

Lactic-Acid Bacteria Supplement Fermented Dairy Products with Human Behavior-Modifying Neuroactive Compounds

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Abstract: Using high performance liquid chromatography, we established that probiotic *Lactobacillus* strains (*Lactobacillus helveticus* 100ash, *L. helveticus* NK-1, *L. casei* K₃III₂₄, and *L. delbrueckii* subsp. *bulgaricus*) grown on two milk-containing nutrient media produce important neuromediators such as biogenic amines, their precursors and deamination products, as well as neuroactive amino acids. The concentrations of biogenic amines (such as catecholamines and, with *L. helveticus* 100ash, also serotonin) equal or exceed those contained in the bloodstream of healthy adult humans, whereas those of most amino acids are comparatively low, except for gamma-aminobutyric acid (GABA). Of paramount importance is the fact that the bacterial cultures can release micromolar amounts of GABA and L-3,4-dihydroxyphenylalanine (DOPA) into the milk-containing media. It is known that DOPA passes through the gut-blood and the blood-brain barrier and converts into major neurotransmitters (dopamine and norepinephrine) that influence important aspects of human behavior. The data obtained suggest that dairy products fermented by live lactobacilli-containing starters are potential sources of human behavior-modifying substances.

Keywords: Microbial endocrinology, fermented dairy products, probiotic lactobacilli, *Lactobacillus helveticus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, neuromediators, catecholamines, neuroactive amino acids, behavior modification, aggressiveness, dominance, biopolitics.

INTRODUCTION

One of the most dynamically developing interdisciplinary areas of research is *microbial endocrinology* [1-10]. Microbial endocrinology is related to both microbiology and medicine and deals with animal hormones, neuromediators, and their analogs/homologues that are involved in intercellular communication in pro- and eukaryotic uni- and multicellular organisms. This work specifically focuses on neuromediators, compounds that carry out or regulate impulse transmission between nervous cells across the synaptic cleft. Neuromediators, including biogenic amines, amino acids, and neuropeptides, perform essential functions in the human organism, especially in the central nervous system where they regulate the sleeping—waking rhythm, hunger and satiation, mood, pain sensitivity, attention, concentration, memory, and sexual desire. They influence decision-making and problem-solving as well as various aspects of social behavior-related personal features including aggressiveness, dominance, and submissiveness.

Importantly, neuromediators are produced by a wide variety of symbiotic and opportunistic bacteria and

fungi [2-4, 5, 9, 11-17]. Since some of these microorganisms are widely spread in nature and represent typical inhabitants of the human/animal organism, the available data suggest that they exert a considerable regulatory influence on the nervous and the endocrine system. For instance, our studies with a commensal strain, *Escherichia coli* MC4100, revealed that its cultural fluid contains tens of nanomols of catecholamines (norepinephrine and dopamine), as well as much higher – micromolar – amounts of the catecholamine precursor L-3,4-dihydroxyphenylalanine (DOPA). *Bacillus cereus* that spoils food and causes food poisoning releases norepinephrine and DOPA into the medium [13, 18, 19]. As for neuroactive (regulatory) amino acids, suffice it to mention that the normal human gastro-intestinal (GI) microbiota produces the inhibitory neuromediator gamma-aminobutyric acid (GABA); if, under pathological conditions, the microbiota fails to supply the organism with GABA, irritated bowel syndrome (IBS) and still more serious health problems may develop (reviewed, [20]).

Foodstuffs consumed by humans exert a strong influence on their physiological, psychological, and behavioral characteristics. Apart from dietetics, medicine, and biotechnology, nutritional issues are of relevance to modern *biopolitics*, an interdisciplinary field that focuses on the relationship between human biology and social behavior as related to political

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activities [6, 7, 21-23]. This relationship is partly due to the impact of neuroactive compounds of microbial origin that are present in food items and influence the functioning of the brain and, therefore, human behavioral traits. Modifying foodstuffs with neurochemicals-producing microorganisms enables us to subtly manipulate the behavior of human individuals and groups, including the level of aggressiveness and the status in dominance-submission hierarchies. This subject is receiving increasing attention in the literature [4, 6, 8, 17].

Of special importance in nutritional terms are dairy products. Recently, it has been revealed that dairy products contain various compounds that are capable of regulating the functioning of the human nervous system [6, 8, 13]. For example, we have established that commercially produced fermented dairy products such as yogurts with live starter cultures contain a number of neuroactive substances that were present at concentrations of 0.2-2 μM (micromoles/l) [24]. They include biogenic amines such as norepinephrine and dopamine and also a number of amino acids including taurine and glycine. Of particular interest was the detection of high (micromolar) concentrations of DOPA in the tested yogurt samples. This catecholamine precursor can cross the gut-blood and the blood-brain barrier [25, 26]. In the brain, DOPA converts to dopamine, which can transform into norepinephrine. The catecholamines are involved in regulating locomotive activity and emotional responses to environmental stimuli; they influence such socially important human qualities as communicability, dominance, aggressiveness, sexuality, and others [6, 27, 28].

These data give grounds for the suggestion that fermented dairy products are a potentially important source of several types of neuromediators (biogenic amines and amino acids) and their microbes produce concentrations that are sufficiently high to modify the pool of neurotransmitters controlling the neurological status of the human organism.

The goal of this work was to determine whether lactobacillus bacteria used as live starter cultures in industrially produced dairy products synthesize and release major brain neuromediators.

MATERIALS AND METHODS

The following strains of lactobacilli were used in this work: *Lactobacillus helveticus* 100ash, *L. helveticus* NK-1, *L. casei* K₃III₂₄, and *L. delbrueckii* subsp.

bulgaricus. These cultures were obtained from the Russian State Collection of Human Normal Microbiota (Gabrichevsky Institute of Epidemiology & Microbiology). Each of the strains of lactobacilli was grown on rehydrated 1% milk with a fat content of 0.5% or on the PHM medium containing pancreatic hydrolysate of milk (500 ml), distilled water (500 ml), sodium chloride (4 g), peptone (2 g), lactose (10 g), cysteine hydrochloride (0.1 g), and agar (0.75 g); pH 6.8-7.2. The milk medium was employed by us because it is industrially used for the microbial production of fermented dairy products including the yogurts tested in our previous studies [24]. The milk hydrolysate-based medium (PHM) contained amino acids and short polypeptide fragments resulting from caseine hydrolysis; additional amino acids and peptides were added by supplementing the PHM medium with peptone. This medium was used because it was enriched in "raw materials" from which living cells enzymatically synthesize biogenic amines, e.g., catecholamines and serotonin that are produced from the amino acids tyrosine and tryptophan, respectively. The other components contained in PHM were responsible for adjusting the osmolarity, redox potential, and acidity of this medium.

The bacteria were cultivated for 6 hours under microaerophilic conditions at 37°C; the inoculum dose was 1.0×10^7 CFU/ml. After 6 hours of cultivation, the medium pH value was 3.5-4.5 and all tested strains formed a more or less dense clot; the cultivation was terminated by cooling the culture to +4-6°C. The CFU number of lactobacilli was determined by diluting culture samples in pre-reduced peptone saline containing 0.5 g/L cysteine/HCl (pH 6.3) and plating them on MRS agar medium; the plates were incubated under microaerophilic conditions at 37°C for 48 hours. The CFU number before the termination of the fermentation process was $4.5-5.5 \times 10^8$ CFU/ml.

The contents of biogenic amines including catecholamines (dopamine, DA, and norepinephrine, NE) and serotonin (5-HT), their precursors (L-3,4-dihydroxyphenylalanine, DOPA, and 5-hydroxytryptophan, 5-HTP), and metabolites (3,4-dihydroxyphenylacetic acid, DOPAC, homovanillic acid, HVA, and 5-hydroxyindolylacetic acid, 5-HIAA) as well as neuroactive amino acids such as aspartic acid, glutamic acid, glycine, taurine, and γ -aminobutyric acid (GABA) were determined in the tested systems (sterile milk; PHM medium; or bacterial cultures grown on these media). In some experiments, bacterial biomass was separated by centrifugation (8000g; 20 min); the

culture fluid supernatant (CF) was passed through a millipore filter (pore diameter 0.22 μ). The wet biomass sediment was sonicated (Braunsonic 1510, USA) at 22 KHz; four to eight thirty-second pulses of sonication were carried out with one-minute spaces between them; the sonication was performed at 0°C. The completeness of biomass disintegration was assessed with a light microscope at a magnification of 1000; the share of broken cells exceeded 90% in all samples.

The sonicated biomass (SM) was centrifuged (8000g; 20 min), and the SM supernatant, the culture fluid supernatant, and the sterile media (milk and PHM) were used for high-performance liquid chromatography (HPLC) with an amperometric detection system. In a complex mixture of organic substances, the amperometric method provides for a high sensitivity of the detection system to extremely low concentrations of compounds of interest while its sensitivity threshold for the other components of the mixture is significantly higher [29].

The amperometric detection of HPLC-separated compounds is based upon measuring the electric current that is caused by oxidizing or reducing the tested compounds on the surface of the working electrode while a certain voltage is generated between the working electrode and the reference electrode [29]. The particular class of compounds that is detected in this work, biogenic amines and their derivatives, are oxidized when they come into contact with the working glass-carbon electrode; for instance, catecholamines, e.g., dopamine, convert into *p*-quinonimines. In addition to the compounds to be chromatographically separated and electrochemically detected, the tested solution contains an internal standard, 3,4-dihydroxybenzylamine (DHBA), at a fixed concentration of 0.5 μ M/l. The location and size of the DHBA peak on the chromatogram helped us calibrate the detection system.

An LC-304T chromatographer (BAS, West Lafayette, CUSA) with a Rheodyne 7125 injector was employed; the volume of the loop used for applying samples was 20 μ l. The tested biogenic amines were separated on a reverse-phase ReproSil-Pur column (ODS-3, 4x100 mm, 3 μ ; Dr. Majsch GMBH, Elsico, Moscow). A PM-80 pump (BAS, USA) was used; the elution rate of the mobile phase was 1.0 ml/min at a pressure of 200 atm. The mobile phase contained 0.1 M citrate-phosphate buffer with 1.1 mM octanesulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH 3.0). The measurements were carried out using an LC-4B electrochemical detector (BAS, USA) with a glass-carbon electrode (+0.85 V

against an Ag/AgCl reference electrode. The samples were scanned with the Multichrome 1.5 (Ampersand) hardware—software system. All reagents used for the assay were analytical grade. The chromatographer was calibrated using a mixture of the tested biogenic amines; the concentrations of all these substances were 0.5 μ M. The amine concentrations contained in the samples were calculated by the internal standard method that is based on determining the ratio between the peak area in the standard mixture and that in the samples, whereas the correct location and size of the peak of the internal standard (DHBA) demonstrated that the detection system was adequately calibrated [30].

The content of neuroactive acids (GABA, glycine, taurine, aspartic acid, and glutamic acid) in the experimental samples was determined by HPLC with a fluorimetric detection system using an LC-304T chromatographer (BAS, West Lafayette, USA) with a Phenomenex column (C18, 4 × 150 mm, 4 μ). A standard method modified by [31] was applied. The tested amino acids are weak chromophores, i.e., they do not emit or absorb UV light. Therefore, in order to reliably detect these amino acids, the samples were derivatized. For this purpose, we used *o*-phthalic aldehyde that forms a fluorescent complex with each of the tested amino acids. Derivatization was performed by supplementing 5 μ l of the dialysate with 10 μ l of 0.1 M borate buffer and 10 μ l of *o*-phthalic aldehyde-sulfate reagent in 0.1 M borate buffer (pH 9.5). 0.01 μ M GABA, aspartic acid, glutamic acid, taurine, and glycine in 0.1 N HClO₄ were used as a standard calibration mixture. After 15 min of incubation at room temperature, 20 μ l of dialysate were applied to an Agilent Hypersil ODS column (5 μ M, 4.6 x 250). The substances separated were determined using an Agilent 1100 fluorescence detector (USA) with excitation and emission wavelengths of 230 and 392 nm, respectively. The mobile phase for determining the neuroactive amino acids contained 0.06 M NaH₂PO₄ x H₂O, 3.2 mM Na₂HPO₄, 0.025 mM EDTA, and 1.24 mM CH₃OH (pH 5.6). The rate of the mobile phase was 1.5 ml/min.

The results obtained were statistically processed; the data given in the tables below are the means of 4-6 independent measurements.

RESULTS

Using HLPC, we established that both milk and the PHM medium contain a number of major neuroactive compounds; generally, their concentrations were higher

in the PHM medium than in the milk. The tested starter cultures (*L. helveticus* 100ash, *L. helveticus* NK-1, *L. casei* K₃III₂₄, and *L. delbrueckii* subsp. *bulgaricus*) significantly changed the neuromediators' concentrations in the two media. Predominantly, they increased these concentrations. Quantitative data on the concentrations of biogenic amines and amino acids are given in Tables 1-2 and Table 3, respectively.

L-3,4-Dihydroxyphenylalanine (DOPA): All tested cultures enriched both milk and PHM with DOPA, the catecholamine precursor (Table 1). This effect is particularly significant with *L. casei* K₃III₂₄ grown on milk and with *L. helveticus* NK-1 grown on the PHM medium. DOPA concentrations detected in total culture and culture fluid samples were similar. The biomass (intracellular content obtained by sonication) lacked DOPA, which suggests that DOPA is an extracellular compound in the lactobacillus cultures.

Dopamine (DA): The DA concentration slightly but statistically significantly increased (from 40 to 60–70 nM) if *L. helveticus* NK-1 and *L. delbrueckii* subsp. *bulgaricus* were cultivated in milk. The PHM medium initially (prior to inoculation) contained ~6 μM DA (Table 1), and the DA concentration decreased to zero

in the presence of *L. helveticus* 100ash cells and to 0.5–2.5 μM with the other tested cultures, i.e. the bacteria seemed to take up DA from the medium. Most DA was contained in the culture fluid; its intracellular content was zero in *L. helveticus* strain 100ash and 10–100 nM in the other tested probiotic cultures.

Norepinephrine (NE): All tested bacterial cultures (except *L. casei* K₃III₂₄) caused a statistically significant increase in the NE concentration in both media. When cultivated on PHM, *L. casei* K₃III₂₄ decreased the NE concentration, compared to the control sample. Like DA, NE was predominantly present in the culture fluid, and only a minor part of its pool appeared to bind to bacterial cell structures (see data on the intracellular content).

3,4-Dihydroxyphenylacetic Acid (DOPAC): In the presence of all tested bacterial strains, the concentration of DOPAC, the product of oxidative deamination of DA, increased 3–5 fold in milk. Conversely, the cultivation of the lactobacillus strains in the PHM medium, which initially contained much DOPAC (~1 μM), resulted in decreasing the DOPAC concentration 1.5–10fold, both in the total culture samples and in the bacterial cell-free culture fluid.

Table 1: Catecholamines, their Precursor (DOPA), and Metabolites in Milk, Pancreatic Hydrolysate of Milk-Containing Medium (PHM), and Cultures of Lactobacilli

| Strains, Substrates & Fractions | | | Catecholamine Pathway | | | | |
|--|------|-------|-----------------------|-----------|-----------|-----------|-----------|
| | | | DOPA | DA | NE | DOPAC | HVA |
| No Bacteria | Milk | | 0.05±0.03 | 0.04±0.01 | 0.02±0.01 | 0.03±0.01 | 0 |
| | PHM | | 0.70±0.10 | 6.17±0.50 | 0.69±0.05 | 1.20±0.25 | 0 |
| <i>L. helveticus</i> 100ash | Milk | Total | 0.42±0.20 | 0.04±0.20 | 0.15±0.04 | 0.15±0.03 | 0 |
| | | Total | 1.50±0.30 | 0 | 0.09±0.02 | 0.18±0.02 | 0.08±0.02 |
| | PHM | CF | 1.82±0.30 | 0.05±0.01 | 0 | 0.22±0.08 | 0.10±0.03 |
| | | Cells | 0 | 0 | 0.10±0.02 | 0 | 0 |
| <i>L. helveticus</i> NK-1 | Milk | Total | 0.08±0.02 | 0.06±0.01 | 0.12±0.03 | 0.14±0.03 | 0 |
| | | Total | 5.37±1.00 | 2.53±0.75 | 2.32±0.50 | 0.98±0.30 | 0 |
| | PHM | CF | 3.71±1.00 | 2.11±0.70 | 1.94±0.45 | 1.48±0.35 | 0 |
| | | Cells | 0 | 0.04±0.01 | 0.10±0.02 | 0 | 0 |
| <i>L. casei</i> K ₃ III ₂₄ | Milk | Total | 0.56±0.25 | 0.03±0.01 | 0.03±0.01 | 0.15±0.04 | 0 |
| | | Total | 1.62±0.25 | 0.59±0.10 | 0.27±0.06 | 1.04±0.20 | 0 |
| | PHM | CF | 2.78±0.90 | 0.22±0.04 | 0.22±0.06 | 1.08±0.25 | 0 |
| | | Cells | 0 | 0.10±0.02 | 0.14±0.03 | 0 | 0 |
| <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> | Milk | Total | 0.11±0.03 | 0.07±0.01 | 0.16±0.05 | 0.11±0.01 | 0 |
| | | Total | 4.00±1.00 | 2.09±0.20 | 3.47±0.55 | 0.86±0.15 | 0 |
| | PHM | CF | 3.40±0.80 | 1.28±0.65 | 2.17±0.25 | 1.08±0.25 | 0 |
| | | Cells | 0 | 0.05±0.01 | 0.09±0.02 | 0 | 0 |

All concentrations are expressed in micromoles per l (μM). Designations: CF, culture fluid supernatant; Cells, intracellular content of the tested substances; DOPA, L-3,4-dihydroxyphenylalanine; DA, dopamine; NE, norepinephrine (noradrenaline); DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid.

Homovanillic Acid (HVA): HVA, another catecholamine metabolite, was only detected in the total sample of the fermented product and in the culture fluid of *L. helveticus* 100ash. It appears, therefore, to function as an extracellular compound.

5-Hydroxytryptophan (5-HTP): 5-HTP, the precursor of serotonin, was not detected in milk prior to inoculation. After cultivating the *L. helveticus* 100ash strain on milk, an atypically shaped peak appeared in the chromatogram close to the point where the 5-HTP peak should be located. These data on the presence of 5-HTP in this sample should be regarded as dubious. Our results concerning the low concentrations of a 5-HTP-similar substance determined in the PHM medium and in PHM-grown bacterial cultures have also been discarded by us as unreliable data.

Serotonin (5-Hydroxytryptamine, 5-HT): 0.4–0.5 μM 5-HT was detected in the total sample and the culture fluid of the *L. helveticus* 100ash strain cultivated on PHM medium.

5-Hydroxyindolylacetic Acid (5-HIAA): Both media lacked this oxidized product of 5-HT. Tens of

manomoles of 5-HIAA were detected in the biomass, the culture fluid, and the total samples of *L. helveticus* NK-1 or *L. delbrueckii* subsp. *bulgaricus* cultivated on these media. Since the cultures of these bacteria lacked 5-HT, it is difficult to account for the accumulation of its oxidized product. Presumably, the sensitivity threshold of the HPLC method was too high to detect 5-HT in the cultures; alternatively, all 5-HT could rapidly convert to 5-HIAA during the first hours of cultivation.

Aspartic Acid: This neuroactive amino acid was present in milk (prior to inoculation) at a concentration of 20–30 nM; the introduction of *L. helveticus* 100ash or *L. helveticus* NK-1 into the milk did not significantly change the concentration of this compound; whereas inoculating the two other strains resulted in completely removing aspartic acid from the medium. Aspartic acid was not detected in PHM whether with or without the tested lactobacilli.

Glutamic Acid was present in the milk medium at a concentration of 0.2 μM ; all tested bacterial strains enriched milk in glutamic acid. The level of glutamic

Table 2: Serotonin (5-Hydroxytryptamine), its Precursor, and Metabolite in Milk, Pancreatic Hydrolysate of Milk-Containing Medium (PHM), and Cultures of Lactobacilli

| Strains, Substrates & Fractions | | | Serotonin Pathway | | |
|--|------|-------|-------------------|-----------|-----------|
| | | | 5-HTP | 5-HT | 5-HIAA |
| No Bacteria | Milk | | 0 | 0 | 0 |
| | PHM | | 0 | 0 | 0 |
| <i>L. helveticus</i> 100ash | Milk | Total | 1.20±0.25* | 0 | 0 |
| | PHM | Total | 0.49±0.05* | 0.40±0.15 | 0 |
| | | CF | 0.47±0.05* | 0.47±0.08 | 0 |
| | | Cells | 0 | 0 | 0.04±0.01 |
| <i>L. helveticus</i> NK-1 | Milk | Total | 0 | 0 | 0 |
| | PHM | Total | 0.77±0.30* | 0 | 0.05±0.02 |
| | | CF | 0.79±0.35* | 0 | 0.07±0.02 |
| | | Cells | 0 | 0 | 0.03±0.01 |
| <i>L. casei</i> K ₃ 111 ₂₄ | Milk | Total | 0 | 0 | 0 |
| | PHM | Total | 0.26±0.10* | 0 | 0 |
| | | CF | 0.17±0.05* | 0 | 0 |
| | | Cells | 0 | 0 | 0.06±0.01 |
| <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> | Milk | Total | 0 | 0 | 0 |
| | PHM | Total | 0 | 0 | 0.08±0.02 |
| | | CF | 0 | 0 | 0.05±0.02 |
| | | Cells | 0 | 0 | 0.05±0.02 |

All concentrations are expressed in micromoles per l (μM). Designations: CF, culture fluid supernatant; Cells, intracellular content of the tested substances; 5-HTP, 5-hydroxytryptophan; 5-HT, serotonin (5-hydroxytryptamine); 5-HIAA, 5-hydroxyindolylacetic acid.

Note: the data marked with an asterisk (*) are unreliable (see text).

Table 3: Neuroactive Amino Acids in Milk, Pancreatic Hydrolysate of Milk-Containing Medium (PHM), and Cultures of Lactobacilli

| Strains, Media & Fractions | | Aspartate | Glutamate | Glycine | Taurine | GABA | |
|--|------|-----------|-----------|-----------|-----------|-----------|-----------|
| No Bacteria | Milk | 0.02±0.01 | 0.20±0.03 | 0.13±0.02 | 0.13±0.02 | 0.02±0.01 | |
| | PHM | 0 | 0.09±0.02 | 0.21±0.02 | 0.88±0.03 | 0.92±0.05 | |
| <i>L. helveticus</i> 100ash | Milk | Total | 0.03±0.01 | 0.52±0.02 | 0.31±0.10 | 0.39±0.03 | 0.24±0.02 |
| | | Total | 0 | 0.15±0.03 | 0.28±0.03 | 0.94±0.10 | 0.63±0.10 |
| | PHM | CF | 0 | 0.15±0.03 | 0.27±0.03 | 1.03±0.10 | 0.88±0.04 |
| | | Cells | 0 | 0.04±0.01 | 0.04±0.01 | 0 | 0 |
| <i>L. helveticus</i> NK-1 | Milk | Total | 0.03±0.01 | 0.59±0.04 | 0.29±0.10 | 0.42±0.03 | 0.16±0.02 |
| | | Total | 0 | 0.18±0.04 | 0.30±0.06 | 0.93±0.10 | 0.82±0.05 |
| | PHM | CF | 0 | 0.16±0.02 | 0.27±0.02 | 1.06±0.06 | 0.80±0.04 |
| | | Cells | 0 | 0.08±0.02 | 0.06±0.01 | 0 | 0 |
| <i>L. casei</i> K ₃ III ₂₄ | Milk | Total | 0 | 0.62±0.10 | 0.24±0.06 | 0.47±0.05 | 0.26±0.02 |
| | | Total | 0 | 0.14±0.02 | 0.26±0.06 | 0.97±0.10 | 0.84±0.03 |
| | PHM | CF | 0 | 0.15±0.03 | 0.22±0.05 | 0.95±0.10 | 0.80±0.10 |
| | | Cells | 0 | 0.08±0.02 | 0 | 0.03±0.01 | 0 |
| <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> | Milk | Total | 0 | 0.53±0.10 | 0.28±0.10 | 0.38±0.07 | 0.32±0.02 |
| | | Total | 0 | 0.17±0.02 | 0.25±0.06 | 0.93±0.08 | 0.90±0.04 |
| | PHM | CF | 0 | 0.16±0.02 | 0.24±0.06 | 0.95±0.10 | 0.85±0.03 |
| | | Cells | 0 | 0.04±0.01 | 0 | 0 | 0 |

All concentrations are expressed in micromoles per l (μM). Designations: CF, culture fluid supernatant; Cells, intracellular content of the tested substances; GABA, γ-aminobutyric acid.

acid in the fermented milk reached 0.5-0.6 μM. Approximately 0.1 μM glutamic acid was detected in PHM; the glutamic acid concentration increased ~1.5fold during the growth of all strains of lactobacilli. Glutamic acid was predominantly present in the culture fluid and apparently was bound to cells to an insignificant extent.

Glycine was present in milk and PHM at concentrations of ~0.1 and ~0.2 μM, respectively. Milk was enriched in glycine to a larger extent than PHM during the growth of all tested bacterial strains. Most glycine was contained in the cultural fluid.

Taurine was detected in milk at a concentration of about 0.1μM; all tested bacteria increased its concentration to 0.4—0.5 μM. Approximately 1 μM taurine was present in PHM initially; its concentration did not significantly change during the growth of all tested cultures. Taurine was mainly contained in the culture fluid.

γ-Aminobutyric Acid (GABA) was present in milk at low concentrations (~20 nM); all tested cultures significantly enriched this medium in GABA; the

maximum increase was attained with *L. delbrueckii* subsp. *bulgaricus* that brought the GABA concentration up to 0.3 μM. GABA was contained in the culture fluid and, therefore, not bound to microbial cells.

DISCUSSION

Our research provides evidence that fermented milk products containing live lactobacilli are considerably enriched in neuroactive compounds including catecholamines (dopamine and norepinephrine), their precursor (DOPA), and their oxidation products; serotonin only appears in the culture of *L. helveticus* 100ash cultivated on the PHM medium.

Interestingly, the concentrations of neuroactive compounds produced by the tested starter cultures are similar to those that are characteristic of the human bloodstream or even exceed these concentrations. Human blood contains [25, 27, 32], on average, ~10 nM DOPA, 0.1-0.5 nM DA (DA in the free form; human blood also contains ~20-30 nM sulfoconjugated DA [32]) and about 1 nM NE. The fermented milk products inoculated by all tested bacteria contained ~0.5-5 μM DOPA, with the NK-1 strain on PHM yielding the

maximum amount of DOPA, $5.37 \pm 1.00 \mu\text{M}$ (Table 1). *L. helveticus* NK-1 and *L. delbrueckii* subsp. *bulgaricus* increased the DA concentration from 0.04 to 0.06 μM , i.e., by 20–30 nM, and most tested strains added some 0.1 μM NE to the milk medium (see Table 1). The blood concentration of the dopamine metabolite DOPAC mostly is within the 10-20 nM range [32]; the DOPAC concentration with the tested microbial cultures was approximately within the same range (with NK-1 and *L. bulgaricus* on PHM) or at a somewhat lower level. Human blood also normally contains 0.5-1.5 μM 5-HT [33], and the 0.4-0.5 μM 5-HT level we detected in the culture fluid of *L. helveticus* 100ash is close to its minimum amount in the bloodstream.

Importantly, microbial cultures accumulate DOPA in milk and the pancreatic milk hydrolysate medium. As mentioned in the Introduction, the catecholamine precursor can penetrate into the brain tissue [25, 26] where it transforms into catecholamines that perform essential functions as major brain neuromediators. Normalizing the brain levels of DOPA and the neurochemicals produced from it helps overcome depression, adynamia, and other negative consequences of stress [6, 27, 28].

Of some interest is the accumulation of neuroactive amino acids (glutamic acid, glycine, taurine, and GABA) during the cultivation of starter cultures of lactobacilli in the milk-containing media. The tested bacteria produce submicromolar (over 0.1 μM) amounts of neuroactive amino acids (except for aspartic acid). However, human blood plasma normally contains tens to hundreds of micromoles of most amino acids [26]. Therefore, the microbially produced concentrations of them seem insignificant in neurophysiological terms.

Nevertheless, GABA is contained in the human organism in amounts (about 0.6 μM in blood plasma and 0.3 μM in cerebrospinal fluid [34]) that are close to those released by the lactobacilli. For instance, our strain of *L. delbrueckii* subsp. *bulgaricus* produced $0.32 \pm 0.02 \mu\text{M}$ on the milk medium. It is known that GABA, an inhibitory amino acid, produces a relaxing and pacifying effect; it is used as a tranquilizer which causes no addiction. GABA improves concentration and memory and normalizes the sleeping rhythm. It promotes the restoration of the locomotion- and speech-controlling neural networks after an injury and stimulates the metabolic processes in the brain that are involved in utilizing glucose and removing toxic metabolites [6, 31, 35].

The results obtained indicate that fermented dairy products and their starter cultures, including those used as probiotics, can serve as sources of behavior-modifying neurochemicals having the potential to modify the operation of the human brain and, therefore, to significantly influence human behavior. This conclusion is in line with the suggestion of a number of microbiologists and clinicians that gut symbiotic microorganisms-based probiotics can be used to modify the neurological and mental state of humans, e.g., for the purpose of decreasing the risk of neurological diseases [3, 4, 15-17, 36, 37]. Our data also demonstrate that functionally specialized food items can be developed that produce significant effects on the human brain, the mind, and social behavior. *Biopolitical* measures should be taken on the global scale that would enable us to efficiently monitor the concentrations of neuroactive compounds in dairy products and other food items.

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