Microbiology, Vol. 73, No. 5, 2004, pp. 565–572. Translated from Mikrobiologiya, Vol. 73, No. 5, 2004, pp. 659–667.

REVIEWS

The Study of the Physiology and Biochemistry of Microorganisms at the Institute of Microbiology, Russian Academy of Sciences

V. K. Plakunov and G. I. El'-Registan

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received May 19, 2004

What's in a name? That which we call a rose by any other name would smell as sweet.
W. Shakespeare

This epigraph is given to emphasize the conventional character of the title of this paper, for there is scarcely a researcher at the Institute of Microbiology who has not dealt, to some degree or other, with the physiology and biochemistry of microorganisms. A researcher will face the physiology and biochemistry of a microorganism even before it is recovered from its natural source since the use of a selective recovery medium suggests a knowledge of the putative biochemical and physiological properties of this microorganism.

Fortunately, in the present issue of the journal, physiological–biochemical characteristics of many microbial groups and communities are described by highly qualified specialists. Therefore, the authors of this review will consider only certain aspects of the inexhaustible subject of microbial physiology and biochemistry.

To proceed further, it is necessary (even though it may seem to be strange) to specify some definitions concerning the terms *physiology* and *biochemistry.* It is not by chance that the word combination *the physiology and biochemistry of microorganisms* has become a stock phrase. This point was even the subject of a thorough discussion in the monograph by Gutina [1]. Actually, it is very difficult to clearly distinguish between the *physiology* and the *biochemistry* of a microorganism. For instance, the study and the description of physiological phenomena related to the thermophily or halophily of microorganisms is impossible without considering relevant physicochemical and biochemical mechanisms such as the stabilization of biomolecules, the formation of osmoprotectants, and so on. There are no distinct boundaries in nature; humans create them for their own convenience.

Therefore, in this paper, we will not strictly demarcate physiological and biochemical phenomena but rather adhere to the idea that good scientific research must treat all the aspects of a problem in close relation. Thus, bearing in mind that the physiological responses of an organism rely on the respective biochemical and, in the final analysis, genetic mechanisms, the study of microbial growth will be considered *mainly physiological*, whereas the study of metabolic processes will be considered *mainly biochemical.* Paying high tribute to Academicians G.A. Nadson and B.L. Issatchenko, who were the first two directors of the Institute of Microbiology, it should be emphasized that the *physiological* line of research at this institute was founded by V.N. Shaposhnikov, who headed the Department of Fermentative Microorganisms (later the Department of Technical Microbiology) from 1938 to 1963. After Shaposhnikov's retirement, the chair of the department was held by his pupil and follower M.N. Bekhtereva.

Leaving apart the biotechnological (in modern terminology) achievements of this department, which are considered in detail in the accompanying paper by E.P. Feofilova, we shall only mention here Shaposhnikov's important theoretical inference about the biphasic character of microbiological processes [2–4]. This inference turned out to be universally valid for all prokaryotes, eukaryotes, and archaea and formed the basis for the subsequent theory of secondary metabolism. We cannot but admire Shaposhnikov's capability for grasping the idea of biphasic microbiological processes at the time when microbial biochemistry was only in its infancy.

The Department of Technical Microbiology served as a stem that branched into new laboratories headed by direct or indirect followers of Shaposhnikov's ideas. Thus, in 1941, A.A. Imshenetskii (a future academician and director of the Institute of Microbiology) founded and became the head of the Department of Plant Materials. This department, which branched off from the Department of Fermentative Microorganisms, was later transformed into the Department of Experimental Variability of Microorganisms and then into the Department of Physiology of Mutant Microorganisms.

In 1961, the Department of Physiology of Microbial Growth and Development (it had branched off from the

0026-2617/04/7305-0565 © 2004 MAIK "Nauka/Interperiodica"

Department of Technical Microbiology) was headed by N.D. Ierusalimskii (a future academician and the first director of the Institute of Biochemistry and Physiology of Microorganisms in Pushchino). Beginning in 1967, the department was headed by I.L. Rabotnova.

In 1960, G.K. Skryabin (a future academician and the second director of the Institute of Biochemistry and Physiology of Microorganisms) founded and became the head of the Department of Microbiological Transformation of Steroids. In 1969, this department was transformed into the Department of Biosynthesis of Microbial Enzymes (headed by E.L. Ruban), and later (1983) into the Department of Regulation of Microbial Metabolism (headed by V.K. Plakunov).

As was already mentioned, the physiological–biochemical approach to the study of microorganisms was not limited to only these departments and was employed in almost all other laboratories of the institute, as is evident from the other papers in this issue. However, we will consider in detail only the research conducted in the aforementioned departments.

The studies devoted to the physiology of microorganisms that are used in biotechnological processes are comprehensively considered in the accompanying paper written by E.P. Feofilova.

Some studies, which initially had an applied character, have now been resumed at an advanced level. These are the studies related to bacterial thermophily (the paper by E.A. Bonch-Osmolovskaya in this issue) and cellulose degradation, which successfully employed the principle of the isolation of microorganisms on optimal media rather than on selective media.

The original view on the physiology of polyploid and mutant microorganisms has stimulated investigations into the mechanisms responsible for the adaptation of bacteria to cultivation conditions due to phenotypic variability via intragenetic rearrangements. The studies of this kind are being conducted under the guidance of T.F. Kondrat'eva in the Laboratory of Chemolithotrophic Microorganisms, headed by G.I. Karavaiko.

Another promising line of research originated from the problem of mutant physiology as studied in the Department of Microbial Adaptation (headed by Yu.N. Karasevich), which was united with the Department of Physiology and Biochemistry of Heterotrophic Microorganisms in 1981. The studies along this line dealt with the adaptation of microorganisms to unusual environmental conditions and persistent substrates (such as unnatural pentoses) and led to the formulation of the conception of preparatory metabolism [5, 6]. This approach is very close to the modern concept of adaptive mutagenesis. Regretfully, the lack of adequate equipment did not allow a good understanding of the mechanisms of this phenomenon.

Interest in the above problems prompted Karasevich to initiate investigations into the degradation and utilization of xenobiotics (pesticides), in particular, chloroanilines and chloro- and fluorobenzoic acids [7]. These studies are in progress under the guidance of E.G. Surovtseva.

The demands of space biology, a science that began to extensively develop in the 1970s, stimulated studies of the physiological and biochemical mechanisms of microbial survival under extreme conditions, such as low temperature, vacuum, hard UV radiation, and desiccation [8, 9]. The principle of searching for Earth-type life on other planets proposed by Imshenetskii at that time [10] is still significant.

A great many of the physiological studies conducted at the Institute of Microbiology in the 1960s were performed in the Department of Physiology of Microbial Growth and Development headed by Ierusalimskii. This department concentrated on developing the principles of the controlled cultivation of microorganisms (chemostat, turbidostat, and other modes of continuous cultivation). It was the period of a boom in investigations of such kind. To the credit of Soviet microbiology, the investigations performed at the institute in this field were appreciable in spite of the lack of adequate instrumentation. In particular, Ierusalimskii formulated the principle of a bottleneck (that is, a liming stage) of metabolic pathways. This principle provides the basis for the continuous cultivation of microorganisms. An important achievement of Ierusalimskii was also the creation of the model (sometimes called the Monod–Ierusalimskii model) relating the growth rate of microorganisms to the accumulation of toxic metabolic products [11, 12]. A comprehensive analysis of the extensive and diverse work of Ierusalimskii in the field of microbial physiology can be found in the review published in 1999 [13].

The ideas of Ierusalimskii were further extended by I.L. Rabotnova, who assumed the chair of the department in 1967. More efforts were concentrated on the investigation of the effect of unfavorable environmental factors (starvation, thermal stresses, extreme pH values, exposure to heavy metals, etc.) on the physiological state of microbial cultures with the aim of controlling their growth and adaptation to unfavorable conditions. Rabotnova paid much attention to creation of the Russian school of microbiologists–physiologists successfully developed the physiological–biochemical approach to the study of microbial growth and formulated an original idea of adaptive metabolism, which helps microorganisms better survive unfavorable environmental conditions [14, 15].

After the Department of Physiology of Microbial Growth and Development had been united with the Department of Regulation of Microbial Metabolism, pupils of Rabotnova (I.N. Pozmogova, S.A. Lirova, Z.V. Sakharova, and E.M. Shul'govskaya) applied her ideas, for instance, to the study of so-called nonspecific stress, which induces similar responses of microbial populations to diverse stress factors [16].

The physiological studies that were conducted at the Department of Microbiological Transformation of Steroids headed by G.K. Skryabin were aimed at obtaining clinically important steroid hormones with the aid of microorganisms that can implement some transformation reactions, such as hydroxylation and hydration. These studies culminated in the development of the respective plant-scale production protocols. When this department was transformed into the Department of Biosynthesis of Microbial Enzymes under the guidance of E.L. Ruban (1969), the major line of research became the study of the enzymes of lipid metabolism (endo- and exolipases). At the time, this research line was very important because of the ever-increasing demand for microbial lipolytic enzymes used in the manufacture of detergents, cheese, drugs, and other products.

In addition to applied aspects, researchers of this department (L.O. Severina and V.I. Duda) studied the location and the export of lipase from *Candida paralipolytica* cells by the methods of cyto- and immunochemistry. These studies confirmed the hypothesis of the involvement of the membrane–ribosome complex in the biosynthesis of this lipase. Parallel investigations were initiated to understand the mechanisms of enzyme regulation by means of protein–lipid interactions [17].

In 1980, V.K. Plakunov became the head of the department, which was later (1983) renamed the Department of Regulation of Microbial Metabolism. Much research effort at that time was concentrated on a comparative study of extremely halophilic archaea from the family *Halobacteriaceae* [18–21], representatives of the genera *Natronobacterium* and *Natronococcus* [22, 23], and slightly to moderately halophilic eubacteria [24]. Primary attention was given to the membrane transport of organic substances and related catabolic reactions (in particular, the energy-driven translocation of substrates through membranes). Advanced methodological approaches (including the use of vesicles with direct and inverted orientations of the cytoplasmic membrane) allowed new regulatory mechanisms to be revealed, for instance, the rarely encountered regulation of the inward transport of substrates by intracellular pools of these substrates (so-called transregulation) and the selective induction and repression of multiple transport systems of a substrate [25, 26]. In recent years, investigations of extremely thermophilic obligately anaerobic archaea of the genera *Thermococcus, Pyrococcus*, and *Desulfurococcus* have been in progress [27].

Advances have been made in studies along an entirely new line of research, that is, the adaptation of halophilic organisms to hypoosmotic stress, which was named *halodeadaptation* by analogy with the well-known term *haloadaptation* that designates bacterial adaptation to hyperosmotic stress. It was found that the lysis products of extremely halophilic archaea may play the role of osmotic alarm signals to enhance the osmotolerance of halobacterial cells [28]. In the case of simultaneous action of two stressful factors (for instance, hypoosmotic shock and infraoptimal pH values), they may interfere with each other in such a manner that protons prevent the osmolysis of halobacterial cells subjected to hypoosmotic shock [29].

Another new regulatory mechanism, named membrane regulation [30, 31], was found to control microbial metabolism by removing metabolic effectors (inhibitors or activators) from cells. Such regulation may provide for the oversynthesis of physiologically active compounds [32].

After the Laboratory of Regulation of Microbial Metabolism had been disbanded, almost all the laboratory staff was employed by the Laboratory of Microbial Biogeochemistry and Biotechnology, headed by Academician M.V. Ivanov, and by the newly organized Laboratory of Oil Microbiology headed by S.S. Belyaev. The latter laboratory concentrated on the study of microflora of oil strata and halotolerant and halophilic microorganisms isolated from various biotopes. These studies confirmed the existence of antagonism between some stressful factors when they act together (e.g., high salt concentration and low partial oxygen pressure). Interestingly, this phenomenon explains the occurrence of aerobic slightly halophilic microorganisms in oil strata with high-salinity anoxic stratal waters and turned out to be sufficiently universal [33, 34].

Of great interest are interactions in microbial communities between microorganisms of the same nutritional level. For instance, the first stage of microbial succession in water-flooded oil deposits is dominated by oil-oxidizing chemoheterotrophic microorganisms (producers), whereas microaerobic zones are dominated by facultatively and strictly anaerobic microorganisms utilizing the products of hydrocarbon oxidation (dissipotrophs). The dependence of the dissipotrophs on the producers is obvious, although the former also influence the latter both nutritionally and regulatorily.

One of the advanced lines of research, the biochemistry of microbial communication, was initiated by two famed Russian microbiologists, Ierusalimskii and Krasil'nikov. As was mentioned in the paper devoted to Ierusalimskii [13], his name is mainly associated with the kinetics of microbial growth and continuous cultivation. However, these problems are only the tip of the iceberg. The scope of Ierusalimskii's research interest was much wider, and we may say that his ideas on the physiology of bacterial cultures, which were considered by him as self-developing ontogenetic systems [35], were ahead of his time and promoted the study of bacterial communication and growth regulation at many scientific centers in Russia. Recently, the conceptions of a culture as an organism and culture ontogeny have been revived by J. Shapiro [36].

On the other hand, Krasil'nikov, who headed the Department of Microbial Interactions and was a great authority in the biology and systematics of actinomycetes, paid great attention to the study of the interaction of microorganisms with each other, with the environment, and with the host organism (in the case of symbiotic interactions) [37]. These interactions are mediated by biologically active metabolites (antibiotics, growth stimulants, etc.), whose chemical nature and conditions of biosynthesis were a subject of investigation by Yu.M. Khokhlova, O.I. Artamonova, A.N. Kozlova, and others. When Krasil'nikov studied the intrapopulation interactions of streptomycetes, he described for the first time the phenomenon of age-related morphocolonial variability [38].

The fact that pro- and eukaryotic organisms developing in cultures, colonies, and other structured ensembles are closely related with each other prompted researchers to study the language through which they communicate with each other, with the environment, and with the host organism.

In the 1970s, Krasil'nikov posed the problem of studying the chemical nature and the mechanism of action of the extracellular metabolites that control the formation of resting fungal forms. Guided by G.I. El'-Registan from its beginnings, the research along this line gradually transformed into the study of the autoregulation of the growth and development of microbial cultures. These studies were performed first in the Laboratory of Physiology of Spore-Forming Bacteria, headed by V.I. Duda, and then in the Laboratory of Classification and Maintenance of Unique Microorganisms, headed by V.F. Gal'chenko, where they are still in progress.

These studies showed the functioning of a universal system for the autoregulation of the growth and development of diverse pro- and eukaryotic microorganisms. This system was found to be based on two varieties of metabolites with the properties of natural structural modifiers of macromolecules and biomembranes. The first variety (d_1 factors, or the autoinducers of anabiosis) is represented in some bacteria and yeasts by alkylhydroxybenzenes (AHBs). The second variety (d_2 factors, or the autoinducers of autolysis) represents free unsaturated fatty acids (FUFAs). The balance of d_1 and d_2 factors in a microbial culture controls its growth, the change of growth stages, and cell differentiation [39]. In their pioneering works, the authors showed that an elevated level of AHBs (d_1 factors) induces the formation of anabiotic cystlike resting cells (CRCs) not only in non-spore-forming gram-positive and gram-negative bacteria, archaea, and yeasts (for which CRCs are the only cell form providing for species survival) [40–42] but also in spore-forming bacteria (for which CRCs are an alternative resting form apart from spores) [40]. It should be noted that studies conducted in collaboration with the Laboratory of Structural and Functional Adaptation of Microorganisms (head, V.I. Duda) at the Skryabin Institute of Biochemistry and Physiology of Microorganisms and the Department of Soil Biology (head, D.G. Zvyagintsev) at Moscow State University revealed similar CRCs (having analogous ultrastructure and composition of biologically important elements) in permafrost soils and Antarctic grounds ranging in age from 0.5 to 3.0 million years [43–46].

Beginning in the 1990s, the Group of Autoregulation of Microbial Processes at the Laboratory of Classification and Maintenance of Unique Microorganisms and the Group of Biochemical Regulation at the Laboratory of Oil Microbiology concentrated their efforts on the study of the regulatory mechanisms that are involved in the physiological stress response of microorganisms and provide for the modulation of their metabolic activity and adaptation to varying environmental conditions. These two research groups obtained experimental evidence that low-molecular-weight extracellular autoregulators (AHBs as the autoinducers of anabiosis [47] and the metabolites released from cells during the partial lysis of a microbial population [28]) are involved in the formation of cell response to various stress factors. These non-species-specific microbial metabolites act as adaptogens and enhance the tolerance of bacterial [47, 28], yeast [48], and animal cells (fibroblasts) [49] to stressful agents. The nonspecificity of these metabolites (AHBs in particular) can be explained by the fact that their protective action is based on their functioning as chemical chaperones [50], in addition to their protective action as well-known molecular chaperones (such as heat shock and other stress proteins). AHBs produce complexes and thus modify the spatial structure of membrane lipids and biomacromolecules (enzymes, peptidoglycans, and polysaccharides) due to hydrophobic and electrostatic interactions and intermolecular hydrogen bonding. This stabilizes cellular biopolymers and membranes, provides control over the catalytic activity of enzymes [50–52] and the functional activity of membranes [53], and modifies the specificity of the biopolymers to their depolymerases [52]. The direction to which the catalytic activity of the modified enzymes changes (either increases or decreases) depends on the concentration and the hydrophobicity of low-molecular-weight ligands [50, 52]. The dynamic equilibrium of AHB homologues and isomers in an aging or stressed microbial culture provides for flexible modulation of the metabolic activity of cells and, hence, their high tolerance to stresses [39].

Another aspect of the involvement of AHBs in stress responses is related to their functioning as scavengers of reactive oxygen species, which are formed in response to almost any stressful action. This was experimentally proved for stationary and pulsed radiolysis [54] and for photooxidation [48]. The involvement of AHBs in the formation of nonenzymatic antioxidant systems is of great importance for actively proliferating cells at the first stage of their stress response (growth retardation); for hypometabolic stationary-phase cells under the conditions of starvation stress; and, especially, for resting anabiotic cells, in which many metabolic activities (including the activity of antioxidant enzymes) are suppressed.

The preliminary addition of exogenous AHBs (or their synthetic analogues) to a growing microbial culture protects cells from gamma radiation, photooxidation, temperature shock, and chemical and biological toxicants [47–49].

It should be noted that the physicochemical mechanisms responsible for the enhanced stress tolerance of subcellular structures, which act in addition to the molecular genetic mechanisms of stress response, are the same both during growth retardation and cell transition to a hypometabolic state and during the development of an anabiotic state.

According to the generalized hypothesis, the physicochemical structural modification of cellular biopolymers and membranes associated with their complexing with low-molecular-weight adaptogens (such as AHBs and some amino acids) includes the following rearrangements: (1) the polycrystallization of the lipid stroma of membranes and the partial or complete inhibition of their functional activity; (2) the dehydration of the cell protoplast; (3) the structural stabilization of enzyme molecules and modification of their catalytic activity; (4) a decrease in (or even loss of) the specificity of cellular biopolymers and supramolecular complexes to the action of cellular and extracellular depolymerases; and (5) the function of a nonenzymatic antioxidant system. In total, these rearrangements cause a reversible metabolic block in cells and provide for their tolerance to various stresses.

Bacterial cultures exposed to sublethal stress may also produce another type of nonspecific extracellular autoregulators with the function of adaptogens [55, 56]. These adaptogens represent nonprotein exometabolites with the characteristics of inducers of enhanced cell viability ($X_{\rm I}$) and cell protectants of direct action ($X_{\rm II}$) [57, 58].

Interesting data were obtained during the study of communication processes in bacterial populations prone to lysis under stressful conditions. One subpopulation in a stressed culture may survive at the expense of the autolytic death of another subpopulation, which is considered to be *bacterial altruism.* The mechanism of this phenomenon, studied with reference to halophilic bacteria subjected to hypoosmotic stress, lies in the action of extracellular nonlipid metabolites with the function of osmotic alarm signals. The incubation of cells with these exometabolites, which are the osmolysis products of halobacteria, enhances the osmotolerance of the cells [28].

The reversible adhesion of cells to solid surfaces can be considered a universal adaptive response of cells [59], in which extracellular adaptogenic metabolites are involved [60]. The desorption of attached cells is controlled by extracellular adaptogens, which are high-molecular-weight paraffins in some bacteria [61] and extracellular proteases in others [62].

The stress response of microorganisms at the population level is detected as a replacement of cell variants and the predominance of the variant that is the most adapted to the altered environment. This phenomenon was called by Ierusalimskii *adaptive modification* [35]. The ability of cells to dissociate is implemented during the germination of their resting forms, the degree of dissociation being dependent on the morphotype of these forms. For instance, the dissociation ability of cystlike cells is considerably higher than that of endospores [63]. This type of stress responses is controlled by the level of the intercellular communication factors that are involved in the regulation of the resting state, namely, acylated homoserine lactones (which control the stationary phase) [64, 65] and alkylhydroxybenzenes (which control the induction of anabiosis and the formation of cystlike cells) [39, 63].

The concentration of AHBs and the proportion between AHBs and FUFAs (i.e., between factors d_1 and d_2) determine the direction of dissociative transitions in some bacilli and pseudomonads and changes in their phenotypes (R, S, M, and D variants and their subvariants). The phenotypes differ in colonial morphology, growth parameters, physiological and biochemical characteristics, stress tolerance, and virulence (in the case of pathogenic bacteria) [47, 63, 66, 67]. The analysis of the mechanisms through which AHBs are involved in the dissociation of bacterial cultures suggests two possibilities: (1) their effect on the conformation and the functional activity of the enzymatic, receptive, or regulatory proteins involved in the control of gene expression and in the regulatory mechanisms of cell dissociation at the transcriptional level and (2) the direct interaction of AHBs with DNA. The latter possibility is confirmed by the weak mutagenic activity of hydrophobic AHBs [67]. This allows AHBs to be considered as endogenous mutagens, which may induce reversible rearrangements in genetic material responsible for the dissociative transitions between different phenotypes. The genetic rearrangements can be detected by PCR with random primers as minor genomic differences [66].

The diverse functional activity of the non-species-specific low-molecular-weight factors responsible for the intercellular communication of microorganisms indicates their important role in the autoregulation of succession and in the stability of microbial communities.

We believe that the further efficient development of the physiological–biochemical line of research at the Institute of Microbiology requires the establishment of a special department (laboratory). This laboratory should be equipped with advanced automated systems for molecular biological and genetic analyses and must concentrate on the study of the physiological, biochemical, and physicochemical characteristics of developing microbial populations, associations, and communities, as well as of newly isolated individual microorganisms. This would allow the metabolic activity of component microorganisms in communities to be studied directly in natural substrates (in addition to the FISH method), which is currently difficult, if at all possible, to accomplish because of the high cost of such investigations and a deficiency of skilled personnel.

REFERENCES

1. Gutina, V.N., *Ocherki po istorii fiziologii mikroorganizmov* (Sketches from the History of the Physiology of Microorganisms), Moscow: Nauka, 1988.
2. Shaposhnikov, V.N., *Tekhnicheskaya mikrobiologiya* (Technical Microbiology), Moscow: Sovetskaya Nauka, 1948.
3. Shaposhnikov, V.N., *Fiziologiya obmena veshchestv mikroorganizmov v svyazi s evolyutsiei funktsii* (The Physiology of Microbial Metabolism in Relation to Its Functional Evolution), Moscow: Izd. Akad. Nauk SSSR, 1960.
4. Shaposhnikov, V.N., *Osnovnye fiziko-khimicheskie zakonomernosti fiziologii obmena veshchestv mikroorganizmov* (The Basic Physicochemical Characteristics of the Physiology of Microbial Metabolism), Moscow: Nauka, 1968.
5. Karasevich, Yu.N., Theoretical Problems in the Study of the Initial Steps of the Microbial Metabolism of Organic Compounds, *Usp. Mikrobiol.*, 1971, no. 7, pp. 156–174.
6. Karasevich, Yu.N., *Eksperimental'naya adaptatsiya mikroorganizmov* (The Experimental Adaptation of Microorganisms), Moscow: Nauka, 1975.
7. Karasevich, Yu.N., *Osnovy selektsii mikroorganizmov, utiliziruyushchikh sinteticheskie organicheskie soedineniya* (The Essentials of the Selection of Microorganisms Utilizing Synthetic Organic Compounds), Moscow: Nauka, 1982.
8. Murzakov, B.G., The Ecology of the Microflora of Desert Soils in Relation to the Problems of Space Microbiology, *Usp. Mikrobiol.*, 1974, no. 9, pp. 96–124.
9. Lysenko, S.V., The Effect of Deep Vacuum on Microorganisms, *Usp. Mikrobiol.*, 1981, no. 16, pp. 231–253.
10. Imshenetskii, A.A., Space Biology, *Usp. Mikrobiol.*, 1971, no. 7, pp. 46–66.
11. Ierusalimskii, N.D., *Osnovy fiziologii mikrobov* (The Essentials of Microbial Physiology), Moscow: Izd. Akad. Nauk SSSR, 1963.
12. Ierusalimskii, N.D., The Theory and Practice of the Continuous Cultivation of Microorganisms, *Mikrobiologiya*, 1961, vol. 30, no. 6, pp. 818–824.
13. Golovlev, E.L., Academician Nikolai Dmitrievich Ierusalimskii, *Mikrobiologiya*, 1999, vol. 68, no. 6, pp. 800–808.
14. Rabotnova, I.L., *Rol' fiziko-khimicheskikh uslovii (pH i rH_2) v zhiznedeyatel'nosti mikroorganizmov* (The Role of Physicochemical Conditions (pH and rH_2) in Microbial Metabolism, Moscow: Izd. Akad. Nauk SSSR, 1957.
15. Rabotnova, I.L. and Pozmogova, I.N., *Khemostatnoe kul'tivirovanie i ingibirovanie mikroorganizmov* (Cultivation and Inhibition of Microorganisms in Chemostat), Moscow: Nauka, 1979.
16. Pozmogova, I.N., *Kul'tivatsiya mikroorganizmov v peremennykh usloviyakh* (Cultivation of Microorganisms under Varying Conditions), Moscow: Nauka, 1983.
17. Lestrovaya, N.N., The Localization of 2-Oxosteroid-Δ^1 Dehydrogenase in *Mycobacterium rubrum* and *Arthrobacter globiformis* Cells, *Mikrobiologiya*, 1981, vol. 50, no. 4, pp. 619–625.
18. Tarasov, A.L., Zvyagintseva, I.S., and Plakunov, V.K., The Effect of Monovalent Cations and Inhibitors on the Transport of C_4-Dicarboxylic Acids in Halophilic Bacteria, *Mikrobiologiya*, 1985, vol. 54, no. 6, pp. 869–875.
19. Pimenov, N.V., Severina, L.O., and Plakunov, V.K., On the Utilization of Glucose and Galactose by Extreme Halophiles, *Mikrobiologiya*, 1986, vol. 55, no. 3, pp. 362–367.
20. Lobyreva, L.B., Fel'dman, R.S., and Plakunov, V.K., The Effect of Aromatic Amino Acids on the Growth of *H. salinarium* and Consumption of [^{14}C]Phenylalanine, *Mikrobiologiya*, 1987, vol. 56, no. 1, pp. 16–20.
21. Severina, L.O., Usenko, I.A., and Plakunov, V.K., Biosynthesis of Exopolysaccharide in the Extremely Halophilic Archaebacterium *H. mediterranei, Mikrobiologiya*, 1989, vol. 58, no. 4, pp. 557–561.
22. Zvyagintseva, I.S. and Tarasov, A.L., *Mikrobiologiya*, 1987, vol. 56, no. 5, pp. 839–844.
23. Kevbrina, M.V. and Plakunov, V.K., Metabolism of Acetate in *N. occultus, Mikrobiologiya*, 1992, vol. 61, no. 5, pp. 770–775.
24. Nikolaev, Yu.A., Matveeva, N.I., and Plakunov, V.K., The Energization of Amino Acid Transport in Some Bacteria Living in High-Salinity Habitats, *Mikrobiologiya*, 1992, vol. 61, no. 2, pp. 183–188.
25. Severina, L.O., Pimenov, N.V., and Plakunov, V.K., Glucose Transport into the Extremely Halophilic Archaebacteria, *Arch. Microbiol.*, 1991, vol. 155, pp. 131–136.
26. Lobyreva, L.B., Kokoeva, M.V., and Plakunov, V.K., Physiological Role of Tyrosine Transport Systems in *Halobacterium salinarium, Arch. Microbiol.*, 1994, vol. 162, pp. 126–130.
27. Usenko, I.A., The Utilization of Amino Acids and Sugars by the Extremely Thermophilic Archaebacteria from the Genus *Thermococcus, Mikrobiologiya*, 1994, vol. 63, no. 5, pp. 783–791.
28. Kokoeva, M.V. and Plakunov, V.K., The Feasibility of Modifying the Osmotolerance of Extremely Halophilic Archaebacteria, *Mikrobiologiya*, 1993, vol. 62, no. 5, pp. 825–834.
29. Shelemekh, O.V., Plakunov, V.K., and Belyaev, S.S., The Protective Role of Protons in the Extremely Halophilic Archaebacterium *Halobacterium salinarum* Exposed to Hypoosmotic Stress, *Mikrobiologiya*, 2002, vol. 71, no. 6, pp. 858–859.
30. Plakunov, V.K., The Membrane Mechanisms of Excretion of Physiologically Active Substances in Bacteria, *Prikl. Biokhim. Mikrobiol.*, 1986, vol. 22, no. 6, pp. 723–735.
31. Plakunov, V.K., *Osnovy enzimologii* (Essentials of Enzymology), Moscow: Logos, 2001.
32. Plakunov, V.K., Volkova, I.M., and Lebedeva, Zh.D., The Role of Excretion in the Oversynthesis of Glutamic Acid

by *Corynebacterium glutamicum, Mikrobiologiya*, 1992, vol. 61, no. 2, pp. 189–193.

33. Arzumanyan, V.G., Voronina, N.A., Geidebrekht, O.V., Shelemekh, O.V., Plakunov, V.K., and Belyaev, S.S., Antagonistic Interactions between Stress Factors during the Growth of Microorganisms under Conditions Simulating the Parameters of Their Natural Ecotopes, *Mikrobiologiya*, 2002, vol. 71, no. 2, pp. 160–165.
34. Geidebrekht, O.V., Arzumanyan, V.G., Plakunov, V.K., and Belyaev, S.S., Influence of the Degree of Aeration on Halotolerance of Yeasts of the Genera *Candida, Rhodotorula*, and *Malassezia, Mikrobiologiya*, 2003, vol. 72, no. 3, pp. 312–319.
35. Ierusalimskii, N.D., The Physiology of Growing Pure Cultures, *Doctoral (Biol.) Dissertation*, Moscow, 1967.
36. *Bacteria as Multicellular Organisms*, Shapiro, J.A. and Dworkin, M., Eds., New York: Oxford Univ. Press, 1997.
37. Gutina, V.N., *Nikolai Aleksandrovich Krasil'nikov,* Moscow: Nauka, 1982.
38. Krasil'nikov, N.A., Microorganisms and Soil Fertility, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1954, no. 2, pp. 14–39.
39. El'-Registan, G.I., The Role of Membranotropic Autoregulatory Factors in the Processes of Microbial Growth and Development, *Doctoral (Biol.) Dissertation*, Moscow, 1988.
40. Mulyukin, A.L., Lusta, K.A., Gryaznova, M.N., Kozlova, A.N., Duzha, M.V., Duda, V.I., and El'-Registan, G.I., Formation of Resting Cells by *Bacillus cereus* and *Micrococcus luteus, Mikrobiologiya*, 1996, vol. 65, no. 6, pp. 782–789.
41. Demkina, E.V., Soina, V.S., and El'-Registan, G.I., Formation of Resting Forms of *Arthrobacter globiformis* in Autolyzing Cell Suspensions, *Mikrobiologiya,* 2000, vol. 69, no. 3, pp. 383–388.
42. Loiko, N.G., Soina, V.S., Sorokin, D.Yu., Mityushina, L.L., and El'-Registan, G.I., Production of Resting Forms by the Gram-Negative Chemolithoautotrophic Bacteria *Thioalkalivibrio versutus* and *Thioalkalimicrobium aerophilum*, *Mikrobiologiya*, 2003, vol. 72, no. 3, pp. 328–337.
43. Mulyukin, A.L., Sorokin, V.V., Loiko, N.G., Suzina, N.E., Duda, V.I., Vorob'eva, E.A., and El'-Registan, G.I., Comparative Study of the Elemental Composition of Vegetative and Resting Microbial Cells, *Mikrobiologiya*, 2002, vol. 71, no. 1, pp. 37–48.
44. Mulyukin, A.L., Sorokin, V.V., Vorob'eva, E.A., Suzina, N.E., Duda, V.I., Gal'chenko, V.F., and El'-Registan, G.I., Detection of Microorganisms in the Environment and the Preliminary Appraisal of Their Physiological State by X-Ray Microanalysis, *Mikrobiologiya*, 2002, vol. 71, no. 6, pp. 836–848.
45. Suzina, N.E., Mulyukin, A.L., Kozlova, A.N., Shorokhova, A.P., Dmitriev, V.V., Barinova, E.S., Mokhova, O.N., El'-Registan, G.I., and Duda, V.I., The Fine Structure of the Resting Cells of Some Non-Spore-Forming Bacteria, *Mikrobiologiya*, 2004, vol. 73, no. 4, pp. 516–529.
46. Soina, V.S., Mulyukin, A.L., Demkina, E.V., Vorobyova, E.A., and El'-Registan, G.I., The structure of resting microbial populations in soil and subsoil permafrost, *Astrobiology*, 2004 (in press).
47. Il'inskaya, O.N., Kolpakov, A.I., Shmidt, M.A., Doroshenko, E.V., Mulyukin, A.L., and El'-Registan, G.I., The Role of Bacterial Growth Autoregulators (Alkyl Hydroxybenzenes) in the Response of Staphylococci to Stresses, *Mikrobiologiya*, 2002, vol. 71, no. 1, pp. 23–29.
48. Stepanenko, I.Yu., Strakhovskaya, M.G., and Belenikina, N.S., Nikolaev, Yu.A., Mulyukin, A.L., Revina, A.A., and El'-Registan, G.I., The Protection of *Saccharomyces cerevisiae* by Alkylhydroxybenzenes against Damage from Oxidative Stress and Radiation, *Mikrobiologiya*, 2004, vol. 73, no. 2, pp. 204–210.
49. Il'inskaya, O.N., Kolpakov, A.I., Mulyukin, A.L., Dreier, F., and El'-Registan, G.I., The Effect of Membranotropic Microbial Autoregulators on the Growth of the Ras-transformed Murein Fibroblast Culture, *Prikl. Biokhim. Mikrobiol.*, 2000, vol. 62, no. 5, pp. 475–480.
50. Kolpakov, A.I., Il'inskaya, O.N., Bespalov, M.M., Kupriyanova-Ashina, F.G., Gal'chenko, V.F., Kurganov, B.I., and El'-Registan, G.I., Stabilization of Enzymes by Dormancy Autoinducers as a Possible Mechanism of Resistance of Resting Microbial Forms, *Mikrobiologiya*, 2000, vol. 69, no. 2, pp. 224–230.
51. Bespalov, M.M., Kolpakov, A.I., Loiko, N.G., Doroshenko, E.V., Mulyukin, A.L., Kozlova, A.N., Varlamova, E.A., Kurganov, B.I., and El'-Registan, G.I., The Role of Microbial Dormancy Autoinducers in Metabolism Blockade, *Mikrobiologiya*, 2000, vol. 69, no. 2, pp. 217–223.
52. Martirosova, E.I., Karpekina, T.A., and El'-Registan, G.I., Enzyme Modification by Natural Chemical Chaperons of Microorganisms, *Mikrobiologiya*, 2004, vol. 73, no. 5, pp. 708–715.
53. Kaprel'yants, A.S., Suleimenov, M.K., Sorokina, A.D., Deborin, G.A., El'-Registan, G.I., Stoyanovich, F.M., Lille, Yu.E., and Ostrovskii, D.N., The Structural and Functional Alterations in Bacterial and Model Membranes Induced by Phenol Lipids, *Biol. Membr.*, 1987, vol. 4, no. 3, pp. 254–261.
54. Revina, A.A., Larionov, O.G., Kochetova, M.V., Lutsik, T.K., and El'-Registan, G.I., The Spectrophotometric and Chromatographic Studies of Radiolysis Products in Aerated Aqueous Solutions of Alkylresorcinols, *Izv. Akad. Nauk, Ser. Khim.*, 2003, no. 11, pp. 2257–2263.
55. Nikolaev, Yu.A., Involvement of Exometabolites in Stress Adaptation of *Escherichia coli, Mikrobiologiya*, 1997, vol. 66, no. 1, pp. 38–41.
56. Nikolaev, Yu.A., Two Novel Extracellular Adaptogenic Factors of *Escherichia coli* K-12, *Mikrobiologiya*, 1997, vol. 66, no. 6, pp. 785–789.
57. Nikolaev, Yu.A., Comparative Study of Two Extracellular Protectants Secreted by *Escherichia coli* Cells at Elevated Temperatures, *Mikrobiologiya*, 1997, vol. 66, no. 6, pp. 790–795.
58. Nikolaev, Yu.A. and Voronina, N.A., Cross-Action of Extracellular Stress Adaptation Factors in Microorganisms, *Mikrobiologiya*, 1999, vol. 68, no. 1, pp. 45–50.
59. Marshall, K.C., Adhesion as a Strategy for Access to Nutrients, *Bacterial Adhesion: Molecular and Ecological Diversity*, New York: Wiley-Liss, 1996, pp. 59–88.
60. Rodionova, T.A. and Nikolaev, Yu.A., The Protective Effect of the Reversible Adhesion of *Bacillus lichenifor-*

mis 603 against *N*-ethylmaleimide, *Mikrobiologiya,* 2004, vol. 73, no. 6, pp. 133–134.

61. Nikolaev, Yu.A., Panikov, N.S., Lukin, S.M., and Osipov, G.A., Saturated C_{21}–C_{33} Hydrocarbons Are Involved in the Self-Regulation of *Pseudomonas fluorescens* Adhesion to a Glass Surface, *Mikrobiologiya,* 2001, vol. 70, no. 2, pp. 174–181.

62. Nikolaev, Yu.A. and Panikov, N.S., Extracellular Protease as a Reversible Adhesion Regulator in *Pseudomonas fluorescens, Mikrobiologiya*, 2002, vol. 71, no. 5, pp. 629–634.

63. Doroshenko, E.V., Loiko, N.G., Il'inskaya, O.N., Kolpakov, A.I., Gornova, I.V., and El'-Registan, G.I., Characterization of *Bacillus cereus* Dissociants, *Mikrobiologiya*, 2001, vol. 70, no. 6, pp. 811–819.

64. Yildiz, F.H. and Schoolnik, G.K., Role of *rpoS* in Stress Survival and Virulence of *Vibrio cholerae, J. Bacteriol.*, 1998, vol. 180, no. 4, pp. 773–784.

65. Fuqua, W.C., Winans, S.C., and Greenberg, E.P., Quorum Sensing in Bacteria: The LuxR–LuxI Family of Cell Density–Responsive Transcriptional Regulators, *J. Bacteriol.*, 1994, vol. 176, no. 2, pp. 269–275.

66. Tsygankova, S.V., Bulygina, E.S., Kuznetsov, B.B., Korotkov, N.I., Khabibullin, S.S., Doroshenko, E.V., and El'-Registan, G.I., The Obtaining of the Dissociants of Some Bacilli and Their Identification by DIR-PCR, *Mikrobiologiya*, 2004, vol. 73, no. 3, pp. 398–405.

67. Il'inskaya, O.N., Kolpakov, A.I., Zelenin, P.V., Kruglova, Z.D., Choidash, B., Doroshenko, E.V., Mulyukin, A.L., and El'-Registan, G.I., The Effect of Anabiosis Autoinducers on the Bacterial Genome, *Mikrobiologiya*, 2002, vol. 71, no. 2, pp. 194–199.