UDC [613.292-021.632]:[579.864+579.873]:159.942.53

PSYCHOBIOTICS – PROBIOTICS THAT ELEVATE MOOD

L. Kapreliants, Doctor of Technical Sciences, Professor
E. Zhuk, Postgraduate
Department of Biochemistry, Microbiology
And Physiology of Nutrition
Odessa National Academy of Food Technologies
112, Kanatna Str., Odessa, Ukraine, 65039

DOI: https://doi.org/10.15673/fst.v15i1.1969

Article history

Received 25.06.2020
Reviewed 11.08.2020
Revised 15.12.2020
Approved 03.02.2021

Correspondence:

L. Kapreliants
E-mail: leonid@onaft.edu.ua

Cite as Vancuver style citation

Kapreliants L, Zhuk E. Psychobiotics – probiotics that elevate mood. Food science and technology. 2021;15(1):
4-14. DOI: https://doi.org/10.15673/fst.v15i1.1969

Цитування згідно ДСТУ 8302:2015

Kapreliants L., Zhuk E. Psychobiotics – probiotics that elevate mood // Food science and technology. 2021. Vol. 15, Issue 1. P. 4-14
DOI: https://doi.org/10.15673/fst.v15i1.1969

Abstract. The human intestinal microbiome includes all microorganisms inhabiting the intestinal tract. One of studies showed that mice raised in a sterile environment exhibited excessive physiological responses to stress, as compared with the normal reference animals. This discovery revealed the involvement of the microbiome in the development of the brain – gut – microbiota axis. Since then, it has been found that intestinal bacteria are involved in regulating diverse and important physiological processes, including immunomodulation, obesity, energy balance, and the function of the nervous system. All these studies have allowed identifying a new type of probiotics – psychobiotics. Psychobiotics can produce a positive effect on a patient's mood. Attempts to reveal the internal mechanisms of positive emotion shifts in people are of a great interest. It has been confirmed that psychobiotics can influence the depth of emotions. The main neurotransmitter that acts as an inhibitor in the brain of humans and animals is γ-aminobutyric acid (GABA). It is involved in metabolic and neurotransmitting processes in the brain. The precursor of GABA is glutamate. The GABA-ergic system of the brain structurally resembles all the others: a number of deep-lying structures in the brain, from which the nerve fibres that secrete GABA run to other parts of the nervous system. Thus, GABA is an inhibitory neurotransmitter that regulates many processes, from muscle tone to emotional reactions. Many studies have shown that some microorganisms can produce GABA that acts on the human body in the same way as GABA produced in the human brain does. The main producers of GABA, according to the studies, are lactic acid bacteria, and among them, the most capable are those of the genus *Lactobacillus helveticus*. According to the WHO standards, new strains of probiotics should be clearly identified to be further used as dietary supplements or drugs. They must be able to survive in the gastrointestinal tract (be resistant to pH, enzymes, cholic acids, etc.), they must also be capable of adhesion, exhibit antagonistic properties, and be genetically stable. So, all strains supposed to be used in the production of probiotics must be clearly identified at the species level and have a genetic passport.

Identifying and introducing new probiotic strains able to produce psychobiotic substances (GABA) is a painstaking task. It is a very important one, though, because stress, depression, and many other mental disorders are becoming more common in the world, and Ukraine is no exception.

Keywords: microbiome, microbiota, probiotic, psychobiotic, axis Brain – Gut – Microbiota, γ-aminobutyric acid.

Introduction. Formulation of the problem

Environmental degradation, constant stress, and other global factors negatively affect people. Taking into account all of the above factors, probiotic functional foods aimed at increasing human metabolism have been produced on an industrial scale for a long time. This market niche is constantly expanding due to high consumer demand. Scientists and manufacturers around the world are actively looking for new strains of probiotic microorganisms with more active biotechnological potential. Ukraine is not the last country in researching and introducing new biotechnologies that allow manufacturing probiotic drugs and dietary supplements.

It has long been established that the human body cannot exist and function without the presence of microorganisms. For every cell in our body, there are ten bacterial cells and 100 bacterial genes for each gene. Most microcultures are bacteria, but yeast fungi are found, too. It is interesting that in a healthy person's body, there are a trillion beneficial microorganisms (85%) and one hundred and fifty billion pathogens (15%). Throughout their lives, they

fight against one another. There are natural ways to restore intestinal microbiota. The simplest method is enriching the diet with natural sources of beneficial microcultures. However, this is not a quick process. To accelerate the restoration of the bacterial balance in the body, it is recommended to resort to preparations containing probiotics [1]. The positive effect of probiotic drugs is due to the action of lactobacilli, bifidobacteria, enterococci, *Escherichia coli*, etc. on the human body. Beneficial microcultures have a variety of physiological functions. They colonise the large intestine with normal microbiota, neutralise pathogenic bacteria, viruses, and fungi, inhibit their growth, prevent relapses of dysbiosis, stimulate the immune response to pathogens (promote production of antibodies), strengthen the intestinal mucosa, block the synthesis of toxins by pathogenic microbiota, lower the concentration of cholesterol in the blood (by improving the breakdown of bile salts), normalise the intestinal motor function, relieve bloating and flatulence, supply the body with vitamins that keep the nervous system functioning and the skin healthy, prevent anaemia, improve the balance between pathogens and normal microbiota in favour of the latter, suppress the activity of the bacteria *Helicobacter pylori*, which cause chronic gastritis and peptic ulcer, help to digest food, protect intestinal tract cells from degeneration, regulate the absorption of vitamins, minerals, gases, and water, prevent eczema, dermatitis, and bronchial asthma. A probiotic not only makes up for the lack of beneficial microbiota in the body, but also has a complex healing effect. It enhances the effectiveness of bacteriophages, antibiotics, protects against carcinogens, allergens, toxins, and improves metabolic processes.

Scientists estimate that each adult carries about 2 kg of microbes. The first bacteria inoculate a newborn organism as early as when the baby is being given birth to, and then the bacterial community does not leave us until the end of life [2]. The system of biomedical sciences views microbiota as a historically formed set of microorganisms, united by a common distribution area, which is, according to modern concepts, one of the leading systemic factors affecting health and morbidity – not only due to the microbiological effect per se, but also because of the inclusion in the relationship of biopsychosocial mechanisms of pathogenesis. The knowledge accumulated for more than a century of studying the human gastrointestinal microbiota has shown that this ecosystem is indeed a forgotten organ of the human body. No doubt, further study of the gastrointestinal microbiota will improve our understanding of this ecosystem [3].

The relationship of the human microbiome with a number of immune and idiopathic diseases is being intensively researched today. This research is a popular trend in the development of functional medicine. It has been established that there is a relationship between the microbiome and recovery processes in many chronic diseases. Assessment of the quality and composition of populations of microorganisms inhabiting us can provide answers to many questions.

According to the WHO report [4], Ukraine ranks first in the number of people suffering from depression, stress, and anxiety (every third Ukrainian is mentally ill). It is noted that more than 6% of the population of Ukraine suffer from depression, and more than 3% suffer from anxiety neurosis. Besides, the WHO notes that the number of people with depression has increased significantly over the past decade. According to the document, more than 300 million people worldwide suffer from this disease and from anxiety neurosis. It is noted that depression spreads to all segments of the population, but it mostly occurs in women after 50 years. Depression is also common in children and adolescents under 15.

The review notes that depressive disorders are characterised by anxiety, loss of interest in anything, feeling of guilt or low self-esteem, disturbed sleep and appetite, constant fatigue. Depression can be persistent or recurrent.

This disease greatly affects a person's performance at work and at school, and in too severe forms can lead to suicide. Doctors note that depression is the result of a complex interaction of social, psychological, and biological factors [5,6].

Considering all the above facts, it becomes clear that today, both throughout the world and in Ukraine, there is a need to search for a new type of probiotic, which, besides the properties characteristic of all probiotics, would also be able to influence a person's psychological state. Thus, **the purpose** of our research is to find, study, and introduce a new probiotic bacterial strain with so-called psychobiotic properties.

The interconnection between intestinal microbiota and the brain

Traditionally, the main functions of the microbiota are maintaining the homeostasis of the internal environment, metabolism [7,8], and immunomodulatory function. However, most often, these functions of the microbiota are considered in the context of its role in gastroenterology, since the microbiotic factor of the gastrointestinal tract (GIT) has traditionally been the subject of research.

The focus is primarily on the immunological aspects of microbiota, which play a fundamental role in the induction, learning, and functioning of the host immune system. This approach is historically justified, since the human immune system itself has evolved as a tool to support the host's symbiotic relationship with extremely diverse microbes. Under optimal conditions, this union of the immune system and microbiota makes it possible to induce protective responses to pathogens and maintain regulatory pathways of tolerance to harmless antigens [9]. In this case, the patterns established of the relationship between the microbiota and the immune system of the human body as a whole belong to such fields study as the

nervous system and psyche. These are considered to be specific levels of human organisation as a systemic subject and are studied both in a healthy state and in the case of disease [10,11]. A fairly broad review by Italian researchers [12] shows the variable role of the intestinal microbial composition in the pathogenetic mechanisms responsible for obesity and inflammation of the systemic, hepatic and adipose tissues. Besides, this research considers some aspects of modulation of the microbial composition of the gastrointestinal tract during dietary exposure and their influence on the pathogenesis of obesity, metabolic syndrome, and type 2 diabetes mellitus.

It should be noted that in recent years, the studies of human microbiota have focused on that of the digestive tract and on the so-called brain – gut – microbiota axis [13] (Fig. 1).

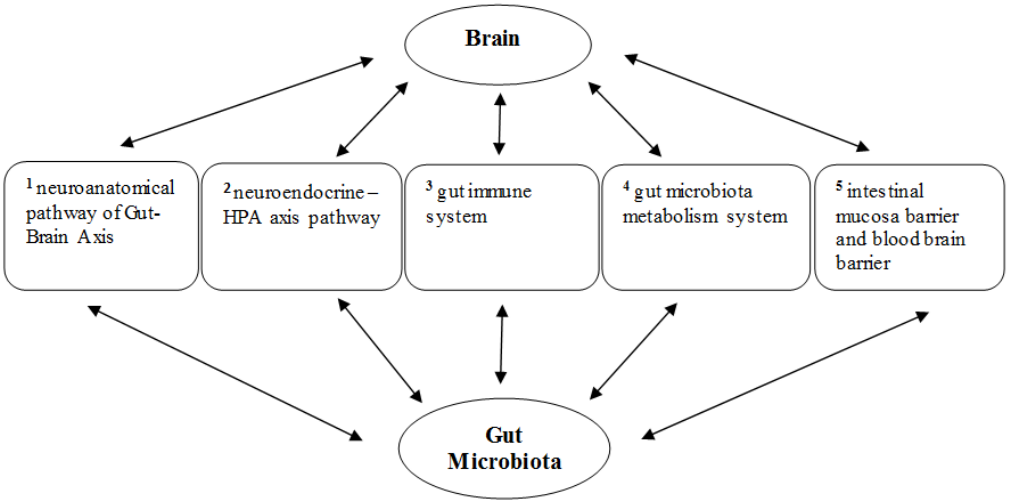

Fig. 1. Brain – gut – microbiota axis

K. Tillisch [14], too, one year after A. J. Montiel-Castro *et al.*, outlined potential pathways of communication between the intestinal microbiota and the brain. She noted that multiple inputs from the periphery can act centrally to modulate mood, pain sensitivity, cognition, and behaviour. One form of vagal-independent communication between the microbiota and the central nervous system is effected through the immune system. Systemic immune modulation of the intestinal microbiota stimulates circulating cytokines, which, in turn, can affect brain function, leading to lower pain threshold, psychomotor slowing, etc. Microbial-induced local immune activation in the intestines may be associated with altered intestinal barrier function, excitation of the enteric nervous system, and modulation of intestinal sensory-motor function. Increasing the sensitivity of the intestines, amplifying interceptive signals can provoke a feeling of pain, anxiety, or depression. A decrease in local and peripheral immune activity leads, on the contrary, to a sensation of digestive and, consequently, general well-being.

One of the prerequisites for the new direction is the fact, proved by numerous studies, that more than 95% of the volume of serotonin produced by the human body is located in the intestinal neurons [15].

This makes it possible to consider this neuronal factor as the "Enteric Nervous System" (ENS), and the intestines themselves to be called the "Second Brain" [16] and "the most sensory organ in the human body" which has a stable effect on the human psyche and behaviour [17].

The basic provisions (described above) of the connection between the intestines and the brain relate mainly to the neuronal organisation of both the brain and the autonomic nervous system and the intestines. In the light of recent studies, an integrated concept of the connection between the microbiome, intestines, and brain (in the normal condition and with neuropsychiatric disorders) is forming [18, 19].

There is a belief that the brain – gut – microbiota axis is the central concept of a new approach to mental health at the intersection of biological psychiatry and postgenomic medicine, and in the next decade, it will play an important role in integrated and holistic studies in the field of health [20]. This specific direction allows researchers to identify certain groups of microorganisms that, due to secondary metabolites (markers), affect in a certain way the human mental behaviour. These groups of microorganisms, psychobiotics, due to the results of fundamental biomedical research have found their further practical development, and medicine has formed a new branch – neurogastroenterology.

Psychiatry also pays attention to several non-traditional influence areas of the development of diseases, including microbiomics (the study of microbiomes), since there is evidence that psychobiotics may be used to treat some patients with mental disorders. For example, taking a cocktail of probiotics leads to a change in brain activity controlled by functional MRI. The mechanisms of signal

transmission in the brain–gut–microbiota axis include bidirectional communications that allow the intestinal microbiome to communicate with the brain and vice versa – the brain can communicate with the microbiota [21]. Many studies have detected dysregulation in the qualitative composition of the intestinal microbiota in the case of some psychological dysfunctions.

The development of the concept of the relationship between intestinal microbiota and the brain led to a paradigm shift in neuroscience and clinical practice [20]. A year earlier, this was scientifically substantiated [22], with special attention to the role of intestinal microbiota variability. Based on the review of published data [23, 24], the authors found it possible to adapt the statement of Hippocrates that all diseases begin from the gut, and postulated in their reviews that thinking and mental health began from the intestines [25]. From the summarised data of South Korean scientists, it also follows that many neuropsychiatric disorders (in particular, autism, depression, anxiety, schizophrenia) are related to changes in the microbiome, microbial substrates, exogenous prebiotics, antibiotics, and probiotics, or are simulated by them [26].

So, recent years have witnessed the development of studies related to correcting the microbiome by its targeted balancing with probiotic microorganisms for the prevention and treatment of mental disorders, for rehabilitation after them, and for a person's good mental state in general.

According to the WHO, probiotics are living microorganisms that, when applied in adequate amounts, improve the health of the host organism [27]. In turn, psychobiotics are defined as living bacteria (probiotics), which, when ingested, improve the psychophysiological status through interaction with commensal intestinal bacteria [28] (Table 1).

Previously, when synthetic antipsychotics were in use, it was believed that the development of an antipsychotic effect was impossible without the appearance of extrapyramidal disorders, and that the therapeutic effect could be correlated and measured by the severity of these neurological side effects. Some authors note that a significant risk of cardiovascular disease, the risk of myocardial infarction and stroke when taking antipsychotics is explained, in particular, by their effect on lipid metabolism with high medical load [29]. Using the available scientific data, today a harmless psychobiotic drug can be obtained that can compete with synthetic antipsychotics.

Table 1 – Potential psychobiotic strains of *Lactobacillus* and *Bifidobacterium* that have been proved to produce an effect on the nervous system

Lactobacillus	***Bifidobacterium***
L. rhamnosus JB-1	*B. longum* NCC3001
L. plantarum C29	*B. longum* R0175
L. helveticus NS8	*B. longum* 1714
L. helveticus R0052	*B. animalis* 01
L. casei Shirota	*B. infantis* 35624

Research in vivo

Psychobiotic studies are conducted on animal models (rats, mice) under conditions of stress induction by testing behavioural responses to evaluate indicators of motivation, anxiety, and depression. When using psychobiotics in animal models, conditions are created for a preliminary assessment of their possible effects in human diseases. Psychobiotics, as shown by experiments on animals, can mitigate or stop the manifestations of anxiety and depression. In one of the studies [30], it was found that early stress caused by the removal of pup rats from the mother manifested itself in decreased performance during the forced swimming test. The introduction of the animal probiotic *B. infantis* with food was accompanied by normalisation of all the studied parameters in the same way as it was in the group of animals subjected to stress and then given the antidepressant citalopram (a selective serotonin reuptake inhibitor). It was found that probiotics could relieve the rats' excessive internal readiness for anxiety and stress. Rats treated with *L. rhamnosus* JB-1 showed a significant reduction in signs of depression and anxiety in tests with forced swimming and solving problems in a maze. The optimisation of the mental state in animals was manifested by a decrease in the corticosteroid level in response to stress, which is indirect evidence of the normalisation of signalling pathways under the action of a probiotic.

Microorganisms, being in symbiosis with the gastrointestinal tract, produce a large number of metabolites, which are extremely important for the normal functioning of the body as a whole, in particular, ɣ-aminobutyric acid (GABA).

Intestinal microbiota has a strong effect on several neurotransmitters and neuromodulators, such as monoamines, serotonin, γ-aminobutyric acid (GABA), and cerebral neurotrophic factor [31]. GABA receptor ligands are considered as potential agents for treatment of various mental and central nervous system disorders. It is one of the most abundant signalling molecules in the nervous system that controls the parts of the brain that are responsible for emotions and the limbic system.

The importance of GABA for the normal functioning of the macroorganism

GABA is an organic compound, non-proteinogenic amino acid, the most important inhibitory neurotransmitter of the central nervous system (CNS) in humans and other mammals. Aminobutyric acid is a nutrient. It is contained in the central nervous system and takes part in neurotransmitting and metabolic processes in the brain. GABA in the body is formed from another amino acid, glutamic, by the enzyme glutamate decarboxylase. GABA is the main inhibitory neurotransmitter. It is soft, level-headed, and not very physically coordinated. Its main job is to regulate

excitatory signals sent by other neurotransmitters. It allows the muscles and blood vessels to relax and the body to sleep normally. Glutamate, the precursor of GABA, is the main excitatory neurotransmitter. Most medications that interfere with GABA are sedatives, including alcohol, gamma-hydroxybutyric acid, barbiturates, and benzodiazepines.

GABA is synthesised in the brain from glutamic acid, another neurotransmitter, by decarboxylation (removal of the carboxyl group from the main chain) (Fig. 2). According to the chemical classification, GABA is an amino acid used for the synthesis of protein molecules, an α-amino acid where the amino group is attached to the first carbon atom in the chain. In GABA, the amino group is linked to the third atom from the carboxyl group (in glutamate, it is the first one before decarboxylation).

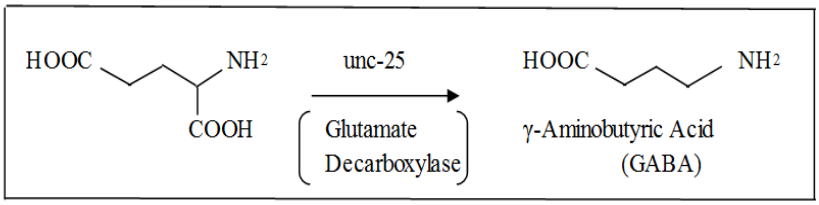

Fig. 2. GABA formation

GABA is synthesised directly in the brain and binds to two types of receptors on the surface of neurons – GABA receptors of types A and B. Type A receptors were previously subdivided into type A and C receptors (found mainly in the retina), but were subsequently viewed as a whole, since they do the same action. This type of receptor is ionotropic: when GABA binds to it, an ion channel opens in the membrane of a nerve cell, and chlorine ions rush into the cell, reducing its reactivity. The nerve cell membrane has a resting potential. There are fewer charged ions inside the cell than outside, and this creates a difference in charge. Outside, the superiority is created by chlorine, calcium, and sodium, while inside, potassium ions and a number of negatively charged organic molecules prevail. Theoretically, the membrane potential has two paths: increase (called depolarisation) and decrease (hyperpolarisation). At rest, the membrane potential is approximately -70 – -90 mV (millivolts), and during the work of the nervous system, a rivalry begins between the two forces – the one exciting the cell (depolarising the membrane) and the one inhibiting it (hyperpolarising). The GABA molecule binds to the ion channel receptor; the ion channel opens and begins to pass negatively charged chlorine ions into the cell. Under the influence of these ions, membrane hyperpolarisation occurs, and the cell becomes less susceptible to excitatory signals from other neurons. This is the first and, perhaps, the main function of GABA – inhibition of the activity of nerve cells in the nervous system.

The structure of the GABA-ergic system of the brain resembles all others. There are a number of structures deep in the brain from which nerve fibres that secrete GABA travel to other parts of the nervous system. Thus, GABA is an inhibitory neurotransmitter that regulates many processes, from muscle tone to emotional reactions.

However, GABA becomes an inhibitory mediator only in the mature brain. In the developing nervous system, GABA-ergic neurons can produce an excitatory effect on cells, also changing the permeability of the membrane to chlorine ions [32]. In immature nerve cells, the concentration of chlorine ions is higher than in the environment, and stimulation of GABA receptors leads to the release of these anions from the cell and subsequent depolarisation of the membrane. Over time, the main excitatory system of the brain, the glutamate, matures, and GABA acquires the role of an inhibitory (membrane hyperpolarising) neurotransmitter.

The maturation of the brain itself is a complex process regulated by many genes at different stages of ontogenesis. Violation of the maturation and migration of neurons leads to various neurological diseases, for example, epilepsy [33]. Epilepsy is one of the most common neurological diseases. With it, the neurons of the brain generate nerve impulses in the wrong way – too often and too strongly, which leads to a pathological focus of excitation in the brain. It is the existence of such a focus that leads to seizures – the most important and dangerous symptom of epilepsy. This “discharge” allows temporarily reducing the excitation in the nervous system. Mutations in a number of genes lead to the fact that GABA-ergic interneurons are out of place and cannot fully perform their inhibitory functions. In mouse models and in the study of the human genotype, a connection was established between mutations, impaired migration and maturation of GABA-ergic neurons, and the development of epilepsy.

Another aspect of the inhibitory effect of GABA is its influence on emotional processes, in particular, on anxiety. Anxiety is a very broad concept. It contains both perfectly healthy human reactions to stressful influences (exam, dark gateway, declaration of love) and pathological conditions (anxiety disorders in the

medical sense of the word). Based on the principles of modern psychiatric science, we can say that there is normal anxiety and anxiety as a disease. Anxiety becomes a disease when it interferes with daily or professional life, blocking the making of any decisions, even the most necessary ones.

The part of the brain responsible for emotional responses is the amygdala, a collection of nerve cells deep in the brain. It is one of the oldest and most important parts of the nervous system in animals. A special responsibility of the amygdala is negative emotions – anger, fear, and anxiety. GABA allows the brain to reduce the intensity of these experiences.

Medicines that are effective in fighting anxiety and seizures must bind to the GABA receptor. They are not direct receptor stimulants; they do not bind to the same part of the molecule as GABA. They are supposed to increase the sensitivity of the ion channel to GABA by changing slightly its spatial organisation. These chemicals are called allosteric modulators.

Thus, GABA is an amazing neurotransmitter. In a developing brain, γ-aminobutyric acid excites nerve cells, and in the developed brain, on the contrary, reduces their activity. It is responsible for the feeling of calmness, lack of anxiety, good mood.

Competent approach to the identification of the studied microorganisms

Multiple studies have shown that the lactic acid bacteria *Lactobacillus helveticus* are the best GABA producers. The WHO standards demand clear identification of new strains that must be able to survive in the gastrointestinal tract (be resistant to pH, enzymes, cholic acids, etc.). Also, they must be able to adhere, exhibit antagonistic properties, and be genetically stable [34]. Therefore, all strains used in the production of probiotics and psychobiotics must be clearly identified at the species level and have a genetic passport [35, 36].

Lactobacillus is a genus of gram-positive anaerobic non-spore-forming lactic acid bacteria. They have a regular rod-like shape; sometimes they are arranged in short chains, or exist singly. In the course of their normal metabolism, lactobacilli can form lactic acid, hydrogen peroxide, produce lysozyme and substances with antibiotic activity: reuterin, plantaricin, lactocidin, lactolin. Heterofermentative lactobacilli species can also produce acetic acid and carbon dioxide as end products.

Lactobacilli are representatives of the normal human microbiota. Due to their properties, they are actively used in the biotechnology of production of probiotics and functional foods.

Identifying lactic acid microorganisms requires studying their morphological, cultural, physiological and biological properties. For successful culturing of this microorganism, growth media must be close to the natural conditions of its existence. Nutrient-rich media (yeast extract, hydrolysed milk, peptone, Tween 80, etc.) and those with low pH are used to cultivate bacteria of the genus *Lactobacillus*. Bacteria of this genus are microorganisms that have complex nutritional needs. Their active development requires the presence of substances necessary for the structure of the bacterial cell (nucleic acids, polysaccharides, etc.). They also need organic forms of nitrogen (because they do not produce it themselves), vitamins, and trace elements. Thus, of the many growth media used to culture lactic acid bacteria, the suitable ones are those with a well-balanced nitrogen, carbohydrate, and vitamin composition, containing all the necessary nutrients and stimulants in a form easily accessible by the microorganisms. From all special growth media, the most common is MPC, which contains yeast and meat extracts, glucose, peptone, sodium acetate, ammonium citrate, and Tween 80, a source of fatty acids needed for normal bacterial metabolism. The acidity of the medium is 6.2–6.4. The MPC medium can be used both to work with probiotic bacilli and to isolate these microorganisms from food or natural habitats.

The ability of lactobacilli to inhibit the development of pathogenic microbiota is one of the most important features of these bacteria [32]. This antagonistic property is possible, because lactic acid microorganisms in the course of their metabolism produce lactic and acetic acids, hydrogen peroxide and bacteriocins. All of these compounds acidify the pH of the medium, which adversely affects other types of microorganisms, including bacteria of the genera *Salmonella* and *Escherichia coli*.

The latest methods to determine microorganisms

In addition, the use of genetic and molecular methods to identify lactic acid bacteria (LAB) is one of the criteria for selecting LAB strains promising for use in biotechnology and food industry. In recent years, approaches based on metagenomic analysis of total DNA or analysis of amplified fragments of 16S rRNA genes from biological samples using high-performance sequencing technologies have been widely used. These technologies make it possible to obtain information on the species diversity of both cultured and non-cultured bacteria in the intestinal microbiota or in the studied samples of microorganisms.

Until recently, biochemical identification was most often used by a number of enzymatic activities involving ready-made test systems, but many well-known species, including *Bifidobacteria* cannot be accurately distinguished by means of this approach [27]. Besides, this method is relatively expensive, which makes it difficult to scale up to a large number of identifiable strains. The problem of high cost and laboriousness also arises when using modern molecular genetics methods of identification, in particular, sequencing of 16S rRNA genes of pure cultures of bacteria.

In recent years, the method of species identification based on matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has been increasingly used in bacteriology. In this method, isolated protein extracts or bacterial cells per se are mixed with a saturated solution of the matrix substance, which leads to their joint crystallisation. The laser pulse causes ionisation and explosive evaporation of the matrix together with the proteins under study. The resulting ions are accelerated in vacuum by an electrostatic field, after which they fly through the area without acceleration and crash into the detector target; the device registers the time of flight of ions, which will reflect their mass-to-charge ratio. Thus, the MALDI-TOF mass spectrometry method allows recording the protein spectrum of a pure culture of bacteria, which, when compared with the database, makes it possible to identify the species accurately. This technology allows analysing a large number of bacterial strains quickly, with high accuracy, and, only considering the cost of consumables, it is much cheaper than other methods [37]. *Lactobacilli*, which produce GABA, are isolated from foods such as cheese, yogurt, sourdough, brine, etc., and are the source of the bioactive properties characteristic of these foods.

Bidirectional communication system of the brain – gut – microbiota axis modulation system

In the course of the same studies in rats, it turned out that in the brain, the probiotic differentially changed the expression of inhibitory GABA receptors in different regions. In particular, in comparison with the reference group, the probiotic reduced the expression of GABA mRNA in the hippocampus and amygdala, but increased its expression in the prelimbic region and the cingulate gyrus. Considering the fact that GABA serves as the main inhibitory neurotransmitter of the nervous system, we can conclude that probiotics can affect the regional balance of the processes of excitation and inhibition, as well as the associated behavioural reactions towards reducing anxiety and depression [28].

Other important metabolites of intestinal microbiota that affect brain function are the so-called microbial signalling molecules represented by short chain fatty acid (SCFA) synthesised by microorganisms. Changes in the microbiota can lead to “leakage” of the intestinal epithelium and the entry of these molecules into the bloodstream. Subsequently, these SCFAs inhibit enzymes involved in epigenetic regulation (including the hypothalamic-pituitary-adrenal axis) and activate some receptors associated with the G-protein of the brain. These proteins serve as a “shuttle” (ferry), moving from the receptor protein to adenylate cyclase, the main enzyme the synthesis of cyclic adenosine monophosphate (cAMP) takes place with.

In turn, cAMP molecules behave as an intracellular mediator and act on target proteins called cAMP-dependent protein kinases (A kinases), the role of which is to open and close ion channels [38].

The mechanisms of signal transmission in the brain – gut – microbiota axis are quite complicated and include bidirectional communications (fig. 3), which allow the microbiome of the digestive tract to communicate with the brain and the brain to communicate with the microbiota [39].

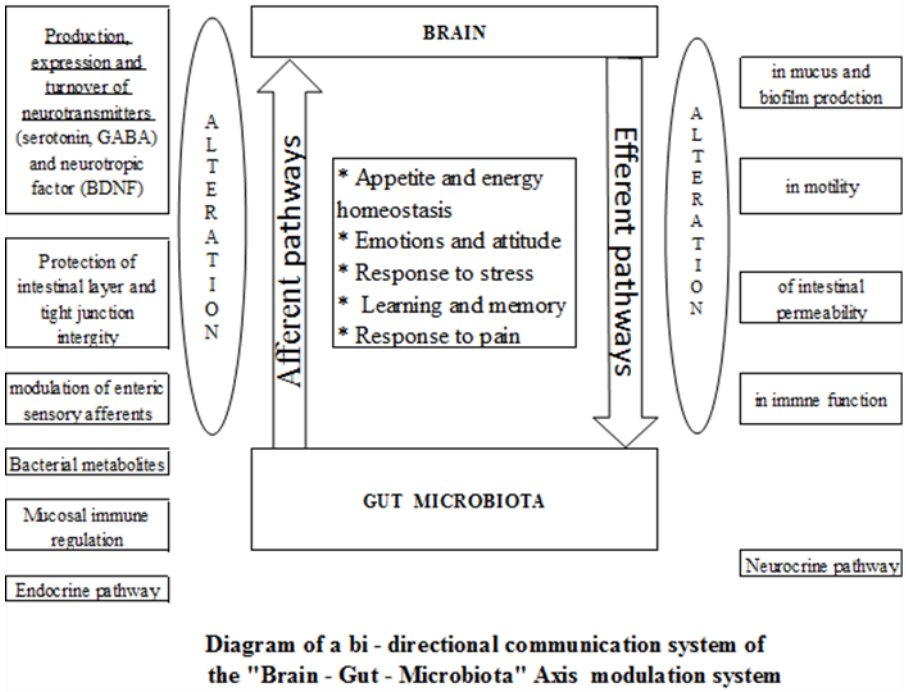

Fig. 3. Signal transmission in the brain – gut – microbiota axis

Various conducting pathways of the central nervous system (CNS) are involved in this axis: afferent, conducting impulses from the receptor to the nerve centre, and efferent, conducting impulses from the nerve centres to the working organ. So, through ascending afferent pathways, information about the state of the intestines from antibiotics, environmental and infectious agents, intestinal neurotransmitters/ neuromodulators, cytokinins, major metabolites, and other substances of the internal environment of the body is transmitted to the central nervous system. In contrast, the hypothalamic-pituitary-adrenal axis, regulatory CNS saturation zones, and neuropeptides (efferent pathway) released from sensory nerve fibres affect the composition of the intestinal microbiota directly or through the availability of nutrients [40].

Conclusion

The effects of probiotics were also studied in a stressful situation (prolonged immobilisation) on Sprague Dawley rats [25]. Animals received *L. helveticus* NS8, citalopram, or placebo. Compared with animals from the reference group, rats treated with the probiotic showed significantly less anxiety and a better memory when recognising objects. Biochemical analysis showed that in the probiotic group, blood corticosterone levels were significantly lower, and the concentration of anti-inflammatory cytokine IL-10 was higher compared with the reference group. In general, the effects of the probiotic *Lactobacillus* and the antidepressant citalopram were unidirectional and comparable.

When comparing the results of studies in animals and humans, significant similarities are found. For example, in a randomised, double-blind, placebo-controlled study, in which healthy volunteers (men and women) were taking a mixture of probiotics (*L. helveticus* R0052 and *B. longum*) or placebo for 30 days, participants from the probiotic group noted a significant decrease in the total duration and the depths of bad mood and distress. In the same group's daily urine samples, the reduction in the cortisol content was statistically significant, which indicates a decrease in the stress level [41].

The data obtained from the expert studies *in vivo* have allowed us to draw conclusions regarding the future introduction of psychobiotic drugs into medical practice [42]. It is very unlikely that probiotics will completely replace synthetic drugs, but there is every reason to hope that due to them, the application of chemical psychotropic drugs can be reduced.

References:

1. Chebaeva SO. Probiotics. Irreplaceable helpers for your body: Moscow: Ripol Classic, Dom. XXI century, 2013.
2. Human Microbiome. Everything you wanted to know about intestinal microflora.School of Rehabilitation and Sports Medicine "Rehabilitologist". Kyiv, 2016. URL:https://rehabilitolog.com/seminars2/mikrobiom-cheloveka/.
3. Rajilic – Stojanovic, Willem M. De Vos.The first 1000 cultured species of the human gastrointestinal microbiota.FEMSMicrobiolRev. 2014. Vol. 38(5): 40-41. URL: pubmed.ncbi.nlm.nih.gov"24861948/. DOI: 10.1111/1574-6976.12075.
4. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva:World Health Organization, 2017.Licence: CC BY – NC – SA 3.0 IGO.
5. Recurrent depressive disorders. Report of the State Expert Center of the Ministry of Health of Ukraine, 2012.
6. Mental health in transition: assessment and guidance for strengthening integration of mental health into primary health care and community-based service platforms in Ukraine. (English). Global Mental Health Initiative. Washington, D.C. World Bank Groupe, 2017. URL: openknowledge.worldbank.org.
7. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded diabetescare. 2010. Vol.33(10):1-2. URL:https://care.diabetesjournals.org/content/diacare/33/10/2277.full.pdf. DOI: 10.2337/dc10-0556.
8. Rajpal DK, Brown JR. Modulation the human gut microbiota as an emerging therapeutic paradigm. Sci. Progr. 2013. Vol.96(3):224–236.DOI: 10.3184/003685013X13691404141587.
9. Belkaid Y, Timothy W. Hand Role of the Microbiota in Immunity and Inflammation. Cell. 2014. Vol.157 (1):121 – 141. DOI: 10.1016/j. cell. 2014.03.011.
10. Fung, Olson & Hsiao. Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci. 2017. Vol.20(2):145-155. DOI: 10.1038/nn. 4476.
11. Thomas S. et al. The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists.Cancer Res. 2017. Vol.77(8):1783-1812. DOI: 10.1158/0008-5472.CAN-16-2929.
12. Federico A. et al. Gut Microbiota, Obesity and Metabolic Disorders. Minerva Gastroenterol Dietol.2017. Vol.63(4):337–344. DOI: 10.23736/S1121- 421X.17.02376-5.
13. Mayer EA. et al. Gut microbes and the brain paradigm shift in neuroscience. J. Neuroscience. 2014. URL: https://www.jneurosci.org/content/jneuro/34/46/15490.full.pdf.
14. Tillisch K. The effects of gut microbiota on CNS function in humanis. Gut Microbes. 2014: Vol.(5):404–410. DOI: 10.4161/gmic.29232.
15. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Current Opinion in Endocrinology, Diabetes, and Obesity. 2013.Vol. 20 (1). DOI: 10.1097/MED. 0b013e32835bc703.
16. Gershon MD. The Second Brain: A Groundbreaking New Understanding of Nervous Disorders of the Stomach and Intestine. New York: HarperPerennial;1998.
17. Enders D. Charming intestines. As the most powerful body governs us. Moscow: BOMBORA; 2020.
18. . Prinsloo S, Lyle RR. The Microbiome, Gut-Brain-Axis, and Implications for Brain Health. NeuroRegulation. 2015. Vol. 2(4). P.158–161. DOI: 10.15540/nr.2.4.158.
19. Latalova K, Hajda M, Prasko J. Can Gut Microbes Play a Role in Mental disorders and Their Treatment. Psychiatr Danub. 2017. Vol. 29(1):28–30. DOI: 10.24869/psyd. 2017. 28.
20. Malan S. et al. The Gut Microbiome and Mental Health: Implications for Anxiety- and Trauma-Related Disorders. Omics: a Journal of Integrative Biology. 2017.Vol. 22(2). DOI: 10.1089/omi.2017.0077.
21. Bauer, KC, Huus KE, Finlay BB. Microbes and the mind: emerging hallmarks of the gut microbiota-brain axis. National Library of Medicine. National Center for Biotechnology Information. 2016.Vol. 18(5): 632–644.URL:pubmed.ncbi.nlm.nih.gov.

22. Rajpal DK, Brown JR. Modulation the human gut microbiota as an emerging therapeutic paradigm. Sci. Progr. 2013. Vol. 96(3):224–236. DOI: 10.3184/003685013X13691404141587.
23. Schmidt, C. Mental health: thinking from the gut. Nature. 2015. Vol. 518(7540):S12-5. DOI: 10.1038/518S13a
24. Hu N. et al. Nutrition and the risk of Alzheimer's disease. BioMed Research Int. 2013. URL: hindawi.com›journals/bmri/2013/524820/.
25. Liang S. et al. Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience. 2015. 561-577. DOI: 10.1016/j. neuroscience. 2015. 09.033.
26. Kim YK, Shin C. The Microbiota – Gut – Brain Axis in Neuropsychiatric Disorders: Pathophysiological Mechanisms and Novel Treatments. Current Neuropharmacology. 2018. Vol. 16(5):559-573.DOI: 10.2174/1570159X15666170915141036.
27. Gorbach S. Probiotics in the third millennium. Dig Liver. Dis. 2002. DOI: 10.1016/s1590-8658(02)80155-48.
28. Ivashkin VT, Ivashkin KV. Psychobiotic effects of probiotics and prebiotics. Russian journal of gastroenterology, hepatology, coloproctology. 2018.Vol.28 (1):4-12. DOI: 10.22416/1382-4376-2018-28-1-4-12.
29. Rybakova SV. Effect of psychotropic drugs on lipid metabolism and the outcomes of cardiovascular diseases in mental patients: author. dis. ... Cand. Of Med. Sciences: 14.00.25: defense17.10.08/scientific director. L. E. Ziganshina, A. S. Galyavich. Kazan: State educational institution of additional professional education "Kazan State Medical Academy of the Federal Agency for Health and Social Development", and State Educational Institution of Higher Professional Education "Kazan State Medical University", 2008. 21 p.
30. Desbonnet L. et al. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience. 2010. Vol. 170(4): 1179-1188.DOI: 10.1016/j. neuroscience. 2010. 08. 005.
31. Bravo JA. et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci U.S.A. 2011. Vol. 108(38):16050-16055.DOI: 10.1073/pnas.1102999108.
32. Ben-Ari Y. et al. GABA: A Pioneer Transmitter That Excites Immature Neurons and Generates Primitive Oscillations. Physiological Reviews. 2007.Vol.87(4):1215-1284. DOI:10.1152/physrev.00017.2006.
33. Bozzi Y, Casarosa S, Caleo M. Epilepsy as a neurodevelopmental disorder. Frontiers in Psychiatry. 2012. P. 1-14. URL:frontiersin.org›articles/10.3389/fpsyt.2012.00019.
34. Khamagaeva IS, Khazagaeva SN. Research of biotechnological potential of Lactobacillus helveticus 17 - 18. Innovative technologies of food products and assessment of their quality: science, education, production. 2016. P. 77-81. URL: esstu.ru ›… Konf/Food_2016… Khamagaeva_Khazagaeva.pdf.
35. Shenderov BA. Medical microbial ecology and functional nutrition. Probiotics and functional nutrition. Moscow: GRANT; 2001.
36. Dunne C. et al. Probiotics: frommythtoreality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie van Leeuwenhoek. 1999.Vol. 76(1):279-292. URL: pubmed.ncbi.nlm.nih.gov"10532384/.
37. Nomura F. Proteome based bacterial identification using matrix assisted laser desorption ionization time of flight mass spectrometry (MALDITOF MS): A revolutionary shift in clinical diagnostic microbiology. Biochem. Biophys. Acta. 2014. Vol. 1854 (6): 528-537. DOI: 10.1016/j. bbapap. 2014. 10. 022.
38. Shervin E. et al. May the force be with you: The Light and Dark Seeds of the Microbiota – Gut – Brain Axis in Neuropsychiatry. CNS Drugs. 2016.Vol. 30(11): 1019 – 1041 DOI: 10.1007/S 40263 – 016 – 0370 – 3.
39. Dinan TG, Cryan JF. Gut Instincts: Microbiota as Key Regulator of Brain Development, Ageing and Neurodegeneration. J. Physiol. 2017. Vol. 595(2):489 – 503. DOI: 10.1113/JP273106.
40. Petra AI. et al. Gut – Microbiota - Brain Axis and its Effect on Neuropsychiatric Disorders with Suspected Immune Dysregulation. Clin. Ther. 2015.Vol. 37(5): 984 – 995. DOI: 10.1016/J/clinthera. 2015.04.002.
41. Messaoudi M. et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticusR0052 and Bifidobacterium longum R0175) in rats and human subjects. British Journal of Nutrition. 2010.Vol.105 (5): 755-764. DOI: 10.1017/S0007114510004319.
42. Alchudzhyan N. et al. Modulating effect of a cocktail of selective probiotic strains on intracellular metabolic changes in the dynamics of bipolar disorder caused by dexamphetamine. Biological Journal of Armenia. 2017. P. 23-29. URL: biology.asj-oa.am ›11427/.

ПСИХОБІОТИКИ – ПРОБІОТИКИ, ЩО ПОКРАЩУЮТЬ НАСТРІЙ

Л. В. Капрельянц, д.т.н., професор, *E-mail*: leonid@onaft.edu.ua
Е. В. Жук, аспірант, *E-mail*: zhukelena1982@ukr.net
Кафедра біохімії, мікробіології та фізіології харчування
Одеська національна академія харчових технологій, вул. Канатна, 112, м. Одеса, Україна, 65039

Анотація. Мікробіом кишечника людини включає всі мікроорганізми, що населяють кишковий тракт. Одне з досліджень показало, що миші, вирощені в стерильному середовищі, демонстрували надмірну фізіологічну реакцію на стрес, порівняно з нормальним контролем. Це відкриття виявило причетність мікробіому до розробки вісі «Мозок – Кишечник – Мікробіота». З того часу було виявлено, що кишкові бактерії беруть участь у регуляції різноманітних і важливих фізіологічних процесів, включаючи імуномодуляцію, ожиріння та енергетичний баланс, а також діяльність нервової системи. Всі ці дослідження дозволили виявити новий тип пробіотиків – психобіотики. Психобіотики здатні надавати позитивний вплив на настрій пацієнтів. Спроби виявити внутрішні механізми позитивних емоційних зрушень людини викликають великий інтерес. Підтверджена здатність психобіотиків впливати на глибину емоцій. Головним нейромедіатором, що чинить тормозну дію в головному мозку людини та тварин є **γ-аміномасляна кислота** (скор. ГАМК). Вона бере участь в метаболічних, а також нейромедіаторних процесах головного мозку.Попередником ГАМК є глутамат. ГАМК-ергічна система головного мозку за своєю будовою нагадує всі інші – ряд глибоко розташованих в мозку структур, звідки нервові волокна, що виділяють ГАМК, йдуть в інші частини нервової системи. Тому ГАМК є гальмівним нейромедіатором, що регулює багато процесів – від м'язового тонусу до емоційних реакцій. Багатьма дослідженнями доведено, що деякі види мікроорганізмів здатні продукувати ГАМК, яка діє на організм людини так само, як і ГАМК, зпродукована у мозку людини. Головними продуцентами ГАМК, як виявили дослідження, є молочнокислі бактерії, а серед них найбільш здатні до цього бактерії роду*Lactobacillushelveticus*. Згідно стандартів ВООЗ, повинна проводитися чітка ідентифікація нових штамів пробіотиків для подальшого застосування їх у якості БАДів чи препаратів. Вони повинні бути здатними до виживання у шлунково - кишковому тракті (бути стійкими до впливу pH, ферментів, холевих кислот та ін.), також вони мають

бути здатними до адгезії, проявляти антагоністичні властивості та бути генетично стабільними. Тому усі штами, що мають бути використаними у виробництві пробіотиків повинні бути чітко ідентифіковані на видовому рівні та мати генетичний паспорт. Робота щодо виявлення, ідентифікації та впровадження нових штамів пробіотиків, що здатні до продукування психобіотичних речовин, а саме ГАМК, є дуже кропіткою, але ж водночас, дуже важливою тому що стрес, депресія та багато які інші види психічних розладів стають все більш поширеним явищем в світі і Україна також не є виключенням.

Ключові слова: мікробіом, мікробіота, пробіотик, психобіотик, вісь «Мозок – Кишечник – Мікробіота», γ-аміномасляна кислота**.**

List of references:

1. Чебаева С.О. Пробиотики. Незаменимые помощники вашему телу: Москва: Рипол Классик, Дом. XXI век, 2013.
2. Human Microbiome. Everything you wanted to know about intestinal microflora.School of Rehabilitation and Sports Medicine "Rehabilitologist". Kyiv, 2016. URL:https://rehabilitolog.com/seminars2/mikrobiom-cheloveka/
3. Rajilic – Stojanovic, Willem M De Vos.The first 1000 cultured species of the human gastrointestinal microbiota //FEMS Microbiol Rev. 2014. 38(5). P. 40-41. URL: pubmed.ncbi.nlm.nih.gov"24861948/DOI: 10.1111/1574-6976.12075
4. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva:World Health Organization, 2017. Licence: CC BY – NC – SA 3.0 IGO. C – 21P.
5. Повторні депресивні розлади. Звіт Державного експертного центру МОЗ України, 2012. С. 457
6. Mental health in transition: assessment and guidance for strengthening integration of mental health into primary health care and community-based service platforms in Ukraine. (English). Global Mental Health Initiative. Washington, D.C.: World Bank Groupe, 2017. URL: openknowledge.worldbank.org
7. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded diabetes care. 2010. 33(10). P. 1-2. URL:https://care.diabetesjournals.org/content/diacare/33/10/2277.full.pdf. DOI: 10.2337/dc10-0556
8. Rajpal, D. K., Brown J. R. Modulation the human gut microbiota as an emerging therapeutic paradigm//Sci. Progr. 2013. 96(3). P. 224–236.DOI: 10.3184/003685013X13691404141587
9. Belkaid Y., Timothy W. Hand Role of the Microbiota in Immunity and Inflammation. Cell. 2014. 157 (1) P.121 – 141. DOI: 10.1016/j. cell. 2014.03.011
10. Fung, Olson & Hsiao. Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci. 2017. 20(2). P. 145-155. DOI: 10.1038/nn. 4476
11. Thomas S. et al. The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists.Cancer Res. 2017. 77(8). P. 1783-1812. DOI: 10.1158/0008-5472.CAN-16-2929
12. Federico A. et al. Gut Microbiota, Obesity and Metabolic Disorders. Minerva Gastroenterol Dietol.2017. 63(4). P. 337–344. DOI: 10.23736/S1121- 421X.17.02376-5.
13. Mayer, E. A. et al. Gut microbes and the brain paradigm shift in neuroscience//J. Neuroscience. 2014. URL: https://www.jneurosci.org/content/jneuro/34/46/15490.full.pdf
14. Tillisch. K. The effects of gut microbiota on CNS function in humanis//Gut Microbes. 2014. Vol. 5, P. 404–410. DOI: 10.4161/gmic.29232
15. Gershon M.D. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Current Opinion in Endocrinology, Diabetes, and Obesity 20 (1). 2013. DOI: 10.1097/MED. 0b013e32835bc703
16. Gershon M.D. The Second Brain: A Groundbreaking New Understanding of Nervous Disorders of the Stomach and Intestine. 1998. 336 p.
17. Очаровательный кишечник. Как самый могущественный орган управляет нами. Эндерс Д. Москва: БОМБОРА, 2020. 352 с.
18. Prinsloo S., Lyle R.R. The Microbiome, Gut-Brain-Axis, and Implications for Brain Health. NeuroRegulation. 2015. 2(4). P.158–161. DOI: 10.15540/nr.2.4.158
19. Latalova K., Hajda M., Prasko J. Can Gut Microbes Play a Role in Mental disorders and Their Treatment. Psychiatr Danub. 2017. 29(1). P. 28–30. DOI: 10.24869/psyd. 2017. 28
20. Malan S. et al. The Gut Microbiome and Mental Health: Implications for Anxiety- and Trauma-Related Disorders. Omics: a Journal of Integrative Biology. 22(2). 2017. DOI: 10.1089/omi.2017.0077.
21. Bauer. K. C., Huus K. E., Finlay B. B. Microbes and the mind: emerging hallmarks of the gut microbiota-brain axis. National Library of Medicine. National Center for Biotechnology Information. 2016. P. 632–644.URL:pubmed.ncbi.nlm.nih.gov
22. Rajpal, D. K., Brown J. R. Modulation the human gut microbiota as an emerging therapeutic paradigm//Sci. Progr. 2013.96(Pt 3). P. 224–236. DOI: 10.3184/003685013X13691404141587
23. Schmidt, C. Mental health: thinking from the gut. Nature. 2015. 518(7540):S12-5. DOI: 10.1038/518S13a
24. Hu, N. et al. Nutrition and the risk of Alzheimer's disease. BioMed Research Int. 2013. URL: hindawi.com›journals/bmri/2013/524820/
25. Liang S. et al. Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience. 2015. P. 561-577. DOI: 10.1016/j. neuroscience. 2015. 09.033
26. Kim Y. K., Shin C. The Microbiota – Gut – Brain Axis in Neuropsychiatric Disorders: Pathophysiological Mechanisms and Novel Treatments. Current Neuropharmacology. 2017. P. 327-341. DOI: 10. 2174/1570159X15666170915141036
27. Gorbach S. Probiotics in the third millennium. DigLiver. Dis. 2002. P. 2 – 7.DOI: 10.1016/s1590-8658(02)80155-4828.Ивашкин В.Т., Ивашкин К.В. Психобиотические эффекты пробиотиков и пребиотиков. Российский журнал гастроэнтерологии, гепатологии, колопроктологии. 2018. 28(1). С. 4-12. DOI:10.22416/1382-4376-2018-28-1-4-12
28. Рыбакова С. В.Влияние психотропных средств на липидный обмен и исходы сердечно-сосудистых заболеваний у психических больных: автореф. дис. … канд. мед. наук: 14.00.25: защита 17.10.08/науч. рук. Л. Е. Зиганшина, А. С. Галявич. Казань: Государственное образовательное учреждение дополнительного профессионального образования «Казанская государственная медицинская академия Федерального агентства по здравоохранению и социальному развитию», и Государственное образовательное учреждение высшего профессионального образования «Казанский государственный медицинский университет», 2008. 21 с.
29. Desbonnet L. et al. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 2010. P. 1179-1188.DOI: 10.1016/j. neuroscience. 2010. 08. 005
30. Bravo J.A. et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U.S.A. 2011. 108(38). P. 16050-16055DOI: 10.1073/pnas.1102999108
31. Ben-Ari Y.et al. GABA: A Pioneer Transmitter That Excites Immature Neurons and Generates Primitive Oscillations. Physiological Reviews. 2007. P. 1215-1284. DOI:10.1152/physrev.00017.2006

32. Bozzi Y., Casarosa S., Caleo M. Epilepsy as a neurodevelopmental disorder. Frontiers in Psychiatry. 2012. P. 1-14. URL:frontiersin.org›articles/10.3389/fpsyt.2012.00019
33. Хамагаева И.С, Хазагаева С.Н. Исследование биотехнологического потенциалаLactobacillushelveticus 17 – 18. Инновационные технологии пищевых продуктов и оценка их качества: наука, образование, производство. 2016. С. 77-81. URL: esstu.ru›…Konf/Food_2016…Хамагаева_Хазагаева.pdf
34. Медицинская микробная экология и функциональное питание. Том 3. Пробиотики и функциональное питание/Шендеров Б.А. Москва: ГРАНТЪ. 2001. 288 с.
35. Dunne C. et al. Probiotics: frommythtoreality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie van Leeuwenhoek. 1999.76(1). P. 279-292. URL: pubmed.ncbi.nlm.nih.gov"10532384/
36. Nomura F. Proteome based bacterial identification using matrix assisted laser desorption ionization time of flight mass spectrometry (MALDITOF MS): A revolutionary shift in clinical diagnostic microbiology. Biochem Biophys Acta. 2014. 1854 (6). P. 528-537. DOI: 10.1016/j. bbapap. 2014. 10. 022
37. Shervin E. et al. May the force be with you: The Light and Dark Seeds of the Microbiota – Gut – Brain Axis in Neuropsychiatry. CNS Drugs. 2016. P. 1019 – 1041 DOI: 10.1007/S 40263 – 016 – 0370 – 3
38. Dinan T. G., Cryan J. F. Gut Instincts: Microbiota as Key Regulator of Brain Development, Ageing and Neurodegeneration. J. Physiol. 2017. 595(2). P. 489 – 503. DOI: 10.1113/JP273106
39. Petra A.I. et al. Gut – Microbiota - Brain Axis and its Effect on Neuropsychiatric Disorders with Suspected Immune Dysregulation. Clin. Ther. 2015. 37(5). P. 984 – 995. DOI: 10.1016/J/clinthera. 2015.04.002
40. Messaoudi M. et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticusR0052 and Bifidobacterium longum R0175) in rats and human subjects. British Journal of Nutrition. 2010.105 (5). P. 755-764. DOI: 10.1017/S0007114510004319
41. Алчуджян Н. и др. Модулирующее действие коктейля из селективных пробиотических штаммов на внутриклеточные метаболические сдвиги в динамике биполярного расстройства, вызванного дексамфетамином. Биологический журнал Армении. 2017. P. 23-29. URL: biology.asj-oa.am ›11427/