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MICROBIAL COMMUNITY OF SOIL: PHYSIOLOGICAL DIVERSITY PATTERNS AND ASSESSMENT

(review)

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Abstract

Study of the taxonomic and functional diversity of soil microorganisms association is of great theoretical significance for understanding the structure of the soil microbial community, the nature of the interaction of individual species of microorganisms belonging to this community, as well as their participation in the processes of soil formation and circulation of substances. This article summarises a brief history of ideas about the functioning of soil microbial complex, providing transformation and mineralization of organic matter in soil formation processes. Soil as habitat for microorganisms is heterogeneous that defines microzonal character of the distribution and activity of microorganisms that live in it. The structure of the association of microorganisms and its physiological profiles vary in time and space (D.G. Zvyagintsev, 1987). This defines methodological difficulties and the significant variability of the results of the evaluation of soil microflora by various authors. This review discusses the methodological approaches in determining the physiological diversity of soil microorganisms association. Traditional methods of elective culture media in over a century allowed to reveal numerous physiological groups of microorganisms and developed an idea about their role in the cycle of matter, processes of soil formation and plant nutrition. However, such work is almost not given anything new in principle, both in environmental studies, as well as in agronomy over the past 20 years. At the end of the 1990s a method of analysis of the carbon source utilization profiles (SUP) of natural microorganisms association by BIOLOG system used previously only in medical and general microbiology was proposed to study the physiological diversity for the test strain (J.L. Garland, A.L. Mills, 1991). This approach was further developed by H. Insam (1997), M.V. Gorlenko and P.A. Kozhevnikov (2005) and others. A number of modifications of this method (Eso-Plates, ECOLOGY and others) characterized by a set of organic substrates, which, as the authors suggest, is most likely present in natural environments were worked out. Instrumentation of ECOLOG (M.V. Gorlenko, P.A. Kozhevnikov, 2005) allows to determine not only the range of organic substrates used by microorganisms, but also to quantify the consumption of each substrate. For the processing and interpretation of a significant amount of information obtained in the course of the analysis of soil samples there are an apparatus of multidimensional mathematical statistics, cluster analysis, rank distribution, and ecological indexes of Shannon and Pielou. SUP method (multisubstrate test) possesses a high performance, good resolution (10^4), a satisfactory reproducibility and is a high-tech and effective tool to assess the physiological diversity. The article deals with the positive and negative aspects of the method. SUP reflects to some extent the potential of aerobic soil microorganisms using low molecular weight organic compounds in the catabolism. However, because of availability of several modifications, as well as some technical problem it is difficult to compare the results obtained by different authors, there is no unified SUP analysis protocol that is required for the comparative environmental studies and the establishment of relevant national and international databases. Thus, analysis of the carbon source utilization profiles (SUP) in BIOLOG system now is under development and testing, and with the accumulation of experimental data and critical analysis, it has good prospects in soil ecology, in research of the relationship between microorganisms and plants, and in assessment of the impact of anthropogenic factors.

Keywords: association, physiological groups, physiological diversity of bacteria, range of consumed substrates, multisubstrate test, system BIOLOG, ECOLOG, EcoPlates.

The founders of soil science V.V. Dokuchaev and P.A. Kostychev were the first to draw attention to the soil as a living system with bacteria as one of the key

factors of its functioning. The soil, as habitat of microorganisms and the product of their activity, is a complex system which includes species various in their physiology that provide the biological circulation of elements, the soil formation and resistance to natural and anthropogenic factors. This determines the theoretical and practical importance of the ecological studies of microbial communities. The key factors of microbial ecology are taxonomic and functional diversity of the microbial community and the nature of interaction of individual microorganism species belonging to this community which provides soil formation and plant mineral nutrition.

This article discusses the models of the soil microbial community functioning and the conditions of microbial activity in the soil. Methodical approaches to the study of soil physiological diversity in microorganism association, and the positive and negative aspects of the methods proposed are considered.

An ecological approach to studying soil microflora, its species and functional diversity is associated with the classical research of S.N. Vinogradsky [1]. Based on own studies and the accumulated experience, he comes to the conclusion that the vital functions of soil microflora are based on the principle of «division of labor, which is expressed <...> in the interoperability of the team members». S.N. Vinogradsky writes: «Microbiological processes in the soil are composed of numerous phases which replace one another, each of the phases being associated with a single pathogen or a small group of pathogens» [1]. Accordingly, he allocated two major consecutive phases of organic matter decomposition in the soil and two large groups (associations) of microbes that differ in their functional roles in these processes. In the first phase, plant and animal residues are decomposed by zymogen microflora which enters the soil with plant and animal residues, while humus is decomposed by soil autochthonous microflora in the second phase [1]. However, he believed that the autochthonous microflora is represented by a specific group of microorganisms using humus as the source of energy nutrition. In his research, S.N. Vinogradsky does not consider the humus formation. But a priori, we can assume that it is derived in the process of plant residue decomposition by zymogen microflora.

Developing S.N. Vinogradsky's concept, N.M. Lazarev [2] considered three large functional groups of microorganisms. The first of them is the actual zymogen microflora which digests protein and monomers that enter the soil with plant residues. The second one is autochthonous microflora A involved in decomposition of a variety of plant biopolymers (with a wide range of the carbon/nitrogen ratio) the transformation of which is followed by the formation of so called α -humates that are rich in nitrogen. As they accumulate in the soil, the activity of the above group fades gradually due to the substrate exhaustion and the accumulation of humates that are the products of these microorganisms' activity and are toxic to them, that is a kind of catabolic repression. Then, a phase of α -humate decomposition by autochthonous microflora B starts. According to N.M. Lazarev, the qualitative composition of organic matter changes in the transformation process, like, accordingly, the structure of the microbial community. In modern understanding, there is a succession of microflora caused by the changes in the sources of nutrition and the physical and chemical parameters of microbial habitat. According to N.M. Lazarev, the surface of minerals, organic matter and microorganisms form a complex which he called a bioorganic mineral complex. Unfortunately, this concept has not been developed further.

Accepting S.N. Vinogradsky's concept [3] in general, E.N. Mishustin introduced two more groups of oligotrophic and chemoautotrophic microorgan-

isms into the system.

S.N. Vinogradsky's ideas were further developed by T.V. Aristovskaya [4] who, basing on the principle of «labor specialization and division» in microbial associations, proposed a concept of elementary soil and biological processes (ESBPs) that include biological transformation of plant residues, humus formation and decomposition, destruction of parent rock minerals, mineralization, hardpan formation and soil salinization, the first five ESBPs of the seven ones listed required for all types of soil formation and reflecting the essence of this phenomenon, in her opinion [4]. Gley, hardpan and bauxite formation, salinization and possibly other transformations involving microorganisms can only characterize the formation of certain specific soil types [4].

G.A. Zavarzin allocates two functional groups as the main differential characteristic of functioning soil microflora, i.e. the microorganisms capable of producing hydrolytic exoenzymes which gives them an advantage in the early stages of biopolymer decomposition and the so-called scattering microflora which utilize the monomers that are the products of biopolymer decomposition [5]. Functionally, these two groups are interconnected in the system acting successively.

Developing G.A. Zavarzin's opinions, V.S. Guzev and P.I. Ivanov [6] proposed a scheme of activity of the zymogen part of the microbial system in the soil where the process of decomposition of biopolymers entering the soil in the form of plant residues is initiated by hydrolytic microorganisms which degrade biopolymers to monomers due to the release of hydrolytic exoenzymes. Being accumulated in the soil, the latter cause the repression of exohydrolase synthesis on the feedback principle, which provides the transition of hydrolytic microorganisms into a resting state. The monomers produced are utilized by another group of copiotrophic organisms, the intensive development of which results in a sharp decrease in monomer content. As a result of the soil monomer pool depletion, copiotrophic organisms go into an inactive state, after which an active phase of oligotrophic organisms starts due to their ability to utilize extremely low amounts of monomers. At the same time, catabolic repression of hydrolase synthesis is removed, and the biopolymer degradation cycle repeats. Thus, the activity of each of the above groups of microorganisms is of pulsating nature. Apparently, the scheme of soil microbial community functioning proposed by these authors can be used for both the zymogen and autochthonous microflora in the same way, since in either case the organic matter consists mostly of biopolymers that are a source of energy and carbon nutrition for the microorganisms.

Thus, steady functioning of soil microbial systems takes place in natural ecosystems with a constant influx of plant residues, including a variety of biopolymers. Both N.M. Lazarev and T.V. Aristovskaya considered the complex chemical composition of plant residues that enter the soil. They noted that transformation of organic matter and minerals by a microbial association includes various sequential and simultaneous biochemical reactions caused by the trophic and other relations in a self-adjusting system. In our opinion, the ESBP concept reflects the essence of microbiological processes of soil formation most fully. T.V. Aristovskaya preferred to consider the taxonomic composition of the microflora involved in organic matter and mineral transformation without going into the details of biochemical substrate processing reactions. N.M. Lazarev and his school were focused on the study of physiological groups of microorganisms, suggesting that the latter may be the indicators of a particular stage in the transformation process.

All the above models suggest that microbial processes in the soil are the result of collective activities of microorganism associations. Performance of indi-

vidual species in the association and their physiological activities are substantially different from those observed in pure culture grown on artificial medium. The formation of these associations and their activities in the soil is greatly influenced by the mineralogical composition, physical and chemical properties and the structure of the soil, as well as by the status and nature of the distribution of organic matter in it.

Soil structure and distribution of microorganisms. Soil, as the microorganism product and habitat, is heterogeneous in its structure and includes particles, micro and macro aggregates which are penetrated by capillaries, have pores and voids of different sizes filled with soil solution, organo-mineral gel and gases composed mainly of nitrogen, oxygen and carbon dioxide [7]. The degree of filling may be different and varies depending on humidity, temperature, specific weather conditions, intensity of biochemical processes in the soil, etc.

Activity of microorganisms is associated with soil aggregates [8-10]. They are distributed in the form of films [11, 12] and microcolonies [13-15] on the surfaces of minerals and organic matter particles which enter the soil with plant residues, in water film on the walls of the pores and capillaries, as single (rarely numerous) cells in the soil solution that fills the pore space of soil aggregates [8]. Typically, formation of colonies and films takes years, sometimes those are formed by a number of microbial species [9, 15].

The interaction of microorganisms with the surfaces of soil aggregates is of a complex nature dependent on the mineralogical composition, the nature of the organic material, the pore space dimensions [9, 10]. Biochemical activity of microorganisms is higher in large pores and is virtually absent in small capillaries commensurate in sizes with the microorganisms [9]. According to D.G. Zvyagintsev [9], bacteria develop better in the films with a thickness over 10 μm . The activity of bacteria adsorbed (immobilized) on the surfaces of soil particles and immersed in organo-mineral gel is generally lower than in the solution [9, 15].

The sizes of the bacterial cells and the fungal mycelium thickness in the soil were noted to be lower compared to the artificial culture of microorganisms grown on nutrient media [16].

Organic matter has various composition and is unevenly distributed at the different stages of plant residue transformation. Accordingly, the topography of microorganism distribution in the soil is characterized by micro zonation and is of mosaic nature both taxonomically and functionally [2, 10, 17, 18]. Therefore, in such a complex heterogeneous environment as the soil, various processes (i.e., decomposition of plant residue organic matter, humus formation and its decomposition, and destruction and formation of minerals) take place simultaneously at different phases of transformation being spatially divided both in the soil profile and horizontally.

Environmentally, the microflora of soil macroaggregates is something like synusia consisting of various microorganism species populations formed as a result of competition for food sources in the specific physical and chemical conditions of soil aggregates. In them, the activity of microorganisms is apparently based on the type of consortium, and the relationship between the populations of individual species are based on cooperation and are manifested in different ways from symbiosis to syntrophy [19], which determines the stability of the system under particular conditions. Changing these conditions leads to competition, restructuring of the association in accordance with the change of physical and chemical soil parameters. We must assume that the taxonomic and functional diversity of microorganisms is subject to significant changes as a result of temporal and spatial variability of physical and chemical properties of soil aggregates.

Among the external factors responsible for these changes, the leading role is played by precipitation, periodic soil drying and the temperature. In waterlogged soil, the pore space of soil aggregates is filled with water, oxygen content is reduced, anaerobic processes develop, and a reorganization of the microbial soil complex takes place [20]. There reduction processes develop that contribute to the formation of ferrous and manganese oxides, hydrogen sulfide, methane, organic acids and toxic compounds [4, 7, 20]. Conversely, soil drying results in aggregate shrinkage, pore space narrowing [7], involving microorganisms in the dehydrated organo-mineral gel matrix, increasing the osmotic pressure of the soil solution concentrated primarily in the capillaries and films [9]. This reduces the biochemical activity of microorganisms and is the cause of their partial death and the restructuring of the microocenosis as a whole.

The main internal factor of the change in taxonomic and functional diversity of the microflora of soil aggregates is the very activity of microorganisms which causes qualitative changes of organic matter and results in the accumulation of metabolic products leading to the restructuring of the microbial complex [2, 9]. Accordingly, its taxonomic composition and physiological diversity change.

Thus, the soil microflora is a self-adjusting system that is formed at the level of soil aggregates and microzones, including mainly plant residues, humus complexes and mineral surfaces. Taxonomic and functional diversity of soil microflora is highly variable and depend on the freaks of nature, the internal regularities of decomposition processes, the synthesis of organic matter, and the physical and chemical properties of the soil.

Physiological groups of soil microflora. Almost all studies of taxonomic and functional diversity of soil microorganisms are based on the analysis of average soil samples and give us some integrated idea of the qualitative composition of microflora for the sufficiently large soil massif. One should clearly understand that these data reflect the dominant processes and dominant microflora at the date of sampling under certain weather conditions and a certain state of vegetation. The studies of soil samples from the same field areas would provide different results under different conditions which can erase the previously made conclusions.

The study of the functional and taxonomic diversity of soil microflora has been performed since the beginning of the XX century quite intensively. Significant progress has been made after the onset of solid culture media proposed by Robert Koch and especially after the introduction of agar-agar in the practice of research [21]. A great amount of bacteria, actinomycetes, filamentous fungi, yeasts, microscopic algae, and protists have been isolated from the soil; these make up the basis of microorganisms represented in the collections of various scientific centers worldwide. An inestimable role in the study of soil microorganisms is played by elective nutrient media and enrichment cultures, the principles for the use of which have been proposed by S.N. Vinogradsky [1] and M. Beijerinck, [21], respectively. A rapid growth in the studies using molecular genetic techniques has been observed in the two last decades. As a result, the number of microorganism species found in the soil has increased more than 10-fold. A significant part of these relates to the so-called uncultivated forms [22].

The use of elective media made it possible to isolate various physiological groups of microorganisms involved in the small biological cycle of substances which reflects the general regularities of these processes in the soil. Physiological groups are the sets of microorganisms that perform the same function in the chain of substance transformation in the soil [23]. However, it is well known that microorganisms are multifunctional systems. Therefore, under the changing

conditions, they can be rearranged, perform another (sometimes opposite) function or be assigned to another physiological group. For example, at deficiency of mineral nitrogen compounds, the so-called denitrifying bacteria are capable of fixing molecular nitrogen [24], and in the presence of protein and amino acids in the medium, they perform the functions of ammonifying [25].

Hence, it is clear that the taxonomic composition of the microorganisms that represent a particular physiological group is unstable and varies depending on the specific conditions in the soil. Therefore, the physiological groups identified for certain elective media do not necessarily exercise the same processes in the soil.

According to D.G. Zvyagintsev [9], the stability of certain physiological and biochemical functions of microorganism associations in the changing physical and chemical parameters of the soil is due to the presence of several overlapping microorganism species, on the one hand, and to their adaptation to the new conditions, on the other hand.

From the perspective of soil science and practical agriculture, physiological groups of microorganisms capable of being functional indicators of biochemical processes in the soil that are important for its fertility and, accordingly, crop productivity, were of interest. The most popular of them and the ones widely used as the objects of research in soil microbiology are summarized in the Table.

Main physiological groups of soil microorganisms and their roles in soil biochemical processes

Physiological group	Biochemical process
Ammonifying bacteria	Decomposition of organic nitrogen compounds to ammonia
Nitrifying bacteria	Oxidation of ammonium nitrogen to nitrites and nitrates
Denitrifying bacteria	Reduction of nitrates and nitrites to nitrogen dioxide and molecular nitrogen
Nitrogen fixing bacteria	Fixation of molecular nitrogen from the atmosphere
Cellulose decomposing microorganisms	Decomposition of cellulose and hemicellulose
Pectin decomposing microorganisms	Decomposition of pectin
Amylolytic microorganisms	Starch hydrolysis
Humus decomposing microorganism	Depolymerization and mineralization of humic acids
Sulfur bacteria	Oxidation of reduced sulfur compounds to sulfuric acid
Sulfate reducing bacteria	Recovery of sulfates to sulfur and hydrogen sulfide
Iron bacteria	Oxidation of iron and manganese oxides
Phosphate solvent microorganisms	Solubilization of poorly soluble calcium, iron, and aluminum phosphates
Microorganisms capable of decomposing organic phosphorus compounds	Mineralization of phosphorus containing organic compounds

These physiological groups often used in microbiological studies include the microorganisms involved in the processes of nitrogen cycle such as ammonifying, nitrifying, denitrifying, and nitrogen fixing bacteria. The key role in carbon cycle is played by a physiological group of cellulose decomposing microorganisms, since cellulose and hemicellulose make up to 70 % of carbon entering the soil with plant residues.

To account for the number and allocate the corresponding physiological group of microorganisms special elective nutrient media are used; these media are described in the respective manuals, workshops and original papers [26-28].

The main results of nearly a century of studies of physiological groups of microorganisms have identified a significant part of organotrophic and chemoautotrophic prokaryotes involved in carbon, nitrogen, phosphorus, sulfur, iron, manganese, and other cycles [4, 23]. Various soils have been shown to have their own profiles of the abundance and composition of physiological groups of microorganisms, the boundaries of which are however blurred and can not be used to classify the soil type. The qualitative composition and the abundance of physiological groups of microorganisms of the same soil were found to depend

on the season, vegetation and weather conditions, and vary for the soil profiles. The impact of agricultural activities on the abundance of different physiological groups of microorganisms depends on the soil type, the composition of agrochemicals, applied agricultural technologies, and tillage intensity [29-32].

Previously, the presence of a particular physiological group of microorganisms and their abundance were suggested, with some reservations, to reflect the intensity of the respective processes of substance conversion and the soil fertility [33]. Indeed, this regularity can be seen in general. However, numerous experiments have demonstrated high variability of the results of these observations. The results of analyses are interpreted differently. There are no clear criteria for soil quality assessment based on the analysis of physiological groups of microorganisms. All these led to the lack of reliability of such data as the indicators of soil fertility and land treatment efficiency. Therefore, these parameters have not been used practically in farming [21, 33].

The main disadvantage of the method lies in the fact that the activity of microorganisms, cultured on artificial culture media does not correspond to their behavior in a complex physical and chemical environment of the soil when interacting with other organisms [9]. In other words, considering the abundance of a particular physiological group of microorganisms, we can discuss some of the potential microflora possibilities in the best case, which do not necessarily appear in the soil at the time of sampling.

The risk is that that further studies of this kind result in the accumulation of contradictory and poor (or even falsely) interpreted data with our limited knowledge about the physiology of microbial associations under specific soil conditions (unlike the physiology of individual species and strains of microorganisms cultivated on nutrient media). The way out is to follow the principle proposed by S.N. Vinogradsky, that is to study microflora and microorganism activity in the natural environment, i.e. in the soil. According to S.N. Vinogradsky, «pure cultures acquainted the researchers with general microbial physiology, but a study like this can only lead to analogies and hypotheses to be verified by direct experiments under the conditions as close to natural as possible» [1]. Elective culture media and identification of physiological groups of microorganisms remain an important tool for detecting microorganisms with specific physiological features for biotechnological application.

Carbon source utilization profiles. In 1991, J.L. Garland and A.L. Mills [34] proposed the BIOLOG system which is widely used in medical microbiology and general microbiology for the identification of bacteria, to assess the functional diversity and developed the general approaches to the interpretation of carbon source utilization profile (SUP) using multivariate statistics [35].

The method uses standard 96-well plates to determine SUPs. Low molecular weight carbonaceous substrates and tetrazolium salts are added in each well as the indicators of source utilization. With the growth of microorganisms in the wells filled with a nutrient solution, colorless tetrazolium salts are reduced to formazan which colors the solution burgundy. Substrate utilization rates are assessed by the intensity of medium color in the wells using an optical reader. Application of the BIOLOG system provided extensive data, 80 % of which relate to soil sample SUP analysis [36]. The impact of mechanical processing, crop residues [37], and heavy metals on soil SUPs have been noted [38]. The efficacy of this method for the characterization of potential carbon source utilization by microbial communities in composts [39], industrial wastewater [40] and other natural environments, and in plant rhizosphere [37, 41-43] has been demonstrated.

Modified methods with more rationally selected sources of carbon nutri-

tion corresponding potentially to those of microorganisms from various natural environments were proposed. In an analytical review, J. Preston-Mafham et al. [36] noted a number of modifications which differ mainly in the set of test substrates. H. Insam [44] proposed the plates which contain 31 substrates to study physiological diversity of a microorganism association (SUP) in the soil and the plant rhizosphere. On this basis, BIOLOG, Inc. (USA) launched the EcoPlates™ plates [45] with a standard set of carbohydrates, amides, amino acids, carboxylic acids, nucleosides and some polymers.

A total of 47 substrates are used in the ECOLOG multisubstrate testing system developed at the Department of Soil Biology of the M.V. Lomonosov Moscow State University [46]. We believe that their composition in the ECOLOG system is mostly reasonable and acceptable to the non-formalized interpretation of the results from an ecological point of view. It contains no exotic chemical compounds. In both cases, the modifications of substrate sets to study the physiological diversity of various natural environments are allowed. It should be noted that the ECOLOG system makes it possible not only to determine carbon source utilization profiles, but also to quantify the utilization individually. Thus, it compares favorably to other BIOLOG modifications. A multidimensional mathematical statistics apparatus, neural network algorithms, the ecological diversity Shannon index, and rank distribution are proposed for processing and interpretation of a considerable amount of information obtained in the analysis of soil samples [46]. The method for multisubstrate testing (MST), or substrate utilization profile (SUP) testing, turned out to be a good tool for a comparative description of the physiological diversity of soil microbial communities and plant rhizosphere, as well as for the assessing the impact of natural and anthropogenic factors on the association of soil microorganisms [46-50].

A number of disadvantages should be noted which, according to some authors [36, 46, 51], limit the scope of SUP based methods and appears to require further development. Thus, the conditions in the wells on artificial liquid culture medium do not meet the conditions of bacterial growth and physiological and biochemical functions in situ [36, 51], that is why the contributions of individual physiological groups in SUPs does not necessarily reflect their relative proportions in the total source utilization profile typical for populations in the soil [52].

Low molecular weight substrates and short-term (2-5 days) incubation of the plates inoculated with soil suspension reflects the growth of R-strategists in an individual well in the given experimental conditions, and not the fact that the activities of these bacteria in the soil is the same under different physical and chemical parameters and in association with other microorganisms. In addition, it is necessary to consider that not a single bacterial species but a number of them may enter the wells with nutrient solution, resulting in a possible manifestation of their synergism or antagonism which would undoubtedly have an impact on the intensity of tetrazolium salt transformation to formazan. One should consider that not all microorganisms are capable of reducing tetrazolium salts [36]. At the same time, K-strategists that provide biopolymer transformation (cellulose, hemicellulose, pectin, lignin, and humic complexes) drop out of sight. There are technical questions for the sample preparation and analysis protocol with regard to timing and methods for soil sampling, methods of preparing soil samples for analysis, nutrient medium composition, buffer solution pH, the temperature and time of plate incubation with the wells inoculated with soil suspension or supernatant, profiles of the substrates used in an experiment, etc., which is to be unified in the case of monitoring studies.

To sum up, we would like to note that the study of taxonomic and func-

tional diversity of soil microbial association is of extremely great theoretical significance for the understanding of the soil microbial community structure, the nature of its constituent species interactions, their involvement in soil formation, soil fertility formation and the circulation of elements. This explains the role of this approach in ecological research and farming practice, especially in the development of effective methods of maintaining and increasing soil fertility.

Analysis of data on the variety of physiological groups of microorganisms in the soil obtained by microbiologists within nearly a hundred years, has shown that the methods of limiting dilution and elective nutrient media used to identify and determine the number of bacteria of different physiological groups are low informative to characterize their physiological diversity in soil. No more than 5-6 of physiological groups of microorganisms were considered in published studies in accordance with the objectives of the studies [30]. The nitrogen cycle (ammonifying, nitrifying, denitrifying, nitrogen-fixing bacteria) and cellulose decomposing bacteria which were considered as the indicators of the relevant processes in the soil were the most common objects of observations. Thus obtained information is used for the analysis of fertility and efficiency of land treatment. However, these methods have not been adequately appreciated in practical farming [21]. Nevertheless, the fact is that the high titers of nitrifying and cellulose decomposing bacteria are typical for active soil organic matter mineralization processes and, consequently, for the favorable regime of plant mineral nutrition. Apparently, the methods for the analysis of physiological groups of microorganisms on elective nutrient media have been exhausted for a comprehensive environmental assessment of the biodiversity of soil microbial associations, however, remaining a good tool to study the role of microorganisms in soil formation and plant nutrition, as well as identification of new physiological groups of microorganisms.

A breakthrough in the research of physiological diversity of soil microorganism associations is related to the methods based on carbon source utilization profiles (SUPs) analysis used with various modifications of BIOLOG [36, 44, 45] and multisubstrate test ECOLOG systems [46] with the appropriate software. J. Preston-Mafham et al. have described the above advantages and disadvantages of these systems critically and in detail [36]. Currently, over 1500 papers have been published which demonstrate the BIOLOG system capabilities to analyze the physiological diversity of the soil, the plant rhizosphere and other natural substrates [46].

The ecological meaning of these data is clear: any (taxonomic or functional) diversity provides a comparative description of the studied ecosystems, so SUP parameters can be used as the indicators of their relevant changes. However, the biological sense is far from being understood, primarily due to the fact that the *in vitro* conditions of bacterial growth and metabolism are different from those *in situ* (in plant rhizosphere or in the soil). As a result, we estimate some physiological potential of soil microorganisms present, but not its implementation in the natural conditions. Therefore, the SUP methods in their present form are, apparently, sufficiently useful and informative as indicators of human impact on the microflora and of physiological diversity in plant rhizosphere microflora. However, according to J. Preston-Mafham et al. [36], they are not applicable for environmental monitoring due to the use of several modifications differing considerably in the set of substrates, and to the lack of a unified analysis and result assessment protocol, which makes them almost disparate. In particular, fresh [53], dried [46], and frozen [54] soil is sampled; according to some protocols, soil suspension is prepared at mechanical shaking [53]; according to other protocols, it is pre-sonicated, centrifuged, and the supernatant is used for

culturing [46]. According to some authors, there are much less species in the liquid fraction compared to the sediment or soil suspension [36]. In frozen soil, the number of bacteria, fungi and actinomycetes reduces considerably, and their ratio changes [55].

Thus, traditional study methods for the functional state of soil microflora revealed the forms involved in the key processes of carbon, nitrogen, phosphorus and other element cycles. Some physiological groups are still used as the indicators of land treatment efficiency and soil fertility, but this area is almost not developing in the latest 20 years. Carbon source utilization profile (SUP) analysis (modified BIOLOG, EcoPlates, and ECOLOG systems) is currently applied to study a more or less limited number of low molecular weight organic compounds and reveals the physiological groups of microorganisms capable of being involved in their catabolism. In other words, SUP analysis reflects to some extent the potential of physiological diversity of organo-heterotrophic aerobic and facultative aerobic soil bacteria that utilize basic carbon chemical compounds. SUPs are applied most efficiently to the study the effects of natural and anthropogenic factors on soil microflora. We believe that multisubstrate testing has good prospects for the study of rhizosphere, especially when combined with the analysis of root exudates, which is a key to understand the mechanism of the formation of rhizosphere bacterial association and its interaction with the plant.

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