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REVIEW PAPERS

Analysis of Soil Bacterial Diversity: Methods, Potentiality, and Prospects

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Abstract—The paper presents a comparative description of the modern molecular genetic and routine culture techniques for assessing bacterial diversity in soils and gives analysis for the different results obtained by these two groups of methods. The necessity of the collaboration of soil scientists, microbiologists, and molecular biologists in integrating different research methods for a proper assessment of soil microbial diversity is discussed. The paramount importance of soil as the source and reserve of biodiversity on the Earth is emphasized.

Key words: bacterial diversity, bacterial communities, soil, molecular genetic methods, culture techniques.

At the 15th World Congress of Soil Scientists, the special symposium *Soils and Biodiversity* paid particular attention to the problems of soil microbial diversity [1, 2] and emphasized the necessity of investigating soils as the source and reserve of biodiversity on the Earth.

The reviews on this subject that have recently been published in Russia [3, 4] and abroad [5] show an increasing interest of researchers in the problems of soil microbial diversity. Nevertheless, as was emphasized by G.V. Dobrovol'skii, the editor in chief of the Russian journal *Pochvovedenie*, the role of soil as the habitat and reserve of microbial diversity in the functioning and self-regulation of the Earth's biosphere has so far been underestimated [6]. R. Tate, the editor in chief of the journal *Soil Science*, lists the following problems needing to be solved by soil microbiologists [7].

How can so many microbial species (up to 4000 species per g soil) coexist in soil?

How does the vast diversity of the soil bacterial community relate to the entire Earth's microbe community?

What soil properties govern the biogeochemical activity of soil microflora?

How do the vast diversity of soil microbial community and the ability of many soil microorganisms to exist in a quiescent state correspond to the paradigm of the importance of biodiversity in the stability of soil microbial communities?

How can the soil microbial diversity be controlled in order to maintain the normal functioning of microbial communities and the homeostasis of the entire soil ecosystem?

We believe that the answer to the last question can be found in Zvyagintsev's conception of the structure and function of soil microbial complexes, which suggests a redundancy of soil microorganisms and their enzymes performing the same biogeocenotic functions under different environmental conditions [8].

As for the last problem, it is reduced to the control of natural microbial populations, which is the future goal of soil microbiology.

The fact that many of the questions raised long ago still remain unanswered can be explained by the paucity of adequate research methods. In Tate's opinion, the development and extensive employment of novel molecular genetic techniques for the analysis of microbial communities should intensify the study of soil microbial diversity. In 1999, Rondon *et al.* published a review paper [9] in which they emphasized the role of soil as the source of vast microbial diversity and highlighted that only 1–5% of soil microorganisms are presently known. One of the reasons for this is the inefficiency of detection of soil microorganisms. The present-day methods of soil microbiology, as well as their advantages and disadvantages, are summarized in the publications [5, 9]. For this reason, we can only mention the most important of them: the inoculation of soil suspensions on nutrient agar plates; the multisubstrate testing of the metabolic potential of microbial communities; analysis of the fatty acid methyl ester profiles; and various molecular genetic methods.

The latest methods assimilated by soil microbiology offer considerable advantages. First, the diversity of soil microorganisms can be evaluated without using the tedious and time-consuming procedures of enrichment, cultivation, and isolation of microorganisms in pure cultures. Second, the microbial diversity determined by molecular genetic methods is often one hundred or more times higher than that determined by routine methods [10–13]. Third, these methods allow the so-called nonculturable microbial forms (i.e., those which

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are unable to grow under laboratory conditions) to be easily detected. And fourth, new evolutionary lineages on the phylogenetic tree of prokaryotes can be revealed [13].

At the same time, molecular genetic and routine methods often give different results, for instance, when determining dominant species. This allowed some researchers to infer that the potentialities of culture techniques have been exhausted, whereas those of molecular genetic methods are as great and may provide absolutely true results.

Before going to the analysis of soil microbial diversity, we shall briefly review the primary molecular genetic methods applied in soil microbiology, try to illustrate the potential new applications of routine culture techniques in assessing soil microbial diversity, compare the results obtained by these two groups of methods, and outline the prospects for researches in the field of soil microbiology.

1. MOLECULAR GENETIC METHODS USED FOR THE ANALYSIS OF SOIL BACTERIAL DIVERSITY

The molecular genetic methods most commonly used in soil microbiology are as follows:

analysis of the DNA extracts of soil samples by the DNA reassociation method [10, 11] and by centrifugation in cesium chloride gradients [14];

extraction of DNA and RNA from soil, amplification of the 16S rRNA genes by the polymerase chain reaction (PCR) technique, and analysis of the amplified PCR products by various molecular genetic methods [15];

in situ analysis of soil microbial communities using oligonucleotide probes labeled with fluorescent dyes [15].

We will dwell on these methods in more detail in order to appreciate their advantages and disadvantages.

1.1. Analysis of the DNA Extracts of Soil by the DNA Reassociation Method and Density Gradient Centrifugation

The DNA reassociation technique, whose theory was developed by Britten and Kohne [16], was primarily used for the analysis of the eukaryotic genome and was first employed for the analysis of bacterial diversity in soil and sediment communities by Torsvik *et al.* [10, 11].

In solutions, the reassociation of denatured DNA obeys the second-order reaction kinetics and is described by the equation: $C/C_0 = 1/(1 + KC_0T)$, where the fraction of reassociated DNA, C/C_0, is a function of C_0T (here C_0 is the molar concentration of the single-stranded DNA nucleotides at the onset of DNA reassociation and T is the time expressed in seconds). The reaction rate constant K depends on the relative concentration of the complementary sequences of DNA and is inversely proportional to the level of C_0T at T equal to the time (in seconds) required for the 50% reassociation of DNA (this time is known as the half-time $T_{1/2}$). Under certain conditions, the term $C_0T_{1/2}$ is proportional to the complexity of the DNA preparation. The reassociation of molten DNA is monitored spectrophotometrically in a standard saline solution using the *Escherichia coli* genome as the reference. According to Torsvik *et al.* [11], the quantity $C_0T_{1/2}$ corresponds to the number of base pairs in nonhomologous DNA, is equivalent to the entire genome size, and can be used, along with the Shannon index, to characterize the diversity of microbial communities and changes induced in them by natural and anthropogenic impacts. The confidence level of the experimental data obtained by this method largely depends on the degree of DNA extraction from soil (estimates show that as low as about 20% of all DNA is extracted from soil samples [15]), the degree of DNA purification from humic substances [17], and the duration of the DNA reassociation process, which ranges from several hours in the case of fairly pure DNA preparations to two weeks in the case of complex soil extracts [11].

The analysis of the soil DNA by centrifugation in cesium chloride gradients allows the total DNA preparation to be separated into individual fractions according to their buoyant density [14]. The buoyant density of DNA is related to its G+C content by the respective formula, which allows the DNA profiles of microbial communities to be constructed by means of simple calculations. In addition, the DNA fractions can be studied by molecular genetic methods, such as DNA hybridization and fingerprinting. The shortcoming of this method is the incomplete extraction of DNA from the soil and the dependence of results on the degree of the preliminary DNA purification and the completeness of DNA separation in the CsCl gradient. As a result, the accuracy of this method allows only considerable changes in microbial communities to be detected.

1.2. Extraction of DNA from Soil, Amplification of the 16S rRNA Gene Fragments by the Polymerase Chain Reaction (PCR) Technique, and Analysis of the PCR Products

It is with this method that most of the recent studies in microbial ecology have been performed [12, 13, 18–22]. The 16S rRNA gene sequencing is now extensively used to evaluate the phylogenetic relations of bacteria [23, 24] and to establish their taxonomic status [25, 26]. The presence of particular bacterial taxa in soil is judged from the analysis of the 16S rRNA sequences of soil bacteria, which consists of the following steps: (1) extraction of DNA from soil; (2) amplification of the 16S rRNA gene fragments by the PCR technique with universal or specific primers; (3) cloning of the PCR products by introducing them into the *E. coli* genome by means of respective vectors (this stage is known as the creation of the library of soil clones); (4) analysis of the soil clones by molecular genetic

methods, such as gene sequencing, hybridization with marker sequences, and various modifications of restriction enzyme analysis followed by electrophoresis; (5) numerical analysis of the derived information with the use of the known nucleotide sequences (at present, several huge gene databases are available through the Internet).

Along with the DNA extraction from soil, some researchers prefer to extract rRNA, which is analyzed similarly to DNA and allows actively growing soil microbial populations to be detected. However, other researchers [5, 15] believe that the application of reverse transcriptase gives less accurate results than the application of Taq polymerase [5].

It should be noted that the method discussed permits only the detection in soil samples of nucleotide sequences specific (or close) to a given bacterial taxon and provides no information on the abundance of this taxon in a microbial community. The potential of this method is also limited by its complexity, so the final results largely depend on the efficiency of DNA extraction from soil, the degree of DNA purification from humic substances, conditions of PCR, and the proper choice of primers and methods used for the PCR product cloning and analysis. Furthermore, PCR often gives rise to the so-called chimeric nucleotide sequences, which do not correspond to any actually existing organism [15]. The different number of the 16S rRNA operons in cells, from one copy per cell in mycoplasmas [27] to ten copies per cell in bacilli [28], augments the yield of the amplified bacillar DNA and decreases the yield of the amplified mycoplasmic DNA. In the final analysis, it remains unclear whether DNA is extracted from the microbial cells present in soil or from a pool of free soil DNA [29].

Noteworthy are data on the occurrence of gram-positive and gram-negative microorganisms in soils. The estimates obtained by the above method suggest that gram-negative bacteria are prevalent in soils, whereas the routine plate methods of analysis indicate the predominance of gram-positive microorganisms. This discrepancy can be explained by the fact that gram-positive microorganisms often occur in soil as spores (bacilli) or thick-walled cells (coryneforms) and, hence, lyse less readily than gram-negative microorganisms. Successful PCR is possible only with properly chosen primers [18, 30], since universal primers do not ensure the amplification of all types of the bacterial DNA extracted from soil, whereas the use of specific primers may lead to erroneous results [19, 31]. The correspondence of primers to the primary structure of DNA, the type of nucleotide sequence at the 3'-terminus of the primers, the presence of conservative nucleotide sequences, and the length of the nucleotide sequence to be amplified are the factors that may influence the composition of clonal libraries and the final results of the analysis [32].

If some microorganisms are present in soil microbial communities in small amounts (less than 1%) [15], their detection requires the use of highly specific primers and particularly careful work of researchers.

1.3. In situ Analysis of Soil Microbial Communities by DNA Hybridization with Specific Oligonucleotide Probes Labeled with Fluorescent Dyes

This method is close to the well-known method of fluorescent-antibody staining and makes it possible to detect the presence of metabolically active or dominant microbial populations in soil based on the analysis of specific interaction between the 16S (or, rarely, 23S) rRNA sequences and the labeled oligonucleotide probes deliberately constructed using pertinent information derived from the available databases. The probe, which is labeled with a fluorescent dye, binds to the target rRNA sequence inside the cells of the analyzed bacterial taxon and thus makes them easily observable under illumination with specific light wavelengths dependent on the dye [15]. This method allows the direct analysis of soil suspensions, the microbial cells isolated from soil, and the DNA preparations extracted from soil. The probes can be labeled with different dyes, such as acridine orange or 4',6-diamino-2-phenylindole (DAPI), and can be constructed for the analysis of higher bacterial taxa, such as *Archaea* and *Bacteria*, or lower taxa, such as sulfate-reducing bacteria [33], the alpha, beta, and gamma subclasses of *Proteobacteria* [34], the *Flavobacterium–Cytophaga* group [33], and others [15]. The limitations of this method are associated with the poor permeability of the cell walls of some microorganisms to oligonucleotide probes and the low content of cells in the analyzed sample (less than 1 million cells per g soil). To avoid the limitation related to a small number of cells, they can be concentrated in soil suspensions. The detection of soil bacteria forming spores, cysts, and thick-walled forms, as well as the detection of resting cells and those containing low amounts of RNA, requires specific conditions. It should be noted that highly specific oligonucleotide probes can be constructed for the analysis of only genetically well-studied microorganisms [15].

2. ANALYSIS OF THE BACTERIAL DIVERSITY OF SOILS EVALUATED BY DIFFERENT RESEARCHERS USING MOLECULAR GENETIC METHODS

Below we will describe and discuss the results of the determination of microbial diversity in different soils by American, Japan, Australian, and English researchers who used the molecular genetic methods.

2.1. Bacterial Diversity of Agricultural Soil in Wisconsin, the United States [21]

The soil was overgrown with clover and grass and used as a pasture at an agricultural experiment station of the University of Texas in Arlington, Wisconsin, the United States. The muddy clay soil was well drained (ground waters at a depth of 25 m), contained 4.4% organic matter in its A1 horizon, and had pH 6.5. Four soil samples were taken from the surface layer 0–10 cm in depth.

Analysis of the 16S rRNA gene sequences of detected soil clones showed the presence in this soil of three major bacterial taxa—*Proteobacteria* (16% of the total number of the soil clones), *Cytophaga–Flexibacter–Bacteroides* (21.8%), and gram-positive bacteria with a low G+C content of DNA (21.8% of the soil clones)—and one minor taxon, *Planctomyces–Chlamydia* (3%). As many as 39% of the soil clones did not belong to any of the microbial taxa present in the evolutionary tree derived by Olsen *et al.* [24]. Thermophilic archaea, green non-sulfur bacteria, fusobacteria, and spirochetes were not detected. Two soil clones were recognized as chimeras.

Let us discuss these results. As the analyzed soil samples were taken from the clover field, it would be reasonable to suggest that they must be dominated by the root-nodule bacterium *Rhizobium trifolii*, a member of the alpha subclass of proteobacteria; however, only 1.6% of all soil clones were found to be related to proteobacteria (a fact that does puzzle). Furthermore, only as low as 0.8% of the soil clones were recognized as actinomycetes (i.e., gram-positive microorganisms with a high G+C content of DNA), although actinomycetes are typical inhabitants of well-drained soils with neutral pH. At the same time, analysis by the culture technique showed that the representatives of *Actinomycetales* and *Arthrobacter* amounted to, respectively, 5–20 and 5–60% of the microbial population of this soil.

Thus, the bacterial diversity of the pasture soil determined by the molecular genetic methods does not correspond to the specificity of this soil: microorganisms of the large taxonomic groups *Proteobacteria*, *Cytophaga–Flexibacter–Bacteroides*, and gram-positive bacteria with a low G+C content of DNA are not specific as they are ubiquitous in diverse environments, whereas actinomycetes (typical soil inhabitants) and the symbiotic nitrogen-fixing rhizobia (which must be present in this soil overgrown with clover) were not detected at all. The low homology (71–85% or lower) of almost all of the soil clones with the known bacterial taxa did not allow their identification to a generic level by the molecular genetic methods used. This could obviously be done by the culture methods, which is tremendous work.

2.2. Bacterial Diversity in Soybean Field Soil of a Farm of the Kyushu University in Japan [20]

Soil was sampled from a depth of 1–5 cm. Soil characteristics were not presented. The analysis of the 17 soil clones detected showed that 3 of them were chimeric. Some of the clones could not be assigned to any of the lineages of the microbial phylogenetic tree [24]. The other clones were identified as gram-positive bacteria with a high G+C content of DNA, green sulfur bacteria, proteobacteria, and archaebacteria of the kingdom *Crenarchaeota*. It should be noted that crenarchaea are obligate extreme thermophiles growing at temperatures from 74 to 103°C and are commonly isolated from hot solfataric waters. Surprisingly, this archae group was also detected in extremely cold environments, such as the Antarctic and Alaskan marine biotopes [36, 37], which, as the authors believe, calls for the revision of the global ecological significance of crenarchaea. However, without having crenarchaea isolated in pure cultures and their versatile characterization, speculation about their possible dwelling in soils seem to be premature.

Three of the soil clones were found to be homologous to *Rhodocyclus gelatinosus*, *Pseudomonas flavescens*, and, to a lesser degree, to *Agrobacterium tumefaciens* (95, 97, and 89.9% homology, respectively). Rhizobia, whose presence in the soybean field would be natural, were not detected. At the same time, Ueda *et al.* revealed the 16S rRNA sequences resembling those of frankia, which are the root-nodule bacteria of bushes but not of soybean.

The authors concluded that the microbial complex of the soybean-field soil contains bacteria and archaea. Except for proteobacteria, the majority of the soil clones exhibited low homology (less than 90%) to any of the known phylogenetic lineages, and some of them presumably represented novel phylogenetic groups. Ueda *et al.* were not satisfied with the molecular genetic methods completely ignoring the physiology and ecology of most soil microorganisms and requiring the reappreciation of the results by invoking the routine culture methods of microbiological analysis.

2.3. Bacterial Diversity of Soil an Australian Terrestrial Environment [18]

Soil, whose characteristics were not presented, was sampled from the subsurface layer 5–10 cm in depth in a locality of a mountain situated near Brisbane, the capital of Queensland, Australia.

The molecular genetic analysis of the soil DNA extracts showed the presence of three nucleotide sequence clusters.

Fourteen soil clones of the first cluster were homologous to the nitrogen-fixing symbiotic bacteria of the alpha subclass of the class *Proteobacteria*, namely, to the genera *Bradyrhizobium*, *Azorhizobium*, and *Photorhizobium*. Seven clones of the second cluster were

found to be close to the unique evolutionary lineage, the family *Planctomycetaceae.* One of these clones was related to *Planctomyces limnophilus* and another, to *Isosphaera pallida.* The degree of homology between the soil clones and the 16S rRNA sequences of the genera *Isosphaera* and *Gemmata* was, however, insufficiently high (63–73%) to reliably assign the soil clones to these genera. Twenty two soil clones of the third cluster could not be assigned to any of the known bacterial phylogenetic lineages and, hence, presumably represented a novel evolutionary group with planctomycetes and chlamydia as ancestors.

Thus, the soil bacterial diversity of the Australian terrestrial environment involves rhizobia (unfortunately, the authors did not report whether or not the soil under study was overgrown with leguminous plants), soil clones close to planctomycetes, and soil clones with planctomycetes and chlamydia as evolutionary ancestors. The presence of planctomycetes, which commonly inhabit aquatic biotopes, in the soil under study does not seem to be amazing if one takes into account its location near the ocean, due to which planctomycetes could have been transferred from the ocean to the coastal soil with the splash borne by oceanic winds.

2.4. Bacterial Diversity in a Temperate Forest Soil in the United Kingdom [13]

Soil overgrown with a beech forest was sampled from a depth of 5–20 cm. The 53 soil clones isolated were found to fall into seven groups, six of which belonged to the actinomycete phylum. Some soil clones exhibited a relationship, albeit weak, with the actinomycete genera *Sporichthya polymorpha* and *Frankia.* Two clones formed a monophyletic actinomycete group. Two other clones were found to be close to the opportunistic erythrocytolytic pathogen *Propionibacterium acnes* and the animal and human pathogen *Corynebacterium renale.* Four clones belonged to the group of slowly-growing mycobacteria, such as *Mycobacterium celatum, M. cooki, M. simiae, M. avium,* and *M. scrofulaceum, et al.* which are known to cause pulmonary infections and lymphadenitis in animals and humans.

Thus, the bacteria detected in this soil by the molecular genetic methods were mainly the slowly-growing mycobacteria, propionic acid bacteria, and corynebacteria that are pathogenic to a variable extent to animals and humans. It is known that these bacteria are difficult to cultivate under laboratory conditions and that soil conditions do not satisfy their growth requirements. At the same time, fast-growing saprotrophic mycobacteria, which are typical inhabitants of steppe litters and chernozem soils [38], were not detected in the soil under discussion. Other common inhabitants of forest soils, such as soil actinobacteria of the genera *Streptomyces, Arthrobacter, Rhodococcus,* and *Promicromonospora* [39], were not revealed either.

Some soil clones were found to be close to *Actinomadura madurae.* It should be noted that forest soils are usually acidic and have a low humus content (1–3%), due to which they are dominated by acidotolerant actinomycetes of the genus *Streptosporangium* [40] and micromonosporas [42], whereas *Actinomadura* predominantly live in the chernozem and chestnut soils [40, 41]. In other words, there is again a discrepancy between the estimates of microbial diversity obtained by modern molecular genetic and routine culture techniques.

2.5. Microbial Diversity of Some Other Environments and General Consideration of the Results

The molecular genetic analysis of microbial diversity in Adirondack mountain lakes showed the presence of only one actinomycete species, *Streptomyces amborfaciens* [43], although it is known that lake waters are usually populated by the genera *Micromonospora, Streptosporangium,* and *Actinoplanes* [44] but not by the genus *Streptomyces.* The nonobservance of actinomycetes by the molecular genetic methods may be accounted for by their low abundance, since the universal bacterial primers used in these methods do not allow actinomycete taxa comprising less than 1% of the total bacterial population of soil to be detected. It should be noted that the detectable level of actinomycetes in soil can be decreased by employing PCR with forward bacterial and reverse actinomycete primers.

A comparison of the actinomycete complexes of soils from Cuba and Warwick showed that actinomycetes were more diverse in the soil from Cuba than in the soil from Warwick [22]. Unfortunately, Heuer *et al.* did not present any data on the taxonomic structure of these actinomycete complexes.

Apart from the publications discussed above, there are about 20 other publications devoted to the estimation of microbial diversity in the soils of Canada, Finland, the Netherlands, the United States, and other countries by molecular genetic methods (see, for instance, the review [9]). These publications, however, add little to the above discussion except that they enlarge the list of microorganisms detected in soils by two new groups, *Holophaga–Acidobacterium* and *Verrucomicrobia* [45, 46], which contain mainly nonculturable microbial forms.

In conclusion, we may state the following.

(1) Molecular genetic methods make it possible to detect in soils large bacterial taxa, such as gram-positive bacteria with a low or high G+C content of DNA, proteobacterial subclasses, green sulfur bacteria, planctomycetes, spirochetes, and crenarchaea.

(2) The majority of soil clones have a low degree of homology with the nucleotide sequences of the known bacterial taxa available in databases. For this reason, the defined positions of the analyzed soil bacteria on

the microbial phylogenetic tree and their relation to the known bacterial taxa are approximate and presumptive. Moreover, some researchers believe that the 16S rRNA homology cannot be used to determine the position of bacteria on the phylogenetic tree (see, for instance, the review [47]).

(3) Due to the low degree of DNA homology with the known bacterial taxa, many soil clones are recognized as new phylogenetic groups; at the same time, molecular genetic methods often fail to detect common soil inhabitants, e.g., actinomycetes, which are easily detected by the culture techniques.

(4) Molecular genetic methods sometimes lead to paradoxes, such as the absence of rhizobia in soils overgrown with leguminous plants or the presence of specific aquatic and marine microorganisms (e.g., thermophilic crenarchaea or green sulfur bacteria) in terrestrial environments [19–21].

(5) When discussing the results obtained by molecular genetic methods, researchers often ignore the ecological aspects of the problem, for instance, whether particular bacteria can live or merely be preserved in particular habitats.

Thus, the data on the microbial diversity of soils obtained by molecular genetic methods are much more ambiguous than those obtained by the culture technique. As for the large bacterial groups, such as proteobacteria and gram-positive bacteria with low or high G+C contents of DNA, they are present in nearly all types of soils and the data on their occurrence in particular soils are of little interest. Furthermore, the detection of particular bacterial groups in soil by molecular genetic methods provides no information on their physiological peculiarities and ecological functions. To obtain such information, soil bacteria must be isolated in pure cultures, which is separate and hard work.

The problems arising during the investigation of soil microbial diversity by molecular genetic methods were formulated by Rondon *et al.* [9].

Is the information on the 16S rRNA gene sequences of culturable bacterial forms available in databases sufficiently comprehensive?

How diverse are bacterial forms recognized by molecular genetic methods as nonculturable? What functions do these forms serve? How can these functions be studied?

To what extent does the phylogenetic diversity of nonculturable bacterial forms reflect their physiological diversity?

3. ASSESSMENT OF SOIL MICROBIAL DIVERSITY: NEW POTENTIALITY OF THE OLD CULTURE TECHNIQUE

The portion of this title after the colon is taken from a publication of Chernov [48], in which he attempted to demonstrate the potentiality of the culture techniques for the assessment of yeast diversity. The appropriateness of this technique for the assessment of the diversity of saprotrophic bacteria was proved in the publications [49–52].

In the present chapter, we shall dwell on the methodological and practical aspects of experimental approaches to the ecological assessment of soil microbial diversity that were developed at the Department of Soil Biology of the Faculty of Soil Science of the Moscow State University. The examples of their application for the analysis of microbial diversity in soils and related substrates will also be presented.

3.1. Theoretical Fundamentals of the Ecological Assessment of Soil Microbial Diversity

The systemic approach to the assessment of soil microbial diversity we developed is based on the concept of the hierarchy of microbial habitats [49, 52]. The current interests of soil scientists shift from the medium-level scales of consideration (average soil samples, soil horizons, and soil profiles) to low-level scales (the meso- and microzonal architecture of soil microbial communities) and to high-level biogeocenotic and global scales.

Of great importance in this regard is the conception of the soil as a multitude of micromedia providing for the growth of diverse groups of microorganisms [8]. Such micromedia may represent mineral soil inhomogeneities (ortsteins, lime nodules, etc.), the rhizosphere and rhizoplane of plants, mycosphere and mycorhizosphere, the drilosphere of earthworms, the coprolites, intestines, the remains of soil invertebrates, and so on. Each of these soil compartments is populated by specific microbial communities; therefore, for the proper analysis of soil microbial diversity, all or at least most of these soil compartments should be analyzed. The growth conditions specific to different soil compartments should be taken into account if bacteria isolated from these compartments are cultivated under laboratory conditions. In this case, different nutrient media and cultivation conditions may comprise hundreds of variants. Clearly, only a small fraction of soil microorganisms will grow on one medium under given cultivation conditions. Therefore, increasing the number of test media and cultivation conditions corresponding to particular soil microhabitats may greatly enlarge the range of detected soil microorganisms.

On the other hand, the concept of the soil as an open system that plays a key role in the development and functioning of particular ecosystems and the biosphere as a whole led to attempts to consider the soil microbial diversity at the biogeocenotic level [53]. At this level, soil microbial communities are analyzed in all layers of the soil ecosystem at a given time: in the overground layer (the phylloplane of woody and herbaceous plants), in the surface layer (litters, mosses, lichens, and algal mats), and in the proper soil, including all its horizons. Each of these layers is characterized by a specific

set of substrates, beginning from the live plant tissues and ending with the mineral soil horizons low in easily metabolizable organic substances.

With this approach, one should take into account that soil microbial complexes undergo both seasonal variations (which are different for different microbial groups) and successional changes (which are analyzed, as a rule, in model experiments). A combination of different approaches (successional, vertical, and microlocal) allowed the main regularities of the spatial and temporal structure of the soil microbial communities to be revealed with reference to some groups of prokaryotic and eukaryotic microorganisms [52].

In relevant studies, we proposed to use genus as the main taxonomic unit of soil microbial diversity and, in case microorganisms are difficult to identify to a generic level, to use higher taxonomic ranks, such as the order *Myxobacterales* or the group *Flavobacterium–Cytophaga*. The use of taxonomic units higher than species is especially justified during the analysis of the microbial diversity of different soils from different geographic and climatic zones. In this case, the conventional synecological indices of diversity, such as the Shannon index of alpha diversity or the Wilson–Schmid index of beta diversity, can be used for the quantitative evaluation of microbial diversity in soil and related substrates. However, other indices that characterize the hierarchic and syntypological structure of microbial communities, namely, the proportion of bacterial genera in a community, the rate of occurrence and abundance of particular genera, the taxonomic composition of ecologotrophic bacterial groups, and the proportion of proteobacteria and actinobacteria, seem to be more informative [50–52]. The data on microbial diversity obtained by these methods can be analyzed in the same way as the results obtained by molecular genetic methods [51].

Thus, soil microbial diversity can be analyzed using the approaches developed by us and those based on the phenotypic identification of bacteria to a generic level. If necessary, the alternative chemotaxonomic and molecular genetic methods can be used.

In assessing the bacterial diversity of soils by the culture technique, one should not try to describe all microbial groups and taxa, including heterotrophs, chemolithotrophs, phototrophs, obligate anaerobes, methanotrophs, etc., as it is practically impossible; instead, one should take a bacterial taxon whose representatives are able to grow on one nutrient medium as a model group. This makes possible the detection of dominant, subdominant, and minor components of a microbial community, or, in other words, its synecological analysis. If the model bacterial group is chosen properly, the spatial and taxonomic structures of the respective bacterial complex can be revealed and the regularities of its adaptation to the ecological conditions of the habitat can be established. It should be noted that all general biological regularities of the spatial and temporal structures of biotic communities and their propagation were derived by analyzing particular taxonomic groups: birds, invertebrates, butterflies, and so on.

In our studies, two model microbial groups were analyzed. The first group included 50 genera of aerobic and facultatively anaerobic soil bacteria that are able to grow on a modified peptone–glucose–yeast extract agar medium [54]. The second group, comprised of mycelial actinomycetes, was used for the analysis of prokaryotic complexes in different biotopes. This group of prokaryotes was grown on casein–glycerol agar and on a medium with sodium propionate. The rare genera of actinomycetes were isolated using auxiliary procedures, such as the preheating of soil samples at 100°C for 1 h and the supplementation of the growth media with 1 μg/ml nalidixic acid, 1.5 μg/ml rubomycin, and 50 μg/ml nystatin to suppress the unwanted growth of nonmycelial prokaryotes, streptomycetes, and microscopic fungi, respectively.

3.2. Analysis of the Soil Prokaryotic Diversity Data Obtained by the Culture Technique

The major regularities of the geographic differentiation of the bacterial communities of entire biogeocenoses were established in the studies of contrasting ecosystems, such as peat lands and deserts [50]. Using the Shannon and Wilson–Schmid indices of diversity, as well as the diversity indices devised by us, we characterized the biodiversity of these ecosystems and revealed differences in the structure of bacterial complexes that are associated with the adaptation of bacteria to the excess or deficiency of water in the environment. Peat land ecosystems were dominated by the facultatively aerobic proteobacteria of the families *Enterobacteriaceae* and *Vibrionaceae* and spirillas, i.e., by typical aquatic microorganisms. At the same time, all arid ecosystems were dominated by bacteria of the actinomycete lineage (ten genera). Most of the isolated actinobacteria produced carotenoid or melanoid pigments, protecting them from increased radiation. The dominant microorganisms of these contrasting ecosystems were different except for the eurytopic gliding bacteria and streptomycetes. Thus, the bacterial complexes of humid and arid ecosystems represent transient communities reflecting the evolution of the microbial world from aquatic to terrestrial life [50].

The mycelial prokaryotic complexes of arid ecosystems were characterized by the presence of monosporous actinomycetes in all horizons and their prevalence in the plant substrates (dead leaves, mosses, and the rhizosphere). The proportion between the polysporous (*Streptomyces*) and monosporous (*Micromonospora*) actinomycetes varied depending on the substrate type and the soil horizon. Pigmented actinomycetes were found in all the substrates of the arid ecosystems. Many streptomycetes produced melanoid pigments. Micromonosporas were represented by the

black-colored *Micromonospora carbonaceae* [55]. The biosynthesis of pigments presumably represents an adaptive response of prokaryotes to the dry and high-insolation conditions of arid regions. Microorganisms have to adapt fairly well to the harsh conditions of deserts. This is clearly seen from the high diversity of actinomycetes and related microorganisms in arid ecosystems: desert soils were found to be populated by 11 series of streptomycetes, whereas forest soils are populated by as few as 2–3 series [56]. In arid ecosystems, actinomycetes predominantly populate zoogenic substrates, such as the feces of invertebrates, especially termites, so that their diversity and abundance appear to be considerably higher than in the surrounding soil.

There is evidence that the digestive tract of termites is substantially overgrown by symbiotic actinomycetes, due to which these insects are able to utilize recalcitrant organic substances [57]. Earlier, we described the accumulation of unicellular saprotrophic prokaryotes in the zoogenic loci of arid soils [58].

Thus, bacterial diversities in different climatic zones can be characterized using the model groups of microorganisms and a set of certain ecological and taxonomic parameters.

On the other hand, the same model groups and parameters can be used for the evaluation of microbial diversity on a micro scale. For instance, the analysis of the microbial community of the burrow walls of earthworms (the so-called drilosphere) by such an approach showed that it differs from the microflora of the surrounding soil in increased bacterial abundance and specific taxonomic composition [59].

Laboratory experiments in which earthworms were introduced into soil showed that these invertebrates are important to the microbial diversity of soils. For instance, the microbial community of the soil populated with earthworms was dominated by gram-negative spirillas, enterobacteria, and myxobacteria, whereas the microbial community of the control soil was dominated by gram-positive bacilli and coryneforms [60].

The digestive tracts of soil invertebrates represent a specific econiche for bacteria. Byzov *et al.* described two types of bacterial communities in the intestines of soil diplopods and manure worms [61]. The inner surface of the gut wall of these animals were found to be populated by the communities of facultatively anaerobic bacteria of the families *Enterobacteriaceae* and *Vibrionaceae*. These communities were stable under different nutritional conditions, including starvation, and were almost the same (at the generic level) in the diplopods and worms, the dominant genera being *Klebsiella*, *Escherichia*, *Enterobacter*, *Plesiomonas*, and *Vibrio*. The hindgut was populated by actinomycetes of the genera *Streptoverticillium*, *Streptosporangium*, *Actinomadura*, and *Micromonospora* and the nocardioform actinomycetes of the group *Promicromonospora–Oerskovia*, whereas the surrounding soil was dominated by streptomycetes. It is believed that the actinomycetes mentioned are involved in the utilization of chitin, a component of the peritrophic membrane of millepedes [62].

The intestinal bacterial community of soil invertebrates is a transit community that passes through the intestines together with food. The taxonomic composition of this community is not constant and is determined by the food composition. Some bacteria lyse in the intestines, whereas others reproduce and are expelled into the surrounding soil with feces, enriching it by active bacterial forms.

Thus, soils contain specific microloci, such as the intestines, feces, and burrows of soil invertebrates, which are populated by bacterial communities differing from those of the surrounding soil in both taxonomic structure and ecologotrophic characteristics.

Another example of accumulation of specific bacteria in specific soil microloci are the microbial communities of Fe–Mn concretions, which are typical of podzolic soils and are populated by the genera *Seliberia*, *Metallogenium*, *Gallionella*, and *Siderocapsa*. The fidelity of these genera to particular soil types and their role in the formation of ortsteins and other specific soil structures were described by Aristovskaya [63, 64].

Analysis of bacterial diversity in various soil mesoloci, such as the rhizosphere, rhizoplane, mycorrhizosphere, and algosphere, is an interesting, but separate, problem whose discussion is beyond the scope of the present review.

To be successful, the analysis of microbial diversity in a particular type of soil should take into account its specificity. For instance, saline soils, such as solonchaks and rice paddy soils, were found to be populated by extremely halophilic archaebacteria [65], which were earlier isolated only from seawater, salt lakes, and lagoons. Zvyagintseva and Tarasov isolated the haloalkaliphilic archaebacteria *Natronobacterium pharaonis* and *N. occultus* from the alkali solonchaks of Armenia, a new hydrocarbon-utilizing halophilic species *Haloarcula distributus* from the sulfate solonchaks of Turkmenistan, and a new halophile *Halococcus turkmenicus* from the salt crust of Turkmenian serozem soil [66].

However, the bacterial communities of solonchaks are not dominated by extreme thermophiles: investigation of the taxonomic structure of the bacterial complexes of solonchak biogeocenoses in the coastal regions of the Aral and Dead Seas, the Kysylkum Desert, and Rostov region showed that they are dominated by a small number of halotolerant bacterial genera [67]. The halophilic archaebacteria *Halobacterium* and *Halococcus* were frequently isolated with (and grew better in) the presence of bacilli. Lysak *et al.* suggested that the extreme conditions of saline soils promote the selection of specific halotolerant and halophilic bacteria, although the later are not dominant in solonchaks. The most halotolerant bacteria capable of growth at a salt concentration of 20% were found to

belong to the genera *Bacillus* and *Micrococcus*. This explains why bacilli are dominant on the halophyte leaves but are typically absent in the phylloplane of plants growing in nonsaline soils.

The microbial communities of rice paddy soils, which, being almost constantly flooded, are characterized by anoxic conditions and algal bloom, are dominated by anaerobic clostridia, sulfate-reducers, and the phototrophic bacteria *Rhodopseudomonas* and *Rhodospirillum* [68]. The latter bacteria are virtually absent in peat bog soils; therefore, their occurrence in the rice paddy soils may be due to their association with algae, which are typical components of these biogeocenoses. It should be noted that the major habitat of phototrophic bacteria is algal mats formed in lakes and thermal spring runoff.

These data illustrate how the composition of soil bacterial communities reflects their adaptation to particular habitats. Therefore, when analyzing the results of the investigation of soil microbial diversity, the consideration of the soil type will help to decide whether particular bacteria are indigenous to this soil or they were occasionally born to it by winds or precipitates.

It should be noted that, like all other methods, the method of analysis of bacterial communities by sample inoculation on specific nutrient media with the subsequent differential count of grown colonies and identification of dominant bacteria to the level of groups or genera has some shortcomings and limitations. First, the bacterial diversity of soils is characterized within the limits of aerobic and facultatively anaerobic heterotrophic bacteria belonging to 50–60 genera (theoretically, up to 80 genera of this bacterial group can grow on the test medium used). Second, to be able to identify colonies to the generic level, researchers must be very experienced and skilled in the field of bacterial systematics. And third, the representatives of only some genera, such as *Bacillus, Cytophaga*, and *Rhodococcus*, can be identified to the species level based on phenotypic characteristics. However, in the majority of cases of analysis to the species level, phenotypic studies should be combined with the molecular genetic and chemotaxonomic methods of analysis of bacterial isolates, such as the determination of the G+C content of their DNA, the DNA–DNA hybridization with reference strains, and the chemical analysis of the cell wall constituents (peptidoglycans, mycolic and teichoic acids, etc.). In ecological studies, when a great number of strains isolated from different substrates and soil microloci are to be analyzed, this is hardly possible.

4. WHY IS THERE A DISAGREEMENT BETWEEN THE RESULTS OF DETERMINATION OF MICROBIAL DIVERSITY BY MOLECULAR GENETIC AND THE CULTURE TECHNIQUES?

To begin with, let us compare the results of the determination of bacterial diversity based on the reassociation rates of DNA preparations extracted from soil and from the bacterial biomass grown on test agar medium (the composition of this medium was not presented) inoculated with soil samples [11]. In the latter case, biodiversity turned out to be 170 times lower than in the former case (analysis of the total DNA extracted directly from the soil). Torsvik *et al.* reasonably explained this discrepancy by the inability of the majority of the diverse bacterial groups potentially present in soil to grow on one test medium under given cultivation conditions. As a result, only a small portion of soil bacteria is detected by this method. In order to evaluate soil microbial diversity as completely as possible, soil samples should be inoculated on different test media and cultivated under different conditions providing for the growth of diverse bacterial groups, such as aerobic and anaerobic cellulolytics, sulfate-reducers, methylotrophs and methanogens, chemolithotrophs and phototrophs, actinomycetes and actinobacteria, and so on. Even if the standard test media for the detection of the so-called physiological bacterial groups (nitrifiers, denitrifiers, nitrogen fixers, cellulolytics, etc.) were used, the bacterial diversity would be much greater than with the inoculation of one universal test medium. Only in this case, will there be grounds to speculate as to the proportion between the known and unknown soil bacterial taxa. In view of this, the estimates reported by some authors for the percentage of known soil bacteria (0.1–10% of the existing ones) and for the percentage of culturable bacterial forms (1% of the nonculturable forms) seem to be greatly underestimated. It should be noted that Rondon *et al.* came to the same inference [9].

Many bacteria occur in soil in a specific state known as *viable but nonculturable* [69]. To transit to the culturable state, bacteria must be incubated for some time in the presence of the key intermediates of the Krebs cycle, such as pyruvate or acetate [69]. If the state of bacteria in the soil samples at the moment of their inoculation on the test medium is not taken into account, this may lead to an underestimation of the known bacterial forms. For instance, the inoculation of the test medium with dry soil samples gave rise to a considerably lower number of colonies than the inoculation with the same soil samples but wetted and supplemented with growth substrates [70].

The bacterial populations estimated by the direct count of cells under a luminescence microscope and by the culture technique may differ by 100–1000 times in the case of soil communities and by 10 times in the case of rhizosphere communities [70]. This example shows that the estimate of a soil bacterial population by the culture technique considerably depends on the state of bacteria. In particular, the culturability of bacterial cells occurring in the rhizosphere or in the soil sample that was wetted and supplemented with a growth substrate is notably higher than the culturability of the cells occurring in dry soil.

On the other hand, the diversity of the 16S rRNA genes of the total DNA extracted from soil will always be higher than the diversity of these genes in the DNA extracted from soil isolates. The reasons for this may be the higher genetic inhomogeneity of natural microbial populations as compared with the populations of collection cultures [10, 11]; the longer reassociation times of the DNA extracted from soil, due to the presence of humic substances [10]; the presence in soil of free (extracellular) partially transformed nucleic acids [29]; the presence in soil of nonculturable bacterial forms, which are not detected by the culture technique [17, 69]; the formation of chimeric nucleotide sequences during PCR [15]; the horizontal transfer of genes between bacteria; and the recombination of the chromosomal genes of allied species [11, 69].

Soil is a reserve of microorganisms, many of which cannot grow in the soil and are not culturable under laboratory conditions but can live in specific environments, such as human and animal organisms, aquatic and marine ecosystems, thermal springs, and food. In other words, analysis of the 16S rRNA gene sequences of the total DNA extracted from soil provides insight into the microbial gene pool of soil rather than knowledge about the true soil bacterial diversity.

Of great interest is the problem that has long attracted the attention of all soil microbiologists: What bacteria are soil and what are nonsoil? Soil microorganisms in relation to their involvement in various soil and biospheric processes, soil bacteria are those that not only are present but also are able to grow in soil, including all its meso- and microloci—the phyllosphere, rhizosphere, mycosphere, drilosphere, mineral soil inhomogeneities, the remains and excrements of invertebrates, etc.

Soil bacteria are the components of diverse biotic associations with plants, invertebrates, protozoans, fungi, and so on, which perform the unique functions of nitrogen fixing, nitrification, denitrification, and sulfate reduction and are involved in the degradation of plant debris.

We attempted to determine the range of bacterial taxa, which can be arbitrarily referred to as soil bacteria based on the ecological consideration of all bacterial genera presented in the last edition of Bergey's manual [71], which describes about 530 genera divided into 35 groups according to diverse characteristics: gram staining, morphology, susceptibility to extreme factors, involvement in the processes of methanogenesis, sulfate reduction, sulfur oxidation, etc. Of these 35 bacterial groups, 15 can immediately be excluded from consideration as undoubtedly nonsoil bacteria, as they require specific growth conditions not inherent in soil. First of all, these are pathogenic bacteria of humans and animals. Although they can survive in soil, these bacteria are commonly isolated from clinical materials (blood, sputum, excrements, etc.) and can grow only in complex nutrient media. In Bergey's manual, they comprise group 1 (spirochetes), group 9 (rickettsiae and chlamydia), and group 30 (mycoplasmas) and are included in some other bacterial groups heterogeneous in habitats and properties. Overall, 20% of all bacteria are pathogenic.

Second, these are aquatic and marine bacteria, many of which are chemo- and lithotrophic bacteria involved in the conversion of sulfur and its compounds and some of which are extremophiles—thermophiles, halophiles, and acidophiles. The group of anoxigenic phototrophic bacteria (28 genera) includes typical aquatic bacteria responsible for the formation of mats in lagoons and hot spring runoff and of colored stratified zones in lakes and seas. These bacteria can also be isolated from rice paddy soils. Recently, the two genera, *Heliobacter* and *Heliobacillus*, of the unique phototrophic, strictly anaerobic, gram-positive, endospore-forming, bacteria synthesizing chlorophyll *g* have been isolated from soil [71].

Most of the obligately anaerobic bacteria producing methane (i.e., methanogens) are also nonsoil organisms that inhabit anoxic marine sediments and muds and saline marshes. Up to 60% of methanogens are halophilic organisms. Only one or two methanogenic genera live in soil. Various extremophiles that live in hot (70–100°C) solfataric waters, acidic mine waters with pH 1–3, and in saline environments can also be referred to as nonsoil bacteria.

A specific group of aquatic bacteria are sulfur oxidizers, filamentous gliding bacteria, pelonemas, and encapsulated bacteria, which inhabit lakes, streams, and springs.

Most of nitrifying bacteria are halophilic, and only some of them were detected in soil.

Once the aforementioned bacteria have been excluded from consideration, the number of potential soil bacterial genera is reduced to about 190, including 35 genera of cyanobacteria, specific oxygenic phototrophic bacteria ubiquitous in nature (they inhabit such diverse econiches as saline waters, hot springs, tree bark, and the surfaces of rocks and soil). The other 155 genera are represented by heterotrophs, predominantly aerobic or facultatively anaerobic, and by the bacteria that can live in the aqueous phase of soil. These are the dissipotrophic bacteria producing buds or appendages of the genera *Caulobacter, Hyphomicrobium, Pedomicrobium, Gallionella*, and others.

As a rule, aquatic ecosystems are dominated by proteobacteria, which are highly diverse in morphotypes and taxonomy and possess various adaptive accessories for aquatic life: flagella, prosthecae, attachment disks, sheaths, vacuoles, and so on. Soil, especially in its solid phase, is dominated by gram-positive bacteria, primarily, actinomycetes, which are fairly adapted to the life on solid substrates due to their mycelial organization [72]. Bergey's manual indicates soil as the main habitat of almost all of the 55 genera of mycelial actinomycetes [71].

Another group of typical soil inhabitants includes the bacteria of the actinomycete lineage that produce, if at all, true mycelium only at their early developmental stages but then are fragmented into rod-shaped or coccoid cells. These are the nocardio- and coryneform bacteria, belonging mainly to the genera *Arthrobacter, Rhodococcus*, and *Cellulomonas*. Like actinobacteria, many of which are phytopathogens and symbionts of plants and animals, various subclasses of the class *Proteobacteria* are closely associated with plants and animals. The fidelity of some bacterial genera to certain soil meso- and microloci, soil types, and ecosystems was discussed above.

Among the remaining 100 bacterial genera, 80 are able to grow on the test medium devised by us, which is an indication of its appropriateness to the evaluation of soil microbial diversity by the culture technique. The subsequent analysis of biodiversity with the use of various synecological indices can characterize the bacterial communities of soil much more adequately than the molecular genetic methods.

5. PROSPECTS OF THE EVALUATION OF SOIL BACTERIAL DIVERSITY THROUGH THE INTEGRATION OF DIFFERENT METHODS

We believe that a successful assessment of soil bacterial diversity is possible with the consensus approach utilizing the molecular genetic and conventional culture methods of analysis and the principles of polyphasic taxonomy, which is now widely used for the identification of microorganisms [26]. The polyphasic taxonomy suggests the obtaining of information about microorganisms based on both their phenotypic and genotypic characteristics. It should be noted that the Ribosomal Database, which now contains almost 10000 complete and partial nucleotide sequences of collection cultures and soil clones [73], is being constantly enlarged and in the near future will allow a more adequate interpretation of the 16S rRNA sequences of the total DNA extracted from soil.

The work of Watts and Wellington [74] can serve as an example of the successful integration of molecular genetic, cultural, and metabolic techniques for the analysis of soil microbial diversity. Such a combined approach allowed the authors to establish a correlation between the metabolic activity, diversity, and abundance of soil bacteria and the degradation rate of soil pollutants.

Of great importance for the proper interpretation of results is the collaboration of molecular biologists and microbiologists with soil scientists, for only the latter can adequately describe the soil samples taken for analysis, specify the soil type and soil horizon from which these samples were taken, as well as determine their mechanical composition, physicochemical properties, and other relevant parameters. It should be noted that most of the publications devoted to the analysis of soil microbial diversity do not indicate the type of soil, its position in the surrounding relief, and the horizon from which soil samples were taken. Nor do they present a description of the vegetation of analyzed soil and climatic and temperature conditions, which largely determine the air and moisture parameters of the soil. Meanwhile, the discussion of the functioning of particular bacterial groups in a particular type of soil makes sense only if such description is available.

Soil should be sampled from all soil horizons and from all its meso- and microloci. Moreover, due to seasonal variations in the taxonomic composition of microbial communities, the soil should be sampled several times a year with consideration for the soil moisture content and temperature. For instance, Luedemann *et al.* took samples from several layers of flooded soil, taking into account the oxygen gradient [75]. The analysis of the 16S rRNA gene sequences of the extracted total DNA showed that oxic soil layers were dominated by proteobacteria, whereas anoxic layers, by gram-positive bacteria of the genera *Bacillus* and *Clostridium*.

Further progress in the study of viable but nonculturable bacterial forms can be expected with two new approaches, the metagenome analysis involving the cloning of the large fragments of soil bacterial DNA and express cloning [9]. The application of these approaches to the analysis of not only the 16S rRNA genes but also structural genes, such as the nitrate reductase, ribulose 1,5-diphosphate carboxylase, and methanol dehydrogenase genes, must allow the relationship between the taxonomic structure of soil bacterial communities and their functioning to be studied. Another promising approach suggests the clamping of the largest PCR product sequences with specific peptides, which must allow rare and new phylogenetic groups to be established [76].

Peacocki *et al.* proposed to evaluate the diversity of soil microbial communities using a set of ecological indices of richness, evenness, and similarity calculated from the molecular genetic data and the fatty acid and phospholipid methyl ester profiles [77]. Earlier, we proposed to use the same indices for the structural analysis of soil microbial communities by the culture technique [50, 51].

The analysis of the ecosystems populated by diverse and unique bacterial forms can be efficiently performed using the aforementioned geographic approach and the vertical profiling of soil developed by us. The necessity of a detailed description of soils and the relative abundance of species in natural ecosystems was also emphasized by other authors [5, 9]. Many researchers have recognized that microbial diversity in nature must be not only investigated but also protected. As G.A. Zavarzin wrote, “reserves for microbes are of no less importance than for other genetic resources” [78].

At present, Russian soil scientists are engaged in compiling the Red Book of Soils, which will contribute to the protection of soils as the source and reserve of

biodiversity on the Earth. The long-term cryopreservation of soil samples containing diverse, including ancient, microorganisms makes sense, since there is evidence that bacteria may retain their viability in permafrost for several million years and can be revived by conventional techniques [79].

Thus, the protection of particular ecosystems (and the biosphere as a whole) calls for the improvement and integration of different methods for the assessment of soil microbial diversity and the collaboration of scientists working in different research areas.

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