM/kg. In Germany, the sales value of functional (probiotic) fermented milks was 5.25 DM/kg in 1995 and 5.40 DM/kg in 1997 and it is estimated to be about 5.55 DM/kg in 1999. This means that the cost of the cyanobacterial biomass amounts to approximately 2% of the average selling price of probiotic fermented milks.

In several countries, consumers are wise enough to give credit to scientifically established and documented health claims. If dairy companies invest heavily in marketing communications, the market of functional products will grow dynamically year by year. A functional fermented milk, launched in the first half of the 1990s in Denmark, owing to its well-advertised health claims, became the biggest and fastest success ever of Denmark's

top dairy producer, despite a 70% price premium over regular yogurt. Under such conditions, an increase of a couple of per cents in the cost of production cannot be regarded as significant and the functional product is supposed to gain a high market share provided that the company has a proper marketing strategy. If the *Spir. platensis* biomass is used for the manufacture of regular yogurt, instead of a probiotic fermented milk product, the increase in the cost of production is relatively higher but the beneficial effect of the cyanobacterial biomass can be better highlighted in this case and thus the cyanobacterial yogurt can also be made marketable. Despite all the foregoing, the most important point is that consumers should be well-informed about nutritional issues, they should also be eager for new products and be able to afford to buy goods at prices higher than usual.

The situation is somewhat different in Hungary because the market of functional dairy products is not so lively as in the European Union or in North America. People do not receive enough information about nutritional issues, that is the reason why some experts have their doubts as to whether the majority of consumers would be ready to buy functional fermented milks possessing specific health benefits. The addition of *Spir. platensis* biomass would increase the cost of production to a greater extent in Hungary than in Germany since the current selling price of yogurt in Hungary is about 2-2.5 DM/kg on average. However, certain fancy yogurts might as well cost 3.5-4 DM/kg. Calculations show that, in Hungary, the cost of the cyanobacterial biomass, employed at a concentration of 3 g l^{-1}, amounts to approximately 5-6% of the average market price of flavored yogurts. Therefore, the *Spir. platensis* biomass should be used for the manufacture of dairy products which cost more than yogurts because people who spend on fancy foods are likely to be able to afford cyanobacterial products. Moreover, it is essential that consumers should be kept informed about nutritional issues through advertisements and newspaper/magazine articles so that functional dairy products will sell well when the purchasing power of people starts to increase.

6 SCIENTIFIC PUBLICATIONS AND LECTURES ON THE TOPIC OF THE Ph.D. DISSERTATION

1 LECTURES GIVEN AT SCIENTIFIC CONFERENCES AND SYMPOSIA

1.1 In Hungarian

1.1.1 Varga, L. (1993) Natúr és ízesített joghurtok hasznos élő mikrobaszámának alakulása és változása a tárolás alatt. *PATE Egyetemi Tudományos Diákköri Konferencia*. Előadás, Élelmiszeripari és Technológiai Szekció, Mosonmagyaróvár, 1993. március 17.

1.1.2 Krász, Á. & Varga, L. (1994) Mikrobiológiai változások hazai joghurtféleségekben. *Az MTA Szabolcs-Szatmár-Bereg Megyei Tudományos Testülete, a Bessenyei György Tanárképző Főiskola, a SCOPE Magyar Nemzeti Bizottsága (MTA Biológiai Tudományok Osztálya) és a Magyar Mikrobiológiai Társaság Mezőgazdasági és Élelmiszer-mikrobiológiai Szekciója IX. Mikrobiológiai Tudományos Ülése*. Előadás, Nyíregyháza, 1994. október 7-9.

1.1.3 Szigeti, J. & Varga, L. (1997) Megnövelt biológiai értékű savanyú tejkészítmények előállítása. *Szívbarát Program Pécsi Regionális Konferenciája* a Baranyatej Rt. rendezésében. Előadás, Pécs, 1997. november 7.

1.2 In English

1.2.1 Szigeti, J. & Varga, L. (1996) Effect of a microalgal biomass and that of its bioactive components on starter cultures used in the dairy industry. *11th Microbiological Scientific Session of the Scientific Body of Szabolcs-Szatmár-Bereg County of the Hungarian Academy of Sciences, Bessenyei György Teacher's College, the National Committee of SCOPE, the Agricultural and Food Microbial Section of the Hungarian Society for Microbiology and University of Nancy 1*. Lecture, Nyíregyháza, October 4-5, 1996.

2 ABSTRACTS OF LECTURES

2.1 In Hungarian

2.1.1 Holkovics, A. & Varga, L. (1992) Laktózmentes és galaktózszegény savanyú tejkészítmények előállításának néhány kérdése. *A MÉTE IX. Országos Tudományos Diákköri Konferenciája*. Az előadások tartalmi kivonatai, III. Mikrobiológiai és Biotechnológiai Szekció, Mosonmagyaróvár, 84-85.

2.1.2 Varga, L. (1993) Natúr és ízesített joghurtok hasznos élő mikrobaszámának alakulása és változása a tárolás alatt. *XXI. Országos Tudományos Diákköri Konferencia*. Agrártudományi Szekció

előadásainak összefoglalói, Élelmiszertudományi Tagozat, Budapest, 116.

2.1.3 Varga, L. (1994) Adatok a savanyú tejkészítmények mikroorganizmus számához és tárolás alatti változásához. *A MÉTE X. Országos Tudományos Diákköri Konferenciája*. Az előadások összefoglalói, I. Élelmiszerkémia és Biotechnológia Szekció, Budapest, 32-33.

2.2 Both in Hungarian and English

2.2.1 Varga, L. (1995) Tejcukormentes joghurtkészítmények előállításának kérdései (Some aspects of manufacturing lactose-free yoghurt preparations). *I. Országos Agrár Ph.D. Konferencia és Találkozó*. Az előadások összefoglalói, III. Élelmiszeripari Szekció, Debrecen, 74-75.

3 PAPERS PUBLISHED IN PROCEEDINGS

3.1 In English

3.1.1 Szigeti, J., Ördög, V., Varga, L. & Pulz, O. (1997) Cyanobacteria enriched with trace elements as additives for production of functional, acidified dairy products. *Mengen- und Spurenelemente, 17. Arbeitstagung*. Proceedings, Friedrich-Schiller-Universität, Jena, 239-247.

3.2 Both in English and Hungarian

3.2.1 Varga, L. & Szigeti, J. (1996) Néhány, biológiailag aktív komponens hatása savanyú tejtermékek jellemző mikroflórájára (Effect of some biologically active agents on the characteristic microbial flora of fermented dairy products). *XXVI. Óvári Tudományos Napok*. Új kihívások és stratégiák az agrártermelésben. Az előadások teljes terjedelemben megjelent anyagai, II. kötet, Élelmiszer-minőség Szekció, Mosonmagyaróvár, 426-428.

3.2.2 Varga, L. & Szigeti, J. (1998) Megnövelt biológiai értékű savanyú tejkészítmények előállítása (Manufacture of fermented dairy products of increased biological value). *XXVII. Óvári Tudományos Napok*. Új kihívások a mezőgazdaság számára az EU-csatlakozás tükrében. Az előadások teljes terjedelemben megjelent anyagai, IV. kötet, Minőségi Élelmiszer-előállítás Szekció, Mosonmagyaróvár, 893-898.

4 PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

4.1 In English

4.1.1 Varga, L. & Szigeti, J. (1998) Microbial changes in natural and algal yoghurts during storage. *Acta Alimentaria* **27** (2), 127-135. — Ref.: *Dairy Science Abstracts* (1999) **61** (5), 314.

4.1.2 Varga, L., Szigeti, J. & Ördög, V. (1999) Effect of a *Spirulina platensis* biomass and that of its active components on single strains of dairy starter cultures. *Milchwissenschaft* **54** (4), 187-190. — Ref.: *Dairy*

Science Abstracts (1999) **61** (8), 627.

4.1.3 Varga, L., Szigeti, J. & Ördög, V. (1999) Effect of a *Spirulina platensis* biomass enriched with trace elements on combinations of starter culture strains employed in the dairy industry. *Milchwissenschaft* **54** (5), 247-248. — Ref.: *Dairy Science Abstracts* (1999) **61** (9), 713.

5 PATENTS

5.1 International

5.1.1 Springer, M., Pulz, O., Szigeti, J., Ördög, V. & Varga, L. (1998) Verfahren zur Herstellung von biologisch hochwertigen Sauermilcherzeugnissen. *Patent* No. DE 196 54 614 A 1, 7 pp.

6 THESES AND SCIENTIFIC ESSAYS

6.1 In Hungarian

6.1.1 Varga, L. (1993) Natúr és ízesített joghurtok hasznos élő mikrobaszámának alakulása és változása a tárolás alatt. *Tudományos Diákköri Dolgozat*. Mosonmagyaróvár, 43 pp.

6.1.2 Varga, L. (1994) Joghurtféleségek mikrobaszáma és változása a tárolás során. *Diplomadolgozat*. Mosonmagyaróvár, 41 pp. — Ref.: *Tejgazdaság* (1994) **54** (1), 47-48.

6.2 Both in Hungarian and English

6.2.1 Varga, L. (1996) Egy új lehetőség komplettált savanyú tejtermékek előállítására (A new way of making fermented dairy products containing various vitamins, trace elements and other beneficial compounds by addition of a dehydrated algal biomass). *Szakmérnöki Diplomadolgozat*. Mosonmagyaróvár, 72 pp.