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REVIEWS

Microbial Diversity Studies at the Winogradsky Institute of Microbiology

G. A. Zavarzin

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

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Abstract—Due to a chain of circumstances, some of them caused by unfavorable events, the Institute of Microbiology, Russian Academy of Sciences (INMI), continued the tradition of the "golden age" of microbiology of Winogradsky's time. Interest was focused on the functional diversity of microorganisms as catalysts of natural chemical processes. The concept of a morphophysiological genus continues to play the key role in general microbiology, serving as an operational entity of the science. Researchers affiliated with INMI pioneered in describing 60 genera, constituting 4.9% of the 1228 validated generic designations as of January 1, 2004, and more than 6% of the nonpathogenic cultivable genera deposited at DSMZ. In addition to formal biodiversity, other aspects of physiological and ecological diversity described by different classification schemes are considered. More recent findings have been mostly in the field of alkaliphiles, acidophiles, thermophiles, and halophilic anaerobes, proliferating in extreme environments. Attempts to work out classifications of microorganisms with prognostic potential are presented.

PROBLEM FORMULATION

The Moscow Institute of Microbiology, Russian Academy of Sciences (INMI) is deservedly recognized as a center of research on the diversity of microorganisms, predominantly bacteria. In the 1980s and 1990s, the number of taxa of microorganisms described at INMI constituted as many as 5% of the total worldwide crop. The new taxa mostly belonged to genera of free-living organisms from somewhat peculiar—extreme—habitats; i.e., they were extremophiles. What gave rise to such a steady tradition of interest in a specific area of microbiology, one may ask. Another question is how it happened that researchers from one institution would compete in the search for new organisms and how the skills to solve problems in this very specific area were developed.

The editorial board of *Mikrobiologiya* asked me to write about the history of microbial diversity research at INMI in connection with the 70th anniversary of the Institute and the interest in jubilees that has recently spread in our scientific community. It would hardly be appropriate to single out one institute in an analysis of progress made in a given branch of science even as a "case study" to understand the development of fairly small research groups, depending so much upon random factors. Taking INMI as an example, it would be interesting to compare the advantages and shortcomings of the research tradition of continuity as compared to the total change of subject and research direction practiced, for example, in Germany, where the retirement of the leader brings forth complete reorganization. The new leader starts with "utterly destroying and then …." A research subject is recognized as having been fully exhausted in the course of the two to three decades of the previous leadership. The subject continues to be supported by a minimal number of staff researchers and postgraduate students, completing their involvement with the subject in the next three years. In the USSR, by contrast, the predominant practice was that of continuity: leaders were changed without changing the research body profile either at the laboratory level or that of the institute. This was mostly done out of the wish to preserve the expensive infrastructure but was also based on the conviction that every sector of science must be supported in a big country needing to stay independent. The actual decision, naturally, depends on the scale of the problem. The answer is completely clear as regards pathogenic microorganisms. For every single infection or type of infections, well-trained professionals must be available and ready to check it, even with no outbreaks of an epidemic. The situation is somewhat similar with the problem of biological diversity: all its major divisions must be provided with staff having the required skills. Today, science in the Russian Federation, the successor of the Soviet Union, has considerable gaps. An example close to microbiology would be the whole field of mycology and, to a large degree, protistology, especially considering their present-day experimental aspects.

THE GOAL OF BIODIVERSITY STUDIES

Biodiversity lacked much respectability in the eyes of leading organizations until the 1990s, when the Convention on Biodiversity was drawn up and adopted. Until that point, it was regarded as something of narrow and special importance for an individual branch of science or even as an amateurs' pastime. This absence of interest was occasionally broken only by sensational discoveries of bacteria in unusual environments (today, such bacteria are called extremophiles) or organisms with unusual functions. The Convention on Biodiversity obligates every country to know and preserve biodiversity on its territory. As a result of this convention, the "Biodiversity" federal program is operative in the Russian Federation, applying also to microorganisms. The convention caused a sharp change in the attitude to scholars compiling and maintaining collections of living organisms as a means to preserve species diversity ex situ—outside the natural environment. This fully applies to microorganisms because, for a new taxon of bacteria (or yeasts) to be validated, its viable culture must be stored in at least one (nowadays, two) internationally recognized collections. For all other organisms, an unchanged specimen has to be preserved in the form of a herbarium leaf or a fixed sample, allowing identification of new finds by means of direct comparison. Today, this requirement looks fairly archaic because identification, particularly of bacteria, can be accomplished by molecular biology methods. However, the advantage of storing viable cultures is that they can be used later to refine our knowledge by new methods and for biotechnological purposes. It should be noted that even though the taxonomic interpretation of biodiversity is widespread it is limited in scope.

The objective of the Convention was to preserve the diversity of living beings on the planet as a basis for stable development of the biosphere. For reaching this goal, it is much more important to preserve biodiversity in situ, i.e., in the habitat and the ecosystem. It follows that the preservation of landscape actually has a higher priority than preserving herbarium leaves and even botanic gardens. Now, is the conservation of the ecosystem important for microorganisms, and how large in terms of scale should their reserve be? Being so small, could not bacteria suffice with a droplet of water? That was exactly the response of zoologists to my article "Microbial Reservations" in *Priroda* [1]. Protecting microbes? Isn't that rubbish? Aren't they all harmful? Better to be concerned with migrating birds and their nesting places. This, obviously, requires natural reserves and these activities were steadily advanced in the Soviet Union (not the Russian Federation, where even water protection zones were squandered and legislation was adopted to the effect that "it's OK to spit into the well whenever bottled water is available"). Microbes, as a matter of fact, depend strongly on the habitat and its microbial community. And even though microbes can be transferred from one hot spring or salt marsh to another, their exploration is a much more costly matter both technically and in terms of funding than watching migrating birds in binoculars. For this reason, reservations for microorganisms as a genetic storehouse for the country are well worth it. The most obvious example is communities developing in thermal springs. Preserving habitats of extremophilic communities of microorganisms requires, above all, that the hydrological regime be kept intact, and this objective goes well beyond a droplet of water and cannot be attained without preserving the landscape.

Biodiversity must be evaluated at different levels. In the context of biosphere stability, the functional diversity is of utmost importance. It was argued that natural biodiversity should be analyzed at the level of (1) species, (2) populations, (3) functional groups, (4) systems, and (5) landscapes belonging to different biological zones and biomes [2]. The INMI microbiologists traditionally focused their interest on levels 1, 3, and 4, treating the term "system" as meaning communities of microorganisms.

A widespread though grossly erroneous conviction is that diversity should be understood as species diversity and that special attention should be paid to rare and endangered species. Within this approach, the species diversity is measured by the number of Latin binomials found in flora and fauna lists. However, in estimating the species diversity, a distinction should be made between taxonomy and nomenclature. The discovery of new taxons and their initial description is supported by the fundamental concept of priority set out in the Bacteriological Code but often bypassed by means of subsequent changes of nomenclature. There are basically two approaches to describing a multitude of organisms: taxon splitting and taxon merging. The phylogenetic taxonomy of bacteria, based on arbitrary quantitative criteria of difference in the nucleotide sequence in the 16S rRNA gene, is responsible for an excessive splitting of taxa, especially on the genus level. Thus, by applying genotypic methods and quantitative criteria of taxonomic distinctions, the genus *Clostridium* was split into multiple independent clusters with genera composed of few species. The splitting of genera results in taxon renaming, which conceals the priority of the discoverer of the organism in favor of that of the author of the new name or even of its spelling. For example, one can hardly hope that the chain of renamings of the well-known agent of sulfuric acid leaching *Thiobacillus ferrooxidans–Ferribacillus–Acidithiobacillus* will preserve the names of Temple and Colmer, who were the first to discover this agent. Moreover, changes in nomenclature, having little to do with the science as such, make it possible to ignore the original descriptions and cite only the recent publications. For changes of nomenclature, one should refer to the DSMZ catalog or visit the website http://www.dsmz.de and Euzeby (2002) site http://www.bacterio.cict.fr.

THE HISTORY OF THE INSTITUTE OF MICROBIOLOGY, RUSSIAN ACADEMY OF SCIENCES

The history of bacterial biodiversity investigations at INMI was strongly influenced by the three nonsynchronous and overlapping sequences of events: (1) the progress of the science per se, (2) the development of the research collective, and (3) external circumstances in the country determining the state of the institute (table).

The advances in science are not, of course, portrayed in this table very accurately. In the USSR, some degree of cooperation and the division of subject matter among research institutions were quite common. Therefore, the need to have a "universal" institute was long gone but the idea of a "head" institute persisted. At the moment, the function of "universal consultant" is being carried out by Moscow State University. This will inevitably lead to the decline of original basic studies in favor of professorial compilations and to buying abroad the results of applied research. The question, therefore, is whether science in Russia is still able to assimilate new results or the lag is irreversible and "Russia will never again be able to compete with the United States," as claimed by American analysts.

The Institute of Microbiology was established in 1934 following the relocation of the Academy of Sciences (AS) to Moscow. Its first director was Academician G.A. Nadson, who assembled at the institute a wide group of microbiologists with quite varied interests—from the chemist the Kizel with his postgraduate student A.N. Belozerskii to the hydrobiologist Uspenskii. But the core of the institute was formed by Nadson's young postgraduate students from his former laboratory in Leningrad. Just three years after the institute had been established, it suffered its first "purge," which, however, did not hit hard on Nadson's postgraduates. For this reason, his research interests remained prominent at the Institute despite the fact that he was, before long, also arrested and perished [3]. The next director of the institute was Corresponding Member of the AS B.L. Issatchenko, the last head of Winogradsky's now closed laboratory at the Institute of Experimental Medicine, Leningrad.

Nadson was a representative of the last wave of the "golden age" of microbiology that occurred before World War I. This epoch is closely connected with the names of Winogradsky and M. Beijerinck and was marked by the discovery of new environmental functions of microorganisms. The basic concept was that chemical processes in nature are catalyzed by specific microorganisms just like diseases have their specific causative agents. The ideology of the epoch was clearly stated by Winogradsky in his well-known but rarely cited lecture of 1896. In Russia, this school was represented by St. Petersburg University's botanists, among whom was D.I. Ivanovsky, the discoverer of viruses. The tradition was botanical rather than chemical. Its beginnings were laid down by the professor of the Department of Lower Plants Kh.Ya. Gobi, whose pupils and followers included Winogradsky, Nadson, Issatchenko, and by the plant physiologist Academician A.S. Famintsin. Students of the Natural Sciences Department were also strongly influenced by the spectacular advances made in geological sciences. In this connection, one should mention V.V. Dokuchaev and the concept of actuality, claiming that the same factors are effective in nature now as in the remote past.

The principles of taxonomy of microorganisms worked out by the beginning of the 20th century were outlined by Fisher [4]. There was general agreement only upon grouping bacteria based on their appearance in the vegetative development stage into sphere-shaped, rod-shaped, spiral, and filamentous. This division was previously used in the system proposed by F. Cohn "System der Bakterien," *Beitr. Z. Biol. Pflanz.*, v. 2; cited by [4]): "The classification of bacteria based on their particularly outstanding functions should not, certainly, be neglected. What it yields, however, are merely *physiological groups,* the most important of which are saprogenic bacteria or bacteria of putrefaction, zymogenic bacteria or bacteria of fermentation, photogenic or phosphorescent bacteria, thermogenic or heat-emitting bacteria, pathogenic bacteria or bacteria causing deceases, nitrifying, sulfur- and iron-oxidizing bacteria, and purple bacteria. It would be entirely wrong, however, to designate organisms based on their physiological functions and to employ such designations in parallel with morphological genera … because the taxonomy of all organisms and, therefore, that of bacteria must be based primarily on their morphological features" (p. 54). This essentially botanic approach was based on the fact that all organisms have a form, which can serve as a universal characteristic. On these grounds, the botanist Fisher contended with the botanists Winogradsky and Beijerinck over the principles underlying the introduction of physiological–morphological bacterial genera, such as *Thiothrix, Nitrobacter*, and *Desulfovibrio.* As a result of research conducted at INMI, the traditional list of bacterial forms was extended to include *budding* bacteria, now present in all manuals, and "new and unusual forms" currently known as *prosthecate bacteria.*

World War I disrupted the normal development of science in all countries (except the United States). In Russia, its consequences combined with those of the revolution, civil war, the breach of all links with international science, and the emigration of many outstanding scientists (including Winogradsky). Nadson entered the 1930s with a store of knowledge that did not extend far beyond conventional microbiology. Out of more recent developments, he appreciated most of all genetics as a source of diversity of forms of microorganisms. The institute was supposed to take care of all nonpathogenic microorganisms that might have some value for the national economy, basically, a potential value, because special institutions were set up to handle

Stages in the development of INMI (against the scientific and social background)

Stages in microbiology	Development of INMI	External circumstances in the country
1880–1914: “Golden age of microbiology”: discovery via pure cultures of representatives of major groups of bacteria. Pioneering works of Russian researchers on chemosynthesis, nitrogen fixation, and methanogenesis.	Winogradsky’s laboratory at the Institute of Experimental Medicine, St. Petersburg; random groups at universities and institutes. Foundation of the Microbiological Society.	High status of science and the scholar in society. The funding of science by the government gradually increases against the background of the general progress of science and growth of industry in Russia.
1920–1970: “Unity in biochemistry”: discovery of universal reactions and metabolic pathways in selected representatives of physiological groups. Typology. Major metabolic pathways are shown in selected representatives of bacteria. Discovery of antibiotics and search for organisms producing physiologically active compounds, especially among actinomycetes.	Accidental persistence of certain research units and personalities. 1934: the Academy moves to Moscow, and the Institute of Microbiology is organized by Academician Nadson. The institute unites microbiologists studying nonpathogenic microorganisms. 1937–1939: Nadson and other INMI microbiologists are arrested. 1939: Issatchenko is appointed to directorship. Diversity studies of microbial ecosystems. Organisms are studied in natural environments (ocean, rock, and soil). Genetic studies are closed. Biodiversity of physiological groups is described by Imshenetskii. Search for organisms producing physiologically active compounds (Krasil’nikov). “Physiology and biochemistry” as the major research direction.	1920: civil war and the ensuing devastation with considerable losses of intelligentsia and young people. Late 1920s: the restoration period; science starts to attract young people. 1930s: repressions and purges. Politically unsafe subjects of investigation are dropped. 1941–1945: the Patriotic War. 1946: microbiological institutes are set up in Soviet republics; research is expanded, but its directions remain under certain reglamentation. 1948: the crushing of genetics. High status of science in the country with young people taking the positions of the age groups perished in the war.
1970–present: discovery of multiple organisms with functions similar to those of the earlier known typical representatives.	Discovery of budding; “new and morphologically peculiar bacteria”; and new representatives of phototrophs, chemolithotrophs, and extremophiles. “Single-celled protein.” “Biogeotechnology.”	High and stable funding of science and delayed reaction to advances in international science. Large gap between the Academy of Sciences and universities. Limited intake of fresh blood in academic institutions. Predominance of female researchers and of reproductive psychology.
Since 1980: “genomics” in microbiology; wide use of genetic criteria to estimate microbial diversity; development of the phylogenetic system. Diversity of genomes rather than of organisms.	Lagging behind in genetics and dependence on international cooperation. Interest in microbial communities and their function in biotopes. Biodiversity remains one of a handful of internationally significant research directions in Russian microbiology.	1985: the USSR goes into crisis, with science going into decline from the early 1990s. Shrinking of research technical base and shortage of research staff. Emigration of active researchers.

groups of microorganisms with obvious applied significance, like those used in food industry. Such institutions were quite competent as regards production technology issues.

The entire diversity of taxonomic groups of microorganisms was sectioned into chunks by Nadson and allocated in pieces to his numerous postgraduates. N.A. Krasil’nikov and A.E. Kriss received actinomycetes [5, 6], B.I. Kudryavtsev received yeasts [7], and A.A. Imshenetskii received “microorganisms of vegetatite raw materials” [8]. This created a “fan of niches” and everyone was busy with his own group without crossing the “borderlines.” To cover the diversity of all groups of microorganisms, this approach would have taken about 30 professional researchers ready to be leaders in their areas. The institute never had that many, and significant unoccupied loci remained. Even so, looking back at the five years from 1934 to 1939, one cannot but be amazed at the pace and scope of revival of microbiology in Russia from sheer ruins.

Issatchenko, the next director of the institute, was a pupil of Beijerinck, with roots in the same school of St. Petersburg University. A traveler and explorer of microorganisms in their natural environments, he, unsurprisingly, continued the tradition of microbial diversity studies, supplementing it with interest in the activities of organisms in natural habitats as opposed to Petri dishes. Biodiversity is often interpreted as the sum of species (“species diversity”), which is basically inadequate. In fact, the diversity of ecological systems has a similar or even greater significance. For Issatchenko, a recognized leader in aquatic and geological microbiology, the ecosystematic approach was important especially in connection with the biogeochemical cycles of elements, although in this area he joined Winogradsky's functional approach with a focus on identifying process catalysts rather than with the quantitative approach of V.I. Vernadsky [9]. Accordingly, Issatchenko encouraged the development of aquatic and marine microbiology at the institute; supported studies of soil microbiology; initiated works on geological microbiology; and, as a botanist, was fairly tolerant to studies of phytopathogenic microorganisms conducted by G.K. Burgvits [10] and, after his arrest, by Corresponding Member of the AS V.L. Ryzhkov [11]. Over two short years before the war and two years after, several microbiologists from Moscow joined the institute on his invitation. Botanist Issatchenko clearly formulated the thesis that the agent of a natural process must be identified and an exact taxonomic designation must be obtained.

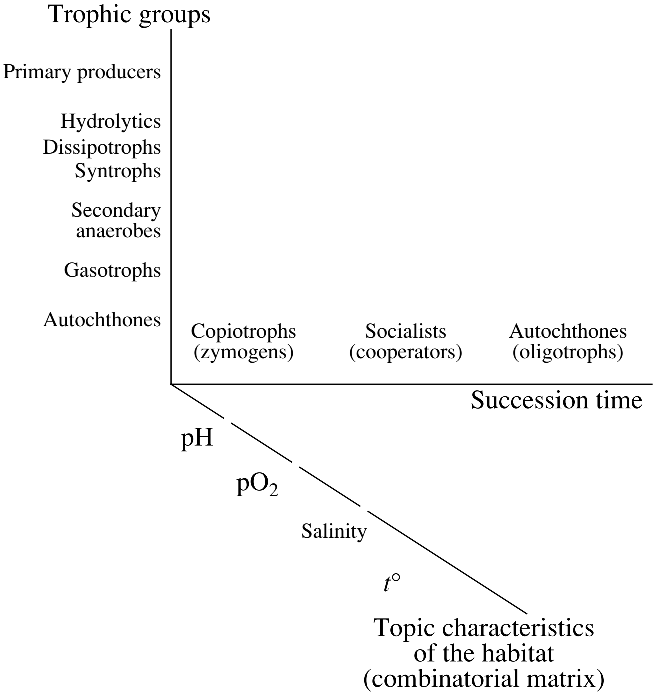
During his directorship of the Botanic Institute in Petrograd, Issatchenko became aware of the applicability limits of a purely floristic approach and recognized, after comprehensive works by A.A. Elenkin, that it made no sense to study the species biodiversity of, for example, blue–green algae on the basis of their morphology. He initiated the study of blue–green algae from the bacteriological viewpoint and requested S.V. Goryunova to describe the biochemistry of pure cultures. Goryunova selected an unfortunate object—*Oscillatoria*—and ran into very difficult problems with pure cultures. As a result, it was only three decades later that blue–green algae, now conventionally known as cyanobacteria, turned into a normal object of bacteriology, after the works by R. Stanier in the 1980s [12, 13]. Fungi were not of much interest for Issatchenko, but he did not object to studies of the species diversity of yeasts, classified on the basis of cultures by V.I. Kudryavtsev. After the war, he contributed much to establishing a collection of yeasts, which he knew well, and gave the name of *Nadsoniella nigra* to a new genus of black psychrophilic yeasts he discovered [7]. The physiology and, particularly, the ecophysiology of fungi failed to gain prominence at INMI, being studied by a small team in the laboratory headed by E.N. Mishustin [14, 15]. Microbiology was first of all understood as bacteriology, and fungi remained the subject of random studies with a bioengineering bias (see E.P. Feofilova, this issue).

Thus, as a result of a peculiar combination of tradition, research interruptions caused by external circumstances, and detachment from international publications (and fashion), the tradition of the "golden age" of microbiology, focused on studies of microorganisms with new physiological functions, was continued at the Institute of Microbiology. Unfortunately, a different tradition, started in 1918 by the work of O. Meyerhof on the metabolism of *Nitrobacter* and formulated by Kluyver in the 1920s as "unity in biochemistry," failed to take root. At that time, the typological approach prevailed in microbiology, implying that just a handful of typical representatives of each physiological group need be known. This approach was provoked in part by the excessive power of the elective media employed: the application of standard media led to the detection of the same species in different samples. Winogradsky's remark that any deviation from the medium prescription is likely to result in new discoveries was forgotten. By the way, by that time he himself had forgotten his contention of 1896 and turned into an ardent partisan of a single typical representative for each function, like *Azotobacter* for aerobic nitrogen fixation.

In the postwar years, the institute became a recognized leader in the field of microbial diversity, as clearly evidenced by the publication of the basic *Determination Manual of Bacteria and Actinomycetes* by Krasil'nikov [16]. It was one of the last such manuals written by a single scholar and not by a collective of authors like *Bergey's Manual.*

The crisis in Soviet biology of 1948 forced Imshenetskii, who was made the director of INMI after the death of Issatchenko the same year, to maneuver, keeping clear of the crazy ideas put forward by Bosh'yan and Lepeshinskaya. Genetics was banished from official science for a long time. On the face of it, Imshenetskii was supposed to be occupied with "heredity and variability" issues, but, actually, he led his own line by encouraging studies of physiological groups, such as cellulolytic bacteria, thermophiles, and nitrifying organisms [17]. This, however, required good knowledge of biochemistry, and this was largely out of date. It should be mentioned that Imshenetskii's role in changing course to studies of the functional diversity of microorganisms as a basis of general microbiology remained underestimated. The atmosphere he created at the institute required that the process agent be described both morphologically and physiologically. Even people like S.I. Kuznetsov, whose original training was quite remote from taxonomy issues, had to follow suit, and it was his pupils V.M. Gorlenko, G.A. Dubinina, G.I. Karavaiko, and N.N. Lyalikova who subsequently made the most significant contributions to microbial diversity studies. Regarded as a symbol of success and one's professionalism, to obtain a pure culture remained the predominant goal of INMI microbiologists.

Starting from the 1950s, I was an eyewitness of microbial biodiversity studies at the institute, and my account of these works that follows is based on my perception. The greatest authority on issues of biodiversity was, of course, Krasil'nikov, and the chunk of science concerned with actinomycetes and related organisms was under his exclusive control thanks to the tireless efforts of his coworker A.I. Korenyako, who isolated and maintained cultures. The basic method consisted in comparing the morphology of cells and colonies grown on solid media. This line of research was taken up by L.V. Kalakutskii [18], while G.K. Skryabin was more interested in microbially produced physiologically



Functional diversity of natural communities (from [38]).

active substances with applied potential. Before long, Krasil'nikov organized the Department of Soil Biology at Moscow State University and his interests moved there, where his research work was continued by D.G. Zvyagintsev, who paid much attention to studies of biodiversity of soil microorganisms. After Skryabin and Kalakutskii left INMI for the newly formed Institute of Biochemistry and Physiology of Microorganisms (IBPM), Pushchino, the work on actinomycetes and related organisms at INMI subsided. Unfortunately, after the death of Kudryavtsev, who had put much effort into establishing the All-Union Collection of Microorganisms (VKM) [19], this work at INMI was suppressed on a strange whim of Imshenetskii, and the Presidium of the Academy of Sciences had to move the collection to IBPM, where it was headed by Kalakutskii, a renowned expert in the field of actinomycetes and gram-positive organisms. VKM, however, does not accept organisms that cannot be cultured on conventional media. In view of this fact, a collection of "unique" organisms cultured under special conditions and on singular media was recently established at INMI by V.F. Gal'chenko. The collection is supervised by INMI experts on extremophilic organisms, and the microorganisms are stored in liquid nitrogen. This collection is not yet internationally validated.

Starting from the early 1960s, the studies conducted at INMI developed in two directions. First of all, the concept that bacteria reproduce by division into sibling cells was basically changed by Zavarzin's work "Budding Bacteria" [20], where the occurrence of mother and daughter cells in *Nitrobacter* and hyphomicrobia was clearly shown. Second, the diversity of bacterial forms found by D.I. Nikitin by electron microscopy of soil samples was broad enough to make inadequate the former concept of rods and cocci, especially so upon the discovery of the six-ray symmetry in *Stella* [21]. The problem assigned to the institute—to develop the process of single-cell protein synthesis—drew attention to hydrocarbon-oxidizing, methane-oxidizing, and hydrogen bacteria. While the first two groups were abandoned before long in favor of IBPM, the hydrogen bacteria were studied in detail in parallel with H. Schlegel's research at Göttingen. Methods of organism culturing on a gaseous substrate had to be mastered [22]. In addition to describing several new hydrogen-oxidizing bacteria, which, notably, included an extremely thermophilic *Calderobacterium*, later shown to belong to the ancient *Aquificales*, a whole series of aerobic carboxydobacteria oxidizing CO was discovered. These included *Seliberia carboxydohydrogena,* with a helical surface structure, and other bacteria the taxonomic rank of which upon revision was raised to that of a genus, like *Carboxydus, Oligotropha*, and *Zavarzinia.* As a result of such studies, gas-utilizing organisms became conventional objects of investigation. This paved the way to subsequent work with anaerobes, although the physiology of hydrogenotrophic sulfate reducers was studied much earlier at INMI by Yu.I. Sorokin, who cultivated these organisms in soldered Wurtz flasks. The discovery of organisms with gas nutrition was an important step to recognizing the leading role of bacteria in generating and maintaining the atmosphere composition [47].

SPECIES DIVERSITY OF BACTERIA

In 1934, microbiologists' awareness of the microbial world diversity was limited to 108 genera included in the fourth edition of *Bergey's Manual*, translated into Russian in 1936 by the Ukrainian Academy of Sciences. This very unfortunate manual contained a characteristic mixture of approaches. It included groups of chemosynthetic organisms with nomenclature drawn by Orla-Jensen, sulfur bacteria taken from the days of Winogradsky and Beijerinck, Cohn's filamentous aquatic bacteria, and a bulk of organotrophic organisms—about 40 genera—classified on their morphology. It is important that this classification was the starting point for research by Soviet microbiologists before their communication with the West was frozen for years.

The next important step was the publication of Krasil'nikov's manual in 1949. This manual also contained about a hundred genera but was tailored to the needs of general microbiology [16]. "We were not much concerned with pathogenic and phytopathogenic bacteria because their determination consists merely in the diagnosis of diseases, needed for fairly narrow practical purposes" (p. 3). The organisms were classified on the basis of their morphology, and one was cautioned against "using physiological features for taxonomy because these tend to change very much with the growth conditions" (p. 10). "The most significant among cultural characteristics is the structure of the colonies" (p. 8). "Colonial identification" in microbiology, especially in soil and sanitary microbiology, has persisted until the present. The botanic approach drew on the 19th century idea that the form is the common property of all organisms. It is worth noting that the contemporary 16S rDNA systematics is based on the same logic of classification based on a single common feature except that it is now the morphology that falls out of line. Krasil'nikov worked with actinomycetes, which are a good object as regards their morphology [23]. "Comparing some mold fungi with actinomycetes, I actually fail to see much difference between them" (p. 276). "Comparing some groups of *Actinomycetales* with fungi, one can observe parallelism in the evolutionary development of these organisms" (p. 277). Krasil'nikov hypothesized that the development of mycelial organisms might have consisted in their degradation to the single-celled branching forms of mycobacteria, analogues of yeast fungi. The idea of degradation from complex to simple in the world of free-living microorganisms failed to be taken in by the scientific community.

At the same time, Kriss turned to the problem of polymorphism in actinomycetes, a very topical issue at the time in view of several extravagant hypotheses voiced abroad and claiming the existence of morphological "pleomorphism" in microorganisms, an idea clearly at odds with the notion of reliability of morphological criteria.

In the 1960s, Soviet microbiologists started to gradually accept the international nomenclature, and Krasil'nikov's systematics was left behind. The transition occurred with the seventh edition of *Bergey's Manual*, which contained as many as 208 genera; 35 of them (17%) had Russian authors (mostly Winogradsky, but also N.G. Kholodnyi, B.V. Perfil'ev, and Krasil'nikov). Later on, two Russian translations of *Bergey's Manual* short version were edited by Zavarzin [24, 25], making it easier for Russian researchers to stick to international nomenclature. Nowadays, the pace at which new genera of microorganisms (bacteria and archaebacteria) are described has increased so much that one can follow them only by using the electronic version. As of January 1, 2004, 1228 genera had been validated, and almost 5% of these were described by INMI researchers. Among nonpathogenic cultivable organisms (in the DSMZ catalogue), this fraction is 6%—a very large figure, the more so that very few nomenclature renamings were suggested by Russian authors and their taxa were actually pioneering discoveries.

The contemporary systematics of microorganisms is highly formalized. To be validated, a new taxon must be described in accordance with "minimal standards," in which genotypic characteristics play an important part, and its type strain must be stored in two internationally recognized collections of type cultures. The designation of the organism must be published in the *International Journal of Systematic and Evolutionary Microbiology* (*IJSEM*; previously, *the International Journal of Systematic Bacteriology*), the editors and reviewers of which keep close watch on the adherence to the International Code of Bacteria. The taxon is not regarded as validated until its publication in *IJSEM*. As a consequence, today the goal in studies of the diversity of microorganisms is not so much to discover the role of microorganisms in natural processes as to comply with the rules set up by the journal. At present, every issue of the journal contains descriptions of more than 20 new taxa, and Russian names can be often met among the authors, mostly in cooperation with researchers from other countries. In connection with the international requirements, a limiting factor is the absence at INMI of laboratories able to perform the molecular identification of organisms, routinely conducted overseas. These functions were undertaken by A.M. Lysenko, who determines the G+C content and DNA–DNA homology by the optical method, and by T.P. Tourova, who performs the 16S rDNA classification.

Let us now discuss the general concepts concerning the diversity of microorganisms. In the pregenomic epoch, the attention of Russian readers was attracted by the *Phenotypic Systematics of Bacteria. The Space of Logical Possibilities* by Zavarzin [26]. The author was guided by the concept that a meaningful taxon in bacterial systematics is a genus, having significant distinctions from others, whereas a species, often described on the basis of a single strain, is not clearly enough defined. With regard to a genus, the concept of morphophysiological unity, formulated by R. Stanier and C.B. Van Niel and their predecessors, starts to make sense. Such a genus can be characterized by a set of genus rank descriptors. The morphophysiological genus describes the functional properties of the organism and as such can be of practical value in studies of natural objects. By considering various combinations of such descriptors, Zavarzin came to the conclusion that they could be arranged into a matrix or a network of combinations, some of which were never observed in genera described by that time. Such combinations were regarded as "forbidden" and gave rise to conditionally forbidden regions in the space of logical possibilities. The discovery of an organism with a combination of forbidden generic features immediately would open up a whole region of high rank taxa in the system. This, in turn, allowed a directed search for taxa to be undertaken having the given generic descriptors in the adopted classification system.

The necessary condition for the emergence of such a combinatorial system, drastically different from that found in higher organisms, is a broad exchange of the genetic material responsible for the observed phenotypic properties. This is how this point was stated by Zavarzin (p. 65): "…single-celled bacteria, as could be expected from the analysis of feature incompatibility, are closely connected with each other, and, what is important, these connections are multiple and versatile: every genus happens to be at the center of a network linking it to representatives of very different groups. This state of affairs cannot be described by dendrograms unless some generic characteristics are arbitrarily assigned prevailing significance." Such a combinatorial matrix was suggested to have a genetic basis in lateral gene transfer, the significance of which in bacteria was viewed in the 1970s with great skepticism. In the 1990s, the attitude to this process made a U-turn. The cover of the book portrayed Zavarzin's perception of the three-level diversity of living beings: two petals of vascular plants and animals; three petals of algae, protozoa, and fungi; and numerous petals of bacteria, lacking analogues in the world of eukaryotes. These roughly correspond to the major trophic groups of osmotrophic autotrophs and organotrophs.

Another concept of combinatorial diversity of prokaryotes originated from the genomic approach. The genotypic systematics based on the 16S rRNA gene produced a "universal tree of living beings," the topology of which as regards bacteria resembles now a sunflower, having many short higher rank branches and a large number of genetic clones for which no cultivable representatives have been found. This is believed to be caused by horizontal gene transfer as a main source of innovations and fast genetic processes. Such transfer involves integration of the acquired gene into the recipient's genome and its preservation in defiance of the mechanism of alien gene removal by domain shuffling in the genome. As the number of completely sequenced genomes increased, it became evident that genomes are mosaic. Today, a set of criteria is worked out to detect alienness. The least likely to be transferred are genes of information systems. More readily transferred are genes connected with metabolism, genes with unknown function, and those lacking orthologs. The integration of new genes is determined by their compatibility with the recipient's genome, and a special selection mechanism is effective within the cell. This gave rise to the concept of a common pool of genes with different probabilities of redistribution, as hypothesized by F. Doolittle, instead of a common pool of abstract descriptors of phenotypic properties. Two theses of fundamental nature were put forward by Academician S.V. Shestakov: (1) natural selection proceeds at the level of organisms rather than genes and (2) microbiology should be rid of the notion of species and should be concerned instead with a common pool of genes.

The latter radical statement is supported by the fact that most bacterial species, especially less readily cultivable ones, were described on the basis of just one or a few strains isolated by similar methods. The space between these reference points remains unexplored. Like in any classification problem, the initially clearly defined criteria become fuzzy as the number of classified objects increases. This represents an inherent feature of extensional classifications based on the comparison of an object with a standard, which, in microbiology, is the name-giving strain.

The first thesis is of a particularly fundamental significance: to understand the function in nature, the organism is important, not the gene. The gene by itself does not operate in nature. Therefore, the activity of microbes in nature starts with organisms (Shestakov is not quite exact in calling them "cells"), not genes. Shestakov believes [27] that "the predominant processes at that time [the initial period of biota existence, G.Z.] were active horizontal transfers, giving rise to the convoluted genomic mosaic that amazed so much microbiologists devising phylogenetic diagrams on the basis of homology of the ribosomal DNA and some other conservative markers" (p. 51). As a result, "the data of comparative genomics based on a large number of proteins allow almost all versions of kingdom branching from a single root but cannot produce clear answers because of the multivariant nature of estimates of molecular and phylogenetic relations even for conservative markers" (p. 52).

If these conclusions are compared with those reached by Zavarzin two decades earlier in his *Space of Logical Possibilities* on the basis of much more limited phenotypic material, one will have to admit that the combinatorial principle, allowing for the existence of several parallel lines, gives the most adequate portrayal of relations between prokaryotes.

In the discussion of Zavarzin's ideas held in 1965 in London on the existence of incompatible features, R. Starkey raised objections against the occurrence of such a possibility in bacteria. This viewpoint implied that phenotypic (=functional) diversity of bacteria actually filled all entries in the matrix of descriptor combinations. History showed that many "forbidden" combinations were just not yet discovered at the time. This is best illustrated by the history of describing new, "forbidden" forms of phototrophic bacteria, e.g., gram-positive, isolated with the use of a bicarbonate medium and the method of N. Pfennig (Gorlenko, this issue). On the other hand, the discovery of an organism with a new property promoted to the rank of a generic characteristic becomes much more valuable, like the capacity to anaerobically oxidize CO with water (V.A. Svetlichnyi) or oxidize rare elements (N.N. Lyalikova-Medvedeva). The number of new organisms increased explosively with the spread of Hungate's technique of anaerobic cultivation (which drew on the method employed by F. von Esmarch in the 1880s; see F. Lafar [42]). In the USSR, this technique was first employed by T.N. Zhilina in studies of methanogens [28]. She discovered obligately methylophilic halophilic methanogens; halophilic acetogens; and, more recently, a wide group of anaerobic alkaliphiles that somehow had escaped the attention of microbiologists. The contributions of INMI researchers to studies of alkaliphiles also include the description of lithotrophic aerobic alkaliphiles by D.Yu. Sorokin, the description of anoxygenic alkaliphilic phototrophs by Gorlenko, and an earlier description of natronobacteria by I.S. Zvyagintseva [46].

PHYLOGENETIC DIVERSITY

The contemporary approach to biodiversity of microorganisms is based on genomic studies. The contemporary genomic view of the bacterial system totally rejects the phenotypic approach by treating phenotypic features as merely an external envelope of a stable genetic core of properties associated with the in-cell-directed functions of the DNA in the ribosomal mechanism of protein synthesis. External features can vary as a result of insignificant genetic changes. Two examples are the change of the cell shape caused by delayed division and the formation of typical aggregates. Such phenomena, to some extent, take us back to the ideas of pleomorphism in regard to external diversity of bacteria. Physiological properties may be governed by a single gene or a small number of genes, like in the case of nitrogen fixation. A 30% difference in the DNA–DNA homology was adopted as a criterion for distinguishing species and a 5% difference in nucleotide sequences in the 16S rRNA gene became the threshold for distinguishing genera. "Phylogenetic systematics" based on firm quantitative criteria started to gain momentum. This period coincided with perestroika in the Soviet Union, and microbiologists in Russia found themselves in the client position, largely depending on their foreign partners to validate the taxa they discovered. The interests and achievements of INMI researchers rested in the traditional area of functional properties of microorganisms. A genus as a taxonomic entity is supposed to have significant distinctions from other organisms, and its discovery, by definition, amounts to an important contribution to the cognition of the microbial world. By "scientometric" standards, the discovery (rather than the emendation of the taxonomic status, let alone nomenclature) means the recognition of the priority and, theoretically, the eternal citing of a discoverer.

Out of 1228 genera validated as of January 1, 2004, as many as 60 (i.e., almost 5% over the entire history of microbiology) were discovered and described by INMI researchers. Thirty of these genera were described during the last decade. Microbiologists affiliated with INMI discovered organisms that gave rise to the following genera: *Acetohalobium, Acidilobus, Ancalochloris, Anoxynatronum, Blastobacter, Calderobacterium, Caldithrix, Carbophilus, Carboxydobrachium, Carboxydocella, Carboxydothermus, Chlorobium, Chloronema, Desulfomicrobium, Desulfonatronovibrio, Desulfonatronum, Desulfurella, Erythromicrobium, Erythromonas, Ferroplasma, Geobacillus, Halocella, Halincula, Halonatronum, Heliorestis, Hippea, Intrasporangium, Labris, Lamprobacter, Methanohalobium, Methylocella, Methylocapsa, Natroniella, Natronincola, Nautilia, Oceanothermus, Oligotropha, Oscillochloris, Promicromonospora, Prosthecochloris, Roseinatronobacter, Roseicoccus, Roseospira, Sandracinobacter, Stella, Sulfitobacter, Sulfobacillus, Sulfurococcus, Tepidibacter, Thermoterrabacterium, Thioalkalicoccus, Thioalkalimicrobium, Thioalkalivibrio, Thioalkalispira, Tindallia*, *Vulcanithermus,* and *Zavarzinia.* The list is not closed.

The interest of INMI researchers was focused on three groups: (1) thermophiles, including extreme thermophiles; (2) anoxygenic phototrophs; and (3) anaerobic and aerobic alkaliphiles. Since 1994, 9 genera of alkaliphiles, 9 phototrophs, 14 thermophiles, and 2 methanotrophs have been described.

Considerable research effort directed at extremophiles was rewarded by the description of many organisms with high taxonomic rank in the phylogenetic system, like *Ferroplasma* and *Caldithrix* (see E.A. Bonch-Osmolovskaya, this issue). Unfortunately, the existing "scientometric" system of ranking of a researcher's scientific achievements in terms of the number of publications and the "impact factor" makes publishing a paper in *IJSEM* an end in itself, despite the fact that such papers merely amount to a form that has to be filled in for taxon registration and validation. The science journals, like *Archives of Microbiology*, carry an explicit warning that purely taxonomic papers will not be accepted. In a scientific investigation, describing a new genus is not an end in itself but a necessary intermediate step in the exploration of the process at hand. Thus, the investigation of organic matter decomposition in hypersaline lagoons resulted not only in the discovery of several new genera of the order *Halanaerobiales* but also in the description of a metabolic pathway of decomposition of osmoprotecting substances specific to halophilic communities. Haloanaerobes are of much interest because some of them are represented by spore-forming gram-negative organisms, a combination of features previously believed to be "forbidden." Haloanaerobes were described as a result of a classical inductive investigation employing elective media and conditions. Anaerobic extreme halophiles were for the first time shown to include methylotrophic methanogens (*Methanohalobium*) and halophilic homoacetogenic bacteria (*Acetohalobium*) [32].

Another example of a purposeful investigation is the discovery of the acidophilic methanotrophs *Methylocella* and *Methylocapsa* by S.N. Dedysh. The goal of this research, undertaken in connection with the Convention on Climate Change, was to identify agents controlling the emission of the greenhouse gas methane from the bog ecosystems predominant in Russia.

Studies in the biotechnology of sulfide ore leaching led to the discovery by Soviet researchers of several important organisms such as the iron-oxidizing *Sulfobacillus, Leptospirillum*, and the above-mentioned *Ferriplasma.* Studies of iron reduction to magnetite resulted in the discovery of a group of thermophilic anaerobic bacteria that includes *Thermovenabulum.* It is pertinent to remind to reader here that the reduction of ferric iron by hydrogen bacteria was first described at INMI and preceded a series of brilliant works by D. Loveley in the United States.

Several new organisms were described during investigation of aerobic oxidation of carbon monoxide (carboxydobacteria) and a new anaerobic process of CO oxidation by water coupled with hydrogen emission [29, 30]. The latter process could be used as a biotechnological substitute for steam–gas conversion.

Two approaches were worked out in microbiology to study abundant microorganisms: the first one relies on direct microscopic observation of organisms in nature and the other employs the deductive method of elective media. Russian microbiologists have made significant contributions to direct studies of microorganisms in nature. The most remarkable are works by Kriss [31] on deep-water microbiology, involving enumeration of organisms on membrane filters and submerged slides, a refinement of the technique first employed by A.S. Razumov and N.G. Kholodnyi. One should also recall the method of direct observation of organisms in bottom sediments developed by the Leningrader Perfil'ev (he and Kriss were Lenin Prize laureates, a rare honor among biologists) and direct electron microscopy studies of soil microorganisms by Nikitin [21]. Until fairly recently, however, the direct methods were generally regarded by microbiologists as inadequate. The breakthrough occurred in the late 1990s with the development of molecular methods and, above all, the emergence of the FISH method for direct taxonomic identification of microorganisms in natural samples based on the use of fluorescent oligonucleotide probes. Earlier attempts at such investigations were based on using fluorescent antibodies to known species (by Zvyagintsev and his pupils at Moscow State University and by Gal'chenko at IBPM to study methanotrophs). Immunochemistry, however, is less reliable with natural samples.

A good example of a solution of the problem of the abundance of forms is the work by Dedysh (2002) on acidophilic methanotrophs. First, the oxidation of methane was shown to proceed in peat samples at low pH. Then, methane oxidation was established in ultrafresh waters devoid of sources of nitrogen. Next, after overcoming severe obstacles, a new acidophilic methanotroph was isolated (until then, only neutrophilic methanotrophs were known), and an oligonucleotide probe was developed on the basis of the pure culture. Next, by using this probe, acidophilic methanotrophs of the new genus *Methylocella* were quantitatively proved to account for a considerable part of the population of methanotrophs under the conditions of Russian landscapes and to control emissions of the greenhouse gas methane to the atmosphere. Finally, the conditions for nitrogen fixation by these organisms were determined. This example shows how the descriptive microbiology of microbial communities in situ is going to be rewritten in the near future. It is obvious that the return in the coming decade to Cohn's century-old microscopic approach will yield a different picture of the microbial world, particularly in regard to natural communities. Investigations of microorganisms in natural environments by means of identification with molecular methods showed that the organisms dominating in natural environments are not those known in pure cultures. The FISH methods of fluorescent microscopic identification can be, actually, regarded as an offshoot of cytochemistry. "Uncultivable microorganisms" came into the limelight and are currently the most attractive objects of investigation. In the crisis that broke out, direct microscopic observations in nature became convincing even for molecular biologists, who now set the tone in biology. The important fact is that the microbial diversity in nature differs considerably from that in cultures and our current understanding of the microbial world in nature is at best very patchy if not false.

Those rejecting the significance of morphological features need to find some way out of the following paradox. First of all, there are areas where only morphological criteria exist. One example is bacterial paleontology, now widely employing scanning electron microscopic assays of geological specimens. The interest is focused on the role of cyanobacteria. Important results in this new field were obtained by L.M. Gerasimenko [48]. The undisputable success of this approach consisted in proving that sedimentary deposits of phosphorites have microbial origin [32]. The important question is whether our understanding of the geochemical role of microbes acquired through a deductive search for agents of familiar geochemical processes and physiology studies of agent cultures is indeed true. It can be claimed that the contemporary knowledge of the physiological functions of bacteria, ascending in its major elements by the end of the 19th century, does produce a consistent picture of the biogeochemical machinery of the planet as regards microbial catalysis of major cycles of biogenic elements. However, we are not at all convinced that the known agents of such processes are indeed predominant. One of the key problems consists in being able to distinguish the functions of an organism in a pure culture from those in the community. Surely, even the most stubborn syntrophs are amenable to cultivation in pure cultures under certain conditions, although this is hardly so with symbionts and members of consortia. To what extent can a microbial community be regarded as an element of biodiversity? My contention is that microbial communities should be regarded as components of biodiversity. One example is biofilms, recently attracting considerable interest but still not amenable to methods of traditional biochemistry, which prefers organisms developing in suspensions (wherefrom aliquots can be taken) at a high rate and producing good yields. In nature, this property need not always go hand in hand with surviving and prolific development. From the fact that *Sphaerotilus* is a tricky object for biochemical assays, it does not follow that its role in nature is insignificant. Moreover, it was found that an organism grown in a biofilm can display different properties and proteins (up to 70%) than when grown in a suspension [44].

FUNCTIONAL DIVERSITY AND COMMUNITIES

In the area of functional diversity of microorganisms, INMI has a long tradition of publishing monographs devoted to physiological groups. The most important of these are the book by Imshenetskii *Microbiology of Cellulose* [8]; several monographs by Imshenetskii [17], E.N. Mishustin [33, 34], and L.G. Loginova [35] on thermophiles (see Bonch-Osmolovskaya, this issue); the works by Zavarzin *Lithotrophic Microorganisms* [36] and *Hydrogen Bacteria and Carboxydobacteria* [22]; the collections of papers *Chemosynthesizing Microorganisms,* and *Microorganisms of Calderas* [45]; and *Methanotrophs* by Gal'chenko. The monographs by Kuznetsov and colleagues [37] and Kriss [31] were devoted to aquatic microorganisms and their habitats (see Ivanov and Karavaiko, this issue).

Starting from the "golden age of microbiology," the notion of physiological groups of bacteria traditionally served as a suitable means to understand their role in nature. An attempt to uncover regularities in the bacterial functional diversity on the basis of Winogradsky's ideas was undertaken by Zavarzin. His starting point was that every environment is populated by a community of microorganisms that make up a cooperative entity: the properties of one group of organisms must agree with those of another group. The prime property of an organism is its nutrition mode. The trophic links in a bacterial community must complement one another in such a way that no unutilized substances are left over. By employing this approach, it becomes possible to predict the properties of organisms expected to occur in the community as soon as the products of the key community members are known. This approach is, essentially, an elaboration of the idea put forward by Winogradsky in 1896: every natural substance must have its consumer (this applies, of course, only to substances of biological origin, not mineral). The proposed trophic system does not apply, e.g., to crenophiles, which inhabit water springs and form biofilms, because in this case the substrates and products travel with the flow of water. The succession of crenophilic communities follows the well-known saprobic classification system.

By combining the trophic system with a topical system that describes habitats and their physicochemical characteristics, one arrives at the space of logical possibilities constituted by ecological niches that host functional groups of organisms, which roughly correspond to morphophysiological genera. Series of such niches can be derived for thermophiles, halophiles, acidophiles, alkaliphiles, crenophiles, etc., populating different habitats. The most amenable in this respect are extreme environments, where, in line with Thienemann's ecological rule, the diversity of organisms is limited. To describe the functional diversity of community microorganisms, Zavarzin had to employ both traditional and new designations for functional groups of organisms, such as hydrolytics (utilizing hydrolyzable polymers), dissipotrophs (utilizing monomers dispersed from their formation site), primary anaerobes (fermentative bacteria), secondary anaerobes (utilizing fermentation products), and gasotrophs (utilizing gases). The functional ecological system of bacteria he proposed is depicted in the figure, taken from [38]. The rows in the horizontal plane correspond to organisms inhabiting biotopes with specific physicochemical characteristics, e.g., psychrophiles and the above-named series. The divisions in the ecological classification differ from physiological divisions and, to a large degree, are determined by growth rates and substance transport in the community. The functional system has nothing in common with the phylogenetic one except for the advantage of exact identification of organisms provided by the latter system. Moreover, Zavarzin came to an empirical conclusion that it is phylogenetically remote organisms that interact most closely in trophic systems. The FISH method gives a vivid picture of such relations with microscopic specimens. According to Zavarzin's views, the level of microbial communities constitutes the most important part of the overall microbial diversity. It is closely related to ecosystems and landscapes [39].

The studies of thermophilic communities with cyanobacterial producers *Mastigocladus laminosus* and *Phormidium laminosus* [45], halophilic communities of cyanobacterial mats [32], and halophilic communities [46] are good examples of investigations of microbial communities as biodiversity components. In all these cases, a trophic pathway was derived for these communities. Isolating an organism and determining its plausible place in an ecosystem do not at all mean that this very organism with its Latin name will be predominant in the given habitat at the given time. It merely represents a group required for the whole system to work. By analyzing its culture, the functional properties of such representatives can be revealed. The discovery of such an organism is based on a deductive approach and either confirms or refutes the initial view of the integral system. The next step of proving the domination of this organism in the ecosystem often does not go beyond enumerating its population. Even acknowledging the importance of such data, the overall picture will not be correct without conducting monitoring at the given site and comparing the data with other habitats. Direct enumerations of microorganisms in the ocean carried out by Kriss at Issatchenko's insistence gave a broad estimate of the number of microorganisms in marine environments. Unfortunately, microscopic examinations of filters stained with erythrosin fail to warrant definitive conclusions on the morphology of organisms, while interpretations of what one sees can (and actually did) lead to errors.

The most reliable method of studying microbial communities consists in the use of minimal ex situ models, known as microcosms, which are not affected by weather and other fluctuations in natural environments. A classic example of a stable microbial microcosm is Winogradsky's column. Microcosms are subjects to cyclical changes, brought about by changes in the internal state of the system and made manifest by blooming and death of the corresponding microorganisms. Enrichment cultures (Beijerinck's *Anreicherungskultur*) unavoidably produce a distorted picture as a result of excessive presence of introduced allochthonous substrates.

Much research has focused on cyano–bacterial mats as representatives of microbial communities. The dash is used here to stress the multicomponent nature of the community. Although it is possible to isolate individual components of a cyano–bacterial community in pure cultures and study their physiology (biochemistry is not that important at this level), in doing so it would be no less difficult to obtain an integral picture of the community than to get a notion of the taiga by growing a shoot of fir tree in a pot. Cyano–bacterial communities are formations with a stable architecture that does not vary with their habitat. By using electron microscopic studies, Gerasimenko was able to show that thermophilic, halophilic, and alkaliphilic cyano–bacterial mats have a common architecture. In studying such communities, it is important to locate the "edifying organism" that determines the key properties of the structure. Communities of mats or biofilms are often compared with tissue in having a very regular arrangement. The studies of modern mats lent more certainty to the fossil interpretation given within the framework of bacterial paleontology [40].

Under the influence of Kuznetsov's school, the attention of INMI researchers was drawn to natural ecosystems understood as one more biodiversity unit. While Kuznetsov himself was more interested in hydrochemistry and the related processes, his pupils tended to follow the tradition of Issatchenko, who insisted that the process under study be exposed to the point of identifying its agent with a decent binary Latin name. Work falling short of this level belongs more to biogeochemistry than microbiology and can serve only as initial material for microbiologists seeking to associate the process with a microbe. Such studies are best performed on biotopes with strong contrasts, e.g., extremely acidic, with acid produced through oxidation of sulfur compounds by microbes. The recognized leader in this field is Karavaiko [41]. Initially, his interest was focused only on *Thiobacillus ferrooxidans*. Later, however, it was extended to other organisms when it became clear that the acidophilic community was much broader and included Markosyan's *Leptospirillum ferrooxidans* and *Sulfobacillus* and other until now not very numerous acidophiles, initially assigned to the heterogeneous genus *Acidiphilum.* A significant attainment in the diversity studies of acidophiles was the discovery by Karavaiko and his coworkers of *Ferriplasmatales*, which gave rise to a new high rank taxon among archaea. A different segment of the sulfur cycle was studied by E.P. Rozanova and T.N. Nazina, who discovered several new sulfate reducers, mostly in connection with studies of oil reservoirs. Large-sized sulfur bacteria forming massive aggregates in natural environments were traditionally difficult objects of study for microbiology. The major problem here was not to describe new taxa—this had been done by Winogradsky at the end of the 19th century— but to obtain pure cultures and investigate their physiology. The recognized leader in this field is Dubinina.

Plausible conclusions on the required medium composition can be made by examining the habitat of the organism. Gorlenko, like other pupils of Kuznetsov, focused his attention on bodies of water, where such observations are easier to do. In this respect, important evidence can be deduced from the stratification of hydrochemical factors and the corresponding occurrence of certain groups of organisms, as is the case with meromictic lakes.

Biodiversity of organisms has several aspects, and its classification can follow different routes depending on the problem at hand.

1. The morphological classification was historically the first to appear and was based on recognition of the form as a universal property of all organisms. Initially, it was based on plain microscopy augmented with several staining methods (Gram staining) and, later, following Zernike's invention, almost exclusively on phase-contrast microscopy. The use of an electron microscope extended the scope of morphological observations in nature, and the pioneering work in this area was done by Nikitin [21]. Later, the scanning electron microscope became the popular instrument, especially in studies of biofilms. All these techniques required their own morphological classifications. A new stage in natural observations was opened recently by fluorescent microscopy, allowing an organism's phylogenetic position to be determined by molecular biology methods. It is now evident that direct observations in situ employing this method will rewrite microbiology in the nearest decade.

2. The physiological classification was based on the use of pure cultures and identification of the type of their metabolism. It had no parallel in other biological disciplines. Being, essentially, a deductive method, it helped considerably in exposing processes that take place in nature.

3. The phylogenetic systematics based on sequence analysis of a single conservative ribosomal gene represented a great step forward, crowned with construction of the universal tree of life. This classification offered a powerful means for identifying microorganisms and introducing order in their diversity. However, it became clear with accumulation of evidence that the universal

tree failed to give an adequate topology of the relations existing between genomes of bacteria.

4. The genomic systematics based on the analysis of complete genomes led, admittedly, to a crisis because genomes happen to be mosaic. It can be predicted that, before long, to describe an organism, one will have to determine the entire genomic sequence and indicate its key genes. This might eventually give rise to a different combinatorial representation of bacterial diversity and to multidimensional matrices as a method of representing the multitude of organisms.

5. The ecological systematics outlined above is intended only for community analysis and employs a nomenclature different from the botanical systematics of vegetative communities (pinete, sphagnete, etc.). The features significant for this systematics are the growth rate and its opposite—the persistence and the conservative stability. The physical state of an organism—whether it is in a suspension or a biofilm—also constitutes a very important property for this classification.

What is the relation between tradition and the abrupt changes of subject and issues of investigation caused by a change of leadership? In answering this question, one has to admit that, in such a wide field as microbial diversity, tradition is a powerful means to maintain a high quality of work. At the same time, of course, one must be prepared to employ up-to-date technical methods and meet the existing requirements. It is my belief that the "nose for microbes" is given by nature and that "microbe hunting" is more of an art than a matter of sticking to the prescribed standards. It is, basically, the skill to pass from observations, often microscopic ones, to nonstandard cultivation procedures. An atmosphere of exploration contributes strongly to the development of such skills. One of their key elements is the ability to observe. For a microbiologist, it is largely to examine and observe in a microscope. That is why, at INMI, the microscope continues to be an instrument of individual research rather than an occasionally used expensive laboratory asset. The new knowledge in the field of biodiversity is above all the result of observing and the ability to make out new things. However, in contrast to morphological disciplines, the microbiologist must know how to design a medium where his objects will grow. And this requires a knowledge of chemistry.

It is worth saying in conclusion that investigations of various aspects of biodiversity constitute the basis of general microbiology. The major contribution of the Winogradsky Institute of Microbiology to world microbiology consists in extending knowledge of the diversity of microorganisms operating in natural environments.

REFERENCES

1. Zavarzin, G.A., Microbial Reservations, *Priroda*, 1990, no. 2, pp. 39–45.
2. *Functional Roles of Biodiversity. A Global Perspective*, Mooney, H.M. *et al.*, Eds., Chichester: Wiley, 1996.
3. Nadson, G.A., *Izbrannye trudy. T. 1, 2* (Selected Works, vols. 1 and 2), Moscow: Nauka, 1976.
4. Fisher, A., *Lektsii o bakteriyakh* (Lectures on Bacteria), Moscow: Izd. Sytina, 1902.
5. Krasil'nikov, N.A., *Luchistye gribki i rodstvennye im organizmy. Actinomycetales* (Ray Fungi and Related Organisms. *Actinomycetales*), Moscow: Akad. Nauk SSSR, 1938.
6. Kriss, A.E., *Ob izmenchivosti u aktinomitsetov* (On the Variability of Actinomycetes), Moscow: Akad. Nauk SSSR, 1937.
7. Kudryavtsev, V.I., *Sistematika drozhzhei* (Systematics of Yeasts), Moscow: Akad. Nauk SSSR, 1954.
8. Imshenetskii, A.A., *Mikrobiologiya tsellyulozy* (Microbiology of Cellulose), Moscow: Akad. Nauk SSSR, 1953.
9. Issatchenko, B.L., *Izbrannye trudy. T. 1, 2, 3* (Selected Works, vols. 1, 2, and 3), Moscow: Akad. Nauk SSSR USSR, 1957.
10. Burgvits, G.K., *Fitopatogennye bakterii. Bakterii vozbuditeli boleznei rastenii* (Phytopathogenic Bacteria. Bacteria Causing Plant Diseases), Moscow: Akad. Nauk SSSR, 1935.
11. Ryzhkov, V.L., *Fitopatogennye virusy* (Phytopathogenic Viruses), Moscow: Akad. Nauk SSSR, 1946.
12. Goryunova, S.V., *Khimicheskii sostav i prizhiznennye vydeleniya sinezelenoi vodorosli Oscillatoria* (Chemical Composition and Lifetime Excretions of the Blue–Green Alga *Oscillatoria*), Moscow: Akad. Nauk SSSR, 1950.
13. Goryunova, S.V., Rzhanova, G.I., and Orleanskii, V.K., *Sinezelenye vodorosli (biokhimiya, fiziologiya, rol' v praktike)* (Blue–Green Alga (Biochemistry, Physiology, and Practical Importance)), Moscow: Nauka, 1969.
14. Mishustin, E.N., *Ekologo–geograficheskaya izmenchivost' pochvennykh bakterii* (Ecological–Geographical Variability of Soil Bacteria), Moscow: Akad. Nauk SSSR, 1947.
15. Mishustin, E.N. and Pertsovskaya, M.I., *Mikroorganizmy i samoochishchenie pochvy* (Microorganisms and Self-Decontamination of Soil), Moscow: Akad. Nauk SSSR, 1954.
16. Krasil'nikov, N.A., *Opredelitel' bakterii i aktinomitsetov* (Determination Manual of Bacteria and Actinomycetes), Moscow: Akad. Nauk SSSR, 1949.
17. *Biologiya termofil'nykh mikroorganizmov* (Biology of Thermophilic Microorganisms), Imshenetskii, A.A., Ed., Moscow: Nauka, 1986.
18. Kalakutskii, L.V. and Agre, N.S., *Razvitie aktinomitsetov* (Development of Actinomycetes), Moscow: Nauka, 1957.
19. Kudryavtsev, V.I., Catalog of Cultures of the All-Union Collection of Nonpathogenic Microorganisms, Moscow: Nauka, 1976.
20. Zavarzin, G.A., Budding Bacteria, *Mikrobiologiya*, 1961, vol. 30, pp. 952–975.
21. Nikitin, D.I., Vasil'eva, L.V., and Lokhmacheva, R.A., *Novye i redkie formy pochvennykh mikroorganizmov* (New and Rare Species of Soil Microorganisms), Moscow: Nauka, 1966.

22. Zavarzin, G.A., *Vodorodnye bakterii i karboksidobakterii* (Hydrogen Bacteria and Carboxydobacteria), Moscow: Nauka, 1978.
23. Krasil'nikov, N.A., *Luchistye gribki. Vysshie formy* (Ray Fungi. Higher Forms), Moscow: Nauka, 1978.
24. *The Shorter Bergey's Manual of Determinative Bacteriology*, Holt, J.G., Ed., Baltimore: Williams & Wilkins, 1977. Translated under the title *Kratkii opredelitel' bakterii Bergi*, Moscow: Mir, 1996.
25. *Bergey's Manual of Determinative Bacteriology,* 9th ed., Holt, J.G. *et al.*, Eds., Baltimore: Williams & Wilkins. Translated under the title *Opredelitel' bakterii Berdzhi,* Moscow: Mir, 1997.
26. Zavarzin, G.A., *Fenotipicheskaya sistematika bakterii. Prostranstvo logicheskih vozmozhnostei* (Phenotypic Systematics of Bacteria. The Space of Logical Possibilities), Moscow: Nauka, 1974.
27. Shestakov, S.V., Early Stages of the Biological Evolution as Considered from the Viewpoint of Genomics, *Paleontol. Zh.*, 2003, no. 6, pp. 50–70.
28. Zhilina, T.N. and Zavarzin, G.A., Trophic Relations between Methanosarcina and Its Satellites, *Mikrobiologiya*, 1978, vol. 52, pp. 266–273.
29. Svetlichnyi, V.A., Sokolova, T.G., and Gerhardt, M., A New Group of Thermophilic Carboxydobacteria, *Dokl. Akad. Nauk SSSR*, 1990, vol. 314, pp. 742–744.
30. Svetlichny, V.A., Sokolova, T.G., *et al.*, *Carboxydothermus hydrogenoformans* gen. nov. sp. nov., a New CO-Utilizing Thermophilic Anaerobic Bacterium from Hydrothermal Environments of Kunashir Island, *Syst. Appl. Microbiol.*, 1991, vol. 14, pp. 254–260.
31. Kriss, A.E., *Morskaya mikrobiologiya* (Marine Microbiology), Moscow: Akad. Nauk SSSR, 1959.
32. Zavarzin, G.A., Gerasimenko, L.M., and Zhilina, T.N., Cyanobacterial Communities of the Hypersaline Lagoons of Sivash, *Mikrobiologiya*, 1993, vol. 62, pp. 113–126.
33. Mishustin, E.N., *Termofil'nye mikroorganizmy v prirode i praktike* (Thermophilic Microorganisms in Nature and Practice), Moscow: Akad. Nauk SSSR, 1950.
34. Mishustin, E.N. and Shil'nikova, V.K., *Kluben'kovye bakterii i inokulyatsionnyi protsess* (Root Nodule Bacteria and the Inoculation Process), Moscow: Akad. Nauk SSSR, 1973.
35. Loginova, L.G., *Anaerobnye termofil'nye bakterii* (Anaerobic Thermophilic Bacteria), Moscow: Nauka, 1982.
36. Zavarzin, G.A., *Litotrofnye mikroorganizmy* (Lithotrophic Microorganisms), Moscow: Nauka, 1972.
37. Gorlenko, V.M., Dubinina, G.A., and Kuznetsov, S.I., *Ekologiya vodnykh mikroorganizmov* (Ecology of Aquatic Microorganisms), Moscow: Nauka, 1977.
38. Zavarzin, G.A., *Lektsii po prirodovedcheskoi mikrobiologii* (Lectures on Environmental Microbiology), Moscow: Nauka, 2004.
39. Zavarzin, G.A., The Microorganisms: A Concept Model in Need of Clarification or Now To Be Rejected? *Microbial Diversity and Ecosystem Function*, Allsopp *et al.*, Eds., Wallingford: CAB Int., 1995, pp. 17–28.
40. The Archaea and Deeply Branching Phototrophic Bacteria, *Bergey's Manual of Systematic Bacteriology*, 2nd ed., Garrity, G.M. *et al.*, Eds., New York: Springer, 2001, vol. 1.
41. Sokolova, G.A. and Karavaiko, G.I., *Fiziologiya i geokhimicheskaya deyatel'nost' tionovykh bakterii* (Physiology and Geochemical Activity of Thionic Bacteria), Moscow: Akad. Nauk SSSR, 1964.
42. Lafar, F., *Bakterii i gribki* (Bacteria and Fungi), St. Petersburg: Brokgauz"-Efron", 1903.
43. Lyakh, S.P., *Adaptatsiya mikroorganizmov k nizkim temperaturam* (Adaptation of Microorganisms to Low Temperatures), Moscow: Nauka, 1976.
44. Protsenko, A.E., *Morfologiya i klassifikatsiya fitopatogennykh virusov* (Morphology and Classification of Phytopathogenic Viruses), Moscow: Nauka, 1966.
45. Zavarzin, G.A., Karpov, G.A., Gorlenko, V.M., Golovacheva, R.S., Bonch-Osmolovskaya, E.A., and Orleanskii, V.K., *Kal'dernye mikroorganizmy* (Microorganisms of Calderas), Moscow: Nauka, 1989.
46. Zavarzin, G.A., Zhilina, T.N., and Kevbrin, V.V., The Alkaliphilic Community and Its Functional Diversity, Mikrobiologiya, 1999, vol. 68, pp. 579–599.
47. Zavarzin, G.A., *Bakterii i sostav atmosfery* (Bacteria and the Composition of the Atmosphere), Moscow: Nauka, 1984.
48. Gerasimenko, L.M., Actualistic Paleontology of Cyanobacteria, *Trudy Winogradsky Inst. Mikrobiol.,* 2004 (in press).