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REVIEW

Winogradsky and Modern Microbiology

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Abstract—The 150th anniversary of the birth of Sergei N. Winogradsky is a special date for general microbiology. Apart from the discovery of chemosynthesis, the development of the elective method, the application of the direct method, and formulation of microbial ecology problems, Winogradsky, in his pioneering studies, addressed fundamental aspects of the naturalistic Weltanschauung from the position of a microbiologist who studies the role of microorganisms in nature. These problems are still important today. Expanding knowledge throws light on them from different angles of view and highlights them with varying degrees of completeness, but the problems remain beyond time. It may be useful for contemporary microbiologists to realize the extent to which they would recognize their own work in the system of questions and answers stated a hundred years ago.

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Discoverers play a special role in the understanding of problems. They formulate the questions that need to be answered. For instance, few epistemological works do not mention Aristotle and his concept of phenomenon understanding. The special position of discoverers consists in the fact that they find themselves face-to-face with the problem itself rather than with somebody's opinion. They have to think it over from the beginning and delineate the choice of the tasks, since the problem is bound to be multisided and associated with many other problems.

Epistemology assumes the application of settled principles and so-called "rigid constructs," presumptions inside the consciousness, that is, of established and generally accepted views. When posing a new problem, the investigator has to develop a new logical system, using established principles of related sciences and avoiding conventionalism in the investigator's own area of research.

Winogradsky started his career as a scientist when medical microbiology was already a well-established science, and bacteria had become objects of microscopical investigations. At the end of the 19th century, Robert Koch's microbiological school imposed strong demands on researchers by postulating the necessity of the logical triad for proving the biological nature of a process: demonstration of contagiosity, obtaining of pure cultures by isolation of single colonies on solid media, and reproduction of the process using the pure cultures obtained. Winogradsky's approach to the study of microbial activity under natural conditions originated from his skepticism about the possibility of existence of an efficient universal method, because interactions between organisms in nature are quite complex. His skepticism was particularly strong regarding the application of universal agarized meat–peptone or glucose–peptone media used by medical and sanitary microbiologists. His skepticism was based on his concept of the specificity of microbial functions and, in this connection, on the necessity of applying different and, of course, nonroutine, approaches to different tasks. Nonetheless, in his work, Winogradsky was forced to combine direct measurements of microbial activity either in situ or using a laboratory model and the analytical approach, which resulted in the isolation of pure cultures. In a broad sense, the problem lay in a compromise between inductive and deductive methods. The search for this compromise is permanent in science and is as important nowadays as it was in the 19th century.

Winogradsky was a pioneer in the branch of microbiology concerned with the investigation of microorganisms in situ. He had to consider the problem of microbial activity in nature from the starting position, simplifying it until its components were simple enough and operationally acceptable and could serve a basis for either descriptive or experimental investigations. Here we are referring to the system of the initial postulates that precede the research. Winogradsky was not inclined to solve universal mysteries as a publicist, being for the most part an experimenter. His Weltanschauung as a naturalist would have remained unnoticed, if it were not for his lecture "The Cycle of Life" delivered at the Imperial Institute of Experimental Medicine in 1896 and the retrospective "prefaces" that he wrote in 1945 to his selected papers collected in *Soil Microbiology*, which he himself viewed as a review of microbial ecology, in the sense that the word "ecology" had in the 1940s. At present, we can formulate the root of the problem as the understanding of the functional role of specialized bacteria in the Cycle of Life. Today, instead of the expression "the Cycle of Life," we use the term "biogeochemical cycles in the biosphere," introduced by V.I. Vernadsky, who studied with Winogradsky at St. Petersburg State University, 30 years after the problem had been posed by Winogradsky. The meaning is the same, but, as a botanist, Winogradsky intended only qualitative analysis of the catalysts that influence the natural processes, while Vernadsky managed to perform a quantitative analysis of the cycles, with emphasis on the geological understanding of biological processes. To evaluate the role of Winogradsky in natural history, it will suffice to perform a mental experiment, leaving chemosynthesis out of natural processes. In such a situation, active catalysts of the cycles of nitrogen, sulfur, and iron, studied by Winogradsky, as well as those of the cycles of hydrogen and methane, discovered using his method and on the basis of his concepts, disappear. Without chemosynthesis, the picture of natural interactions based on chemical processes and their biological agents, goes to pieces. It is beyond doubt that, if it were not Winogradsky, these ideas would have been expressed by other scientists, most probably by the members of the Delft school of microbiology established by Martinus W. Beijerinck; however, it was Winogradsky who actually introduced them.

THE FUNCTIONAL ROLE OF MICROORGANISMS IN NATURE

Modern general microbiology, which considers investigation of nature as its major objective, has adopted the method of elective cultures as its main method. This method was devised by Winogradsky in his studies of nitrifiers and described in the paper devoted to the discovery of free-living nitrogen-fixing bacteria, published in 1895. The method is based on the concept of functional specialization of lower organisms.

> The culture will be elective if it is favorable for the detection of the sole function … By supporting the microbe in question, engaged in the life contest with other microbes, we will have a significant prevalence of this microbe in our cultures … It is obvious that this approach is diametrically opposite to the widespread approach based on the use of substrate (meat–peptone gelatin) that has been considered as universal. Thus, in order to apply the elective culture method, it is essential
>
> (1) to determine all specific properties and conditions of the culture;
>
> (2) to study the microbe morphology so as to keep it in view until it has been isolated in pure culture, which is sometimes difficult to achieve.
>
> The first requirement, a good choice of culture conditions, presents few difficulties; the second one requires some experience and acquaintance with bacterial forms [1, p. 344].

This brief passage includes the whole philosophy of the microbial world cognition. It begins with "the concept of functional specialization." The biological or nonbiological nature of each geochemical process must be determined. If it is biological, the question arises as to the specific nature of its agent. Winogradsky says that the good choice of culture conditions presents no special problems. Actually, it requires the formulation of a hypothesis about the necessary functions. The sum of particular hypotheses concerning particular reactions gives an overall picture of the processes operating in nature. Such a general hypothesis forms the basis of the area of natural sciences named later "the biosphere concept." So, the work begins with the advancement of a hypothesis that must then be verified experimentally. A hypothesis about the functional (first of all, trophic) ecological niche and its physical parameters (temperature, aeration, etc.) precedes the search for a particular species. In this regard, general microbiology deviates from the biological tradition, according to which investigators go from the species in question to its function (or forget about it, being satisfied with species isolation and comparison). The nature of the approach used in general microbiology is deductive; it demands a general consideration of the problem and the understanding of which particular reaction of the whole system is to be investigated.

Competition is the next key word. The elective method is an experimental realization of Darwin's (or, more exactly, Spencer's) principle of "survival of the fittest"; the main objective of the method is, however, not to reveal how species arise but to search for existing species that remain to be discovered. In the elective culture, the best-reproducing organisms predominate. However, in elective batch enrichments, organisms that do not grow rapidly enough are not discarded, as occurs in a chemostat. Growth is not the sole factor of survival, which is based on the opposition to extinction. In practice, the washout from the culture occurring in chemostat is traditionally replaced by subculturing of serial dilutions to isolate organisms dominating in a particular medium and under particular conditions.

Finally, the last detail. General microbiology, the main tool of which is the method of elective cultures for identifying their specific functions, is opposite, in its natural scientific direction, to medical microbiology based on the use of universal media. Winogradsky never ceased to emphasize this difference. The areas of knowledge overlap when the objective is to reveal general and universal properties of live objects, like general properties of bacterial cells, expressed now by the term "cell biology."

A century of microbiological research was dedicated to the search for specific organisms for each combination of conditions; this work continues to the present day. The whole structure of the knowledge system associated with general microbiology arises from the ideas described above. This is an answer to the question about the significance of Winogradsky for modern microbiology. Modern microbiology, as a part of natural science, originated from Winogradsky's concepts, and, it should be said in justice to Beijerinck, from the concepts of this great microbiologist as well. A search for the only specific organism has become a basic idea of the deductive method of research. Maybe, even too much.

In nature, in addition to specific reactions, there are a number of "biotically-mediated" ones. In this regard, the main processes, e.g., decomposition of organic matter, leads to the establishment of domains of stability to which the status of other components, e.g., trace elements with variable valency, must correspond. They will assume forms corresponding to the thermodynamic conditions resulting from microbiota activity. The concept of "geochemical barriers" formed and supported by the activity of nonspecific organotrophic bacteria proved to be productive as well. For example, the establishment of reducing conditions is associated with oxygen removal during aerobic respiration. This sum of nonspecific reactions is the concern of geochemists rather than microbiologists, although the active agent here is the microbial community.

The pathos of the following idea of Winogradsky, formulated in the prefaces to *Soil Microbiology* chapters, is directed against overestimation of the role of pure cultures. Under natural conditions, microorganisms live in communities, of which cooperation and competition are characteristic. To study microbial communities, Winogradsky proposed the "direct method" of direct microscopical observations in situ, based on the inductive approach, which begins from observations and only then comes to an experiment. Hypotheses here do not arise a priori; quite the contrary, they emerge a posteriori from observations. Winogradsky applied this method to his first studies of sulfur bacteria; but he developed this idea only in the second period of his scientific career. At that time, the capabilities of cytochemical methods were extremely limited, so the method was used primarily for determination of bacterial numbers. The result was a huge gap of several orders of magnitude between the results of direct count and cultural methods. The perception of this gap is the chief cause of the contemporary crisis in microbiology, formulated as "the problem of nonculturable microorganisms."

Modern molecular methods, in conjunction with cytochemical ones, allow determination of "phylogenetic" and "functional" genes of nonculturable microorganisms. Contrary to genomic studies, in which microbial communities are substituted for by a "metagenome" without consideration of the physical picture of the community (e.g., a biofilm), cytochemical methods allow adjusting the organism's properties with its physical position in the community or on a solid substrate. Due to the use of modern cytochemical methods in microbiology, the homogeneous chemical data are supplemented with a heterogeneous physical pattern at the level of tens of microns. A break into the needed but previously inaccessible world of initial observations occurs. In such a manner, an inductive, generalized, rather than fragmented concept of the microbial world emerges. Is it possible that communities could be substituted by metagenome, the sum total of microbial genomes? Not entirely so.

I believe that the main link between community components is trophic, which thus far can be revealed only by using laboratory cultures, the obtaining of which is a significant stage from which we can return to communities by designing species-specific probes. An understanding of the biodiversity of microorganisms constitutes the fundamental part of general microbiology.

BIODIVERSITY

Winogradsky began his work in 1885 studying the biodiversity of aquatic bacteria and dedicated his last note (1952) to the principles of bacterial classification. He was a first-rate observer; by 1934, the bacterial genera described by him comprised 15% of all the genera included in *Bergey's Manual*. In 1925, Buchanan wrote sarcastically, "To the present time the account given by this author is the only observation of certain of these forms" [2, p. 37]. Some time later, all observations made by Winogradsky as a morphologist were proven to be correct. What significance does morphology have according to the modern views?

Chemists do not deal with form, and the era of physicochemical biology has removed the functional significance of form from the minds of microbiologists. Actually, the form determines the adaptation of an organism to the physical conditions of the environment. This fact is especially evident when we deal with aquatic organisms developing either in plankton or in the surface layer of silt (pelophiles) or in springs (crenophiles). Soil microorganisms, such as streptomycetes, are well-adapted to life in the air. They developed a mycelial way of existence and by this means solved the problem of cell to cell transportation and spreading. In eukaryotes, such as phototrophs with their relatively uniform physiology, or organotrophs, the form has assumed great importance with regard to the biodiversity development and adaptation to the living conditions. Bacteria gain energy mainly by catalyzing energy-yielding chemical reactions; in this connection, the discovery of chemosynthesis indirectly resulted in a cardinal change in our understanding of life.

Winogradsky proceeded from an assumption that the biological characteristics of bacterial species are fixed and do not change during experiments. He got the idea from a university course on lower plants, which he assimilated from Kh.Ya. Goby and later from A. de Bary. The principle of continuity of the subjects of investigations is a necessary condition for taxonomy. The establishment of the limits of variability within the given taxon is complementary to this principle. This variability must be such that one taxon is not linked to another by a continuous series of transitions, and there is a gap between two complexes of characteristics, the so-called “hiatus.” The reader has a good chance of appreciating the consistency between this principle and the principles of microevolution, which form the basis of the theory of the origin of species. For instance, if we consider lactose consumption as a species character, should we, in practical classification, connect the loss or acquisition of this character with speciation? This concrete example has attracted considerable attention. In early microbiology, with its taxonomy based on morphology, the wide variability of single organisms was named “pleomorphism”; this term should be distinguished from the term “polymorphism,” applied to organisms that change their morphology depending on the stages of development, e.g., cytophagas. Winogradsky struggled with the pleomorphism concept and, correspondingly, the concept of permanent bacterial speciation, over his whole life. The importance of microscopic studies of bacterial forms cannot be overestimated when we talk about zoogleal forms, some of which “form real minute thalli” [1, p. 83], which can be observed in natural environments rather than in pure cultures. Needles to say, morphology changes according to conditions, so, in order to identify successive transitions between developmental stages, long-term observations are necessary. During his last years, Winogradsky was engaged in research on *Azotobacter* polymorphism. However, as far back as at the end of the 19th century, the botanist A. Fisher [3] criticized Winogradsky and Beijerinck for their morphophysiological characterization of the genera *Nitrobacter* or *Azotobacter* and the retreat from the purely morphological, botanical, principle, since the form is a universal property of all organisms. It would be interesting to compare this thesis with our present, chemical, concept of nucleic acids as a universal property of all living organisms. Polymorphism makes morphological properties unsteady and dependent on conditions, particularly, on the conditions of in vitro cultivation. This problem remains pressing in the taxonomy of the physiologically homogeneous group of cyanobacteria, which is still based on the morphology, traditional for algology.

Until recently, microorganisms were studied only in pure cultures and classified almost exclusively according to chemical reactions produced by cell metabolism. Nonculturable organisms, despite their wide distribution in nature, were practically ignored and left to hydrobiologists. At present, microbiologists consider morphological characteristics as minor in relation to genetic ones, first of to the properties of the universally present of a protein-synthesizing apparatus and, above all else, the 16S rRNA gene. At the end of the 20th century, according to C. Woese [4], this approach exploaded the old taxonomy due to its universality for all living organisms, which has enabled the construction of a “universal Tree of Life.” The functional properties, determining the way and possibility of the existence of microorganisms, are currently considered of secondary importance as compared to microorganism origin.

In 1952, not long before his death, Winogradsky, according to his daughter Elena (see Waksman in *Short Stories about the Great Bacteriologist*), literally seethed with anger when looking through the edition of *Bergey’s Manual* published in 1948 and the paper by Stanier and van Niel stating that only the system based on phylogenetic data can be considered as “natural.” Winogradsky was in doubt about the possibility of creating Linnaean bacterial taxonomy, since phylogeny (which, by the way, relates to Darwin and his followers rather than to Linnaeus) is based on paleontology. “Unfortunately, such reconstruction is impossible when the matter concerns bacteria, doomed to disappear without leaving any trace.” As a matter of fact, Winogradsky’s appeal to phylogeny is surprising, because he based his Weltanschauung on present-day events and put the evolutionary constructs made in the style of Haeckel’s solution of “The Riddles of the Universe” beyond the limits of the facts available to bacteriologists.

At the beginning of the 21st century, the phylogenetic reconstruction based on the results of molecular analysis looks most reliable for all groups of living organisms and, therefore, Winogradsky’s objection seems to have been overcome. The last edition of *Bergey’s Manual of Systematic Bacteriology* (2005) [5] can be considered the closest approximation to the phylogenetic principle and to “natural” taxonomy. Is phylogenetic taxonomy the only natural system of living organisms?

I am doubtful [6] about the universally accepted principle of phylogenetic taxonomy. I believe that there can be plenty of natural classifications of living organisms by their objective positions in nature, which, in turn, are dictated by the mode of existence in the natural environment rather than by the origin, and there can also be many classification systems dependent on the goal set by the scientist, including classification by the origin of organisms. Whatever classification system is adopted, it is a product of consciousness rather than nature. Thus, “natural” classification is generally impossible. “Natural classification” is a combination of logically incompatible words, designed to substitute value perception for veritable truth in accordance with the Protestant pragmatic philosophy and to give an immanently absent meaning to the adjective "natural." This method is widely used now to inspire consumer's confidence in the value of the product offered.

The classification according to the 16S rRNA gene opened up possibilities for the application of an experimentally justified system that includes phylogenetic classification of taxonomic units by quantitative criteria (species of one genus should show at least 95% similarity, and organisms that show at least 97% similarity are members of one species) and construction of a phylogenetic tree for higher taxa. When a wide range of sequences had been studied and the number of genera had considerably increased, such a great number of higher taxa were to be introduced that it was found impossible to construct the classic phylogenetic tree of all known prokaryotes, and a great many of radiating lineages emerged [4]. Now, when more than a thousand complete prokaryotic genomes have been decoded, we are in an even more difficult situation. There is also another fundamental difficulty: gene mosaicism or combinatorial effects within the genome. We are forced to a priori pay more attention to some gene categories, and thus to return to subjective evaluation of the significance of properties. Botanists argued over the principle of independent evolution of *merons*, i.e., such parts of the plant as flowers and leaves. This principle, if applied to bacteria, would mean that the evolution of the system for protein synthesis is independent of the evolution of the energy-generating system. Alternative approaches are needed for the formalization and systematization of complete genomes. The novel phylogenetic system, used as a basis for the latest edition of *Bergey's Manual*, has already raised doubts.

"It is possible that some microbiologists would be scandalized by any idea to abolish the Linnaean system of classification as applied to bacteria, being accustomed to using it in all cases when classification is needed," wrote Winogradsky in 1952 [7].

In bacterial classification, under the influence of Stanier, the use of pure cultures of type strains of species as standards for comparison was adopted. This principle is valid for many description systems for natural objects, for example, in botany, paleontology, and mineralogy. A reference sample or specimen of the described taxon must be deposited with a museum or collection. Bacteriologists describe organisms according to their physiological properties and cannot resort to the help of reference samples unless they have living and, necessarily, pure cultures. In order to put into effect the principle of type culture (strain), the cumbersome and expensive system of pure culture collections was constructed in the last third of the 20th century. This system is absolutely necessary, since it eliminates chaos and uncertainties in our understanding of the biodiversity of bacteria. However, we must not think that we can gain full and correct understanding of the biodiversity of bacteria in nature by working with type cultures. We can understand the biochemists' and geneticists' longing to work with well-known bacterial strains, since, in that case, they can achieve reproducibility of results and gain insight into some universal regularities. At the same time, it would be very naive to believe that such an approach could satisfy a naturalist interested in understanding natural processes, for which biodiversity is indeed of fundamental importance. The pragmatic American approach to classification by reference specimens, as well as to identification of similarities on the basis of DNA–DNA homology, protein profiles, or another method of finger-printing suggests abandoning the attempts to understand the essence of the objects of knowledge, which are construed as taxa and thus transferred to operative consciousness in the form of nomenclature terms.

"On the whole, however, we cannot register all isolated species as natural ones, identical to their wild prototypes, without any critical evaluation, because it is probable that they are mere *ecological species* whose properties depend on nutrient conditions. In short, they may be *artifacts*" (resulting from the conditions of in vitro cultivation of pure cultures). These are the words of Winogradsky [7], expressing his views on the principles of microbiology. It would be good for readers of the journal *Microbiology* to familiarize themselves with the last article by Winogradsky "Sur la classification des bactéries" ("On the Classification of Bacteria") and appreciate the results of half a century of efforts undertaken to overcome the doubts expressed in that paper.

Accumulation of new facts, based on the immeasurably increased accuracy of identification of bacteria, which can be achieved without cultivation, raised strong doubts about the universality of pure cultures as the tools for understanding the world of microbes. First, it has become evident that the diversity of bacteria in nature differs dramatically from the sum of all pure cultures deposited in culture collections. Furthermore, microorganisms deposited in these collections do not necessarily dominate in nature. The latter fact may be due to the possibility of a systematic error resulting from the application of standard methods for obtaining pure cultures. Any microscopist, being an attentive observer of nature, is able to see the difference between an artificial collection of validated species and the variety of species that can be found in nature. It is assumed that we can isolate all microbes as pure cultures or as syntrophs or obligate parasites in certain combinations. They say that it is a matter of time and toil. Second, a clear understanding of the differences between *ribotype* and *ecotype* has evolved. The genetic characteristics of the ecotype are well-defined even in physiologically stable microorganisms such as *Synechococcus* [8, 9]. At the most general level, the definition of the problem of this sort coincides with Winogradsky's doubts about the understanding of bacterial species. A ribotype is a number consisting of one-and-a-half thousand digits, which is suited to the identification requirements. However, it conveys nothing of the functional nature of the objects if it is not supported by any additional data correlating with this number. Therefore, our hope that codification of bacterial ribotypes would replace the requirement of cultures is fallacious. It is precisely the functional properties of organisms that are pragmatically needed to naturalists and experts; in the ribotype classification, directed towards phylogeny, these properties are quite reasonably considered to be accessory. There are far more reasons to successfully use complete genomes for the identification where sequences in a computer may replace cultures. However, ecotype variability presents a potential threat here. We can characterize a type strain by sequencing its genome; but what difficulties may we face when determining the limits of the variability of species? How many strains' genomes must we sequence to define species boundaries?

Finally, it should be admitted that the methods of molecular biology applied to "unculturable microorganisms," which, in the 21st century, have become the focus of microbiologists' attention, allow us to give a rather complete description of these microorganisms without resorting to pure cultures, as has been demonstrated by the example of the filamentous aquatic organism *Crenothrix*, described by F. Cohn [10] at the dawn of microbiology.

SPECIFICITY

"Microbial functions in nature are governed by competition for energy resources; this biological factor cannot be entirely replaced by chemical factors. The method for investigation of actual processes mediated by microbes in nature should be based on the study of microbial communities as a whole, in nature, rather than on the study of species isolated from nature" [1, p. 789]. With these words, Winogradsky introduces his understanding of the role that microorganisms play in nature. He makes a reservation that, certainly, there are a number of problems that cannot be solved without pure cultures. However, it should be remembered that a laboratory culture is an artifact. For instance, one can grow plants on a nutritious solution with glucose, but plants cannot be considered "facultatively heterotrophic" because of this fact [1, p. 788]. However, microbiologists almost automatically make such a conclusion when describing pure microbial cultures. During the second half of the 20th century, microbiologists expelled microbial communities as the subject of investigations from their minds and laboratories.

Winogradsky himself introduced a new method for the isolation of pure cultures, based on elective media. He described the method and applied it to the isolation of nitrogen-fixing bacteria; that is, he dealt with the competition for a substrate of anabolism rather than an energy source. Winogradsky developed the method when he was isolating nitrifiers. The elective method is closely associated with Darwin's concept of the survival of the fittest organisms and Spencer's interpretation of it, with which, judging from Winogradsky's inadvertent admission in his autobiography, he was familiar. In any case, Winogradsky, like Louis Pasteur, attached no significance to the evolution of microbes, and his struggle against polymorphism was the struggle for the constancy of species. He contrasted his method of elective cultures with Koch's nonselective method for colony isolation on universal solid media. In his *History of Microbiology*, H.-G. Schlegel ranked the housewife's proposition to use agar for preparing solid media for culturing with the major methodological achievements [11], and for good reason. We can say that the overwhelming majority of pure cultures, on which modern bacteriology is based, have been obtained with this kitchen method. It was opposed strongly by Winogradsky, whose arguments resulted from his general views about the role of microorganisms in nature, based on their specificity. In soil microbiology, investigations based on the methods of isolation and counting colonies "were carried out by scientists not familiar with agrochemistry and chiefly concerned with sanitary problems" [1, p. 781]. In our times, Academician E.N. Mishustin and his numerous disciples in soil microbiology were the followers of Koch's method. D.G. Zvyagintsev [12] justified the use of the colony method by indicating that, according to his direct observations, soil microorganisms grow in soil in the form of microcolonies, and, therefore, the principle of growing colonies on solid media is adequate to the growth conditions of microorganisms: in nature, microorganisms occur in pure microcultures.

Here, we ought to address biofilms, which have proved to be a difficult object for traditional chemical analyses due to the homeomorphic differentiation even in pure cultures. In the form of a film and in the form of a suspension (plankton), the cells of *Pseudomonas aeruginosa* differ by the activation of 70% of the genome [13]. I agree with Winogradsky that agar alone is a strong selective factor, and its overuse has led to systematical distortion of our knowledge of microbial diversity. In our investigations, especially with extremophiles, we, as far as possible, have avoided using agar and used it only at the last stage, in order to assure reviewers that the single colony ensures the purity of our cultures (strains). In our practice, colonies of especially specific, slow-growing organisms have too often turned out to be mixed. Unfortunately, scientists' attempts to "be like the others" serve to increase the numbers of publications rather than to move to unknown territories. Winogradsky [14] emphasized that each new technique provides new information about microbes. Routine is disastrous for science.

In a broader sense, this issue is about whether bacteria are social or individual objects. There are plenty of organisms, from reeds to meadows (Swissen-German "Matte"), for which the social way of life is natural. One can certainly grow such an individual organism in a flowerpot, and the evidence that he will obtain will be important but incomplete, like that derived when using any reductionist method. First of all, the obtained information would say little about plant life in nature. Chloroplasts occur universally in all photosynthetics and perform more or less the same functions in different plants; the results of their operation depend on the structure of the plant tissue in which they are incorporated, while the behavior of the plant depends on its ecophysiology. At the next level, the results depend on the natural consortia of plants. Our attempts to content ourselves with simple methods for gaining insight into natural phenomena will be futile in the case of the highest levels of the hierarchy with its own patterns of interactions. However, without understanding its mechanisms, the system turns into a "black box," a kind of computer: I type on the keyboard, knowing the effect of my actions, but not needing to analyze the events occurring inside.

Winogradsky's investigations of sulfur bacteria also produced another result, standing apart from the rest. This was the method that today is referred to as the microcosm method, the Winogradsky column, in which microbial communities develop, being involved in biogeochemical cycles. Schlegel [11, p. 67] referred to the Winogradsky column as the "totem" of general microbiology, and with good reason. The column, based on the interaction between various specific groups of microorganisms, is a model of natural processes.

Winogradsky himself, especially when his expertise made him an indisputable authority, insisted on applying a strictly inductive approach and rejected standard approaches. Having started in 1885 with duplication of A.S. Famitsyn's method for investigation of organism physiology in microcultures, based of the study of accumulation and disappearance of storage materials, he made an *epochenmachende Entdeckung* (an epochmaking discovery, according to one of the first hearers) that demonstrated the possibility of using inorganic matter as an oxidizable substrate. Investigations of this sort especially correspond to the modern phase of investigation of "nonculturable" microorganisms. The possibilities for "cytophysiology" have greatly expanded owing to the molecular methods for identification of genes and their products.

The method of elective cultures, to which Winogradsky was forced to switch because his investigations based on inpure cultures were strongly criticized, initiated a new era of microbiology. The method was based on the vision of function specificity, which Winogradsky later converted into the simplified concept of the uniqueness of functions. In modern terms, the method is based on the concept of the ecological (functional) niche inhabited by a microbial species adapted to life in this niche. Later, in another field of biology, the concept was shaped as *Gauze's competitive exclusion principle.* However, it is obvious that Winogradsky based his method of elective cultures on this concept.

Actually, the method of elective cultures, based on the concept of competition, struggle for existence, and the whole ideology of the end of the 19th century, including social Darwinism, originated from the concept of selection. The elective method is used to achieve selection, when only the fittest can survive. However, preparation of elective media is a deductive process: the selection conditions are specified by investigators on the basis of their hypotheses about the nature of the objects of study. This takes more mental efforts then just "picking up the colony." Winogradsky attached great importance to this stage (which, in the hands of Beijerinck, received the name *enrichment culture*), i.e., to the selection of the minimal community tolerant of repeated subculturing on media enriched with the substance in question and subsequent observations of the dominant forms. Only then could he proceed to the isolation of pure cultures, in order to meet the logical requirements of Koch's triad. Beijerinck's school did not have Winogradsky's idiosyncrasy about agar plates and platinum loops and succeeded in the isolation of many microbial catalysts of specific natural processes. However, the most important discoveries, such as Beijerinck's sulfate reducers and Soengen's methanogens and methanotrophs, were made with the use of elective media.

To what extent is Winogradsky's concept about the extreme specificity of microorganisms, or the uniqueness of the function carrier, confirmed by today's microbiologists? In fact, elective media allow us to isolate microorganisms from a great diversity of ecotopes. The application of these media also promoted Winogradsky's idea that "everything is everywhere and the environment selects." Beijerinck also shared this idea. Later, these ideas, expressed by Winogradsky in 1896, were picked up by other authors [15]. Nevertheless, for Russian microbiologists, these ideas have been fundamental since then. However, in all fairness, we must admit that such ideas were implicitly inherent to the whole "Golden Age" of microbiology.

The gradual retreat from the concept of monopolists occupying specific econiches has taken place in modern microbiology. We may illustrate the transfer from the unique to the multiple by the example of nitrogen fixation. In Winogradsky's time, there was the assumption, upon which he insisted, that *Rhizobium* is involved in symbiotic fixation, *Azotobacter* participates in aerobic fixation, and *Clostridium pasteurianum* is involved in anaerobic fixation. Not without opposition from teachers in microbiology, it was gradually realized that the function of nitrogen fixation is highly widespread. Various organotrophs and some lithotrophs have this ability.

There is the well-known and well-forgotten warning against the application of pure cultures given by Winogradsky: "The application of pure cultures eliminates the most essential ecological factor, I mean competition, since it is precisely this factor that determines the distribution of the processes implemented by microbes in nature, and automatically directs the succession of these processes" [1, p. 783]. In this connection, Winogradsky makes suggestions that would horrify any modern physiologist or biochemist:

(1) to give up using pure cultures everywhere;

(2) to avoid using collection cultures;

(3) to take the cultures in question directly from natural habitats;

(4) to avoid exposing cultures to strong stresses during isolation, as well as isolating on media to which they are not adapted;

(5) to search for an appropriate medium from the very onset of the experiment, in order to cultivate only on this medium [1, p. 783].

For today's scientists, whose aim is to publish their papers in scientific journals with high impact factors, it is obvious that if they followed these recommendations, they would get negative reviews from their "peer reviewers," guided by generally accepted, that is, commonplace, requirements of the audience. We would need extraordinary approaches and exceptional reviewers for such a paper to be published. As for ordinary papers, they must follow "minimal standards" and data that, when identification of new organisms is concerned, are reduced to a questionnaire preferred by Western scientists. One of the postulates of pragmatic philosophy states that the simple accumulation of facts is by itself the development of science. However, the true progress of science is beyond common facts, especially predictable and prearranged ones.

We should not think that Winogradsky denied Koch's logical triad, the requirements of which he brilliantly fulfilled when he described chemosynthesis by the example of nitrifying bacteria. He convinced everybody of the validity of his ideas in his the experiments with sulfur bacteria, where he used other approaches, which met the criteria established by the scientific community.

As Winogradsky commented on his method of "spontaneous cultures" when he studied soil organotrophs, "is not possible to investigate all these heterotrophic forms without isolation of pure cultures. But before isolation, we should discover and study the functions to which they are adapted, by using spontaneous cultures" [1, p. 786]. To tell the truth, I do not quite comprehend the fundamental difference between Winogradsky's "spontaneous culture" and Beijerinck's "enrichment culture." Does the difference mean that, in the former case, a silica-based biofilm is used in contrast to the liquid culture in a flask, as in the latter case? The difference is significant, especially as applied to soils, but not fundamental.

Another aspect is important. In view of the state of public opinion at that time, Winogradsky assigned primary importance to competition ("contest") between species. In nature, however, cooperation plays an equally important role to competition. It appears in a variety of fashions, from symbiosis to syntrophism. A less-studied aspect of traditional microbiology is the role of edificators, which participate in the development of the physical environment in the form of colloid matrices, as occurs in biofilms. The most illustrative examples of this phenomenon are cyanobacterial mats, where interaction occurs between organisms with different complementary functions, methane-tanks, and "marine snow" particles. A community is the natural habitat of species. Winogradsky referred to this postulate, but it never became the central principle of his understanding of microbial ecology. The direct inductive methods necessary for examination of the interactions were not available. Now, these methods are available, and they have led to the invention of a new morphological (physical) approach.

THE ROLE OF MICROBES IN THE WHOLE CYCLE OF LIFE

Our understanding of the world and its laws is based on our pool of knowledge and the subsequent range of interests. However, the fund of knowledge may be profound and narrow or wide. In the latter case, the possibility of sweeping generalizations occurs. The range of interests of microbiologists working at the end of the 20th century was quite different from that of biologists of the end of the 19th century. Nevertheless, the range of interests may change under the influence of changing understanding of the biosphere in the context of global ecology, or new approaches, such as Global Environmental Changes (Global Change). In addition to all these aspects, we must mention our attempt to reconstruct the primordial biosphere on the basis of accumulating data. Is it possible to see the biosphere from the "microbocentric" point of view, and if so, to which extent?

The end of the 20th century was marked by profound concern about the global ecological crisis, produced, above all else, by changes in the carbon cycle. This concern arose from the threat of climate change. Although the association between the atmospheric composition and climate was hypothesized by Winogradsky's contemporary, S. Arrhenius, the scientific community became aware of the problem only towards the end of the 20th century. The quantitative assessment of the cycles has become possible owing to new investigation techniques, as well as to an extensive network of climatological stations and time-series monitoring of global changes. Global ecology has become one of the main problems of natural science. The Gaia hypothesis arose, which states that biota regulates its habitat in the direction favorable for its growth and survival. Some new terms, such as "global physiology," appeared. Microorganisms play the key role in changing the atmospheric composition [16].

The qualitative picture of the cycles of biogenic elements had been created much earlier by Winogradsky, who presented it during his lecture in 1896. This lecture was familiar to Russian scientists, but it was not included in *Soil Microbiology* and remained almost unknown outside the country, unlike *The Biosphere* [17], published in 1926. Meanwhile, the influence of the ideas stated in that lecture can be regarded as very significant. In his lecture, Winogradsky was trying to show the general implications of new facts and "to define their place in the worldview by the application of logic that he (as a naturalist) is accustomed to searching for and finding in natural phenomena". He observed this logic in the purposiveness of microbial activity. The word purposiveness, emphasized by Winogradsky, was important not only at that time, when chance and natural selection reigned in natural science; it is no less significant today. With what purpose do living things conform (if I may use this word in a metaphorical way) with each other, taking on the properties that can secure their existence? What is goal-orientation when applied to the phenomena driven by organisms not endowed with consciousness? The question is still of fundamental importance for the development of the Weltanschauung. As for me, I have answered this question by asking a counter question of whether the existence of the inexpedient is possible.

Having studied the carbon cycle, where the organic matter produced in plants is passed down to animals through consumption, Winogradsky stated as follows: "the great natural process of transformation of organic substances into inorganic ones is the result of microbial activity." "No one, of course, expects the figures. Who would measure billions of poods of the matter which microbes decompose at any time interval? We will limit ourselves to a simple indication to the extreme *qualitative* multiplicity of the task" (p. 9). The multiplicity of the task corresponds to the diversity of specific functions of microorganisms. Microorganisms fulfill these functions as catalysts—a term introduced later by the chemist V.L. Omelyanskii. However, he also used the term "microbial reagents," and Winogradsky applied Pasteur's term *caractére ferment.*

Microbial specificity is shown in an abstract table that contains the list of organic substances; in front of each substance, we place the name of the microbe capable of decomposing this substance and products of decomposition. To every natural substance, there is a corresponding microbial species capable of decomposing this substance—this statement constitutes "Winogradsky's rule," which has become the presumption of general microbiology. This rule was the foundation for the most vivid expression of the division of labor principle in the microbial world: "At all times, each kind of effect that living enzymes exert on the given body depends on and is limited by some close correspondence between the physiological properties of enzymes and the molecular structure of the given body." We are forced to admit that the hundred-year history of general microbiology is filled with gathering of facts that corroborate this principle. From this, it was inferred that "*microbial functions are specialized in nature*; each job is conducted by its own specialist, whose chemistry is adapted to this job." "The combined presence of species, most notably, of functionally similar ones, in the substrate inevitably results in a *struggle for existence*, which, in the world of infinitely small organisms, is more persistent and intense than it is in the world of the large." One can draw such a conclusion "even not seeking support from Darwin's theory" (NB!).

Further, Winogradsky writes about the universality of microbes and their selection by living conditions, the very same "everything is everywhere" and "the environment selects" that form the basis of modern arguments about the biogeography of microbes [9]: "What microbial properties provide for *spontaneity* and *inevitability* of one or another process at every spot on Earth?"

Making his final conclusions, Winogradsky wrote:

> Microbes are the major factors of the cycling of matter, caused by life and necessary for the regular changing of lives; they are the living carriers of countless numbers of reagents; one can even say that they are the incarnate reagents, without which many necessary processes, involved in this cycle, would be impossible; and it is clear that only the main properties of living beings, such as the capacities for reproduction, expansion, adaptation, and heredity, make these processes supple enough, spontaneous, and inevitable.
>
> Due to this association, we perceive living matter as a single whole, as a single giant organism, which takes its elements from the reservoir of inorganic nature and governs all the processes of its progressive and regressive metamorphosis, and returns, at last, all that it took to lifeless nature [14].

The final words of Winogradsky are very significant for our understanding of the naturalist's outlook. We may leave aside such words as "a single giant organism," with which the supporters of the Gaia hypothesis amuse themselves; however, these words were written a century ago with complete understanding and substantiation of their sense, although without referring to the global climate. At present, the word "organism," indicating an organized complete system, is replaced by the word "system." Essential are the understanding of nature as a single whole and the word purposiveness, which sharply contradicts the private individualistic approach, the fundamental part of the theory of evolution. The matter concerns, in fact, the ideology, originating, in particular, from the Orthodox axiology (system of values), which Winogradsky, judging from his autobiographical manuscript *The Record of our Life,* subscribed to when he wrote these words, having accepted the traditional views of Orthodox Kiev during his childhood.

Presently, we can take a step into the past, to the field of paleontology, which Winogradsky considered not relevant to bacteria. The world of the past, previously inaccessible, uncovers itself before our eyes. The

Precambrian life, as far as can be judged from stromatolites, i.e., lithified cyanobacterial communities, or microfossils of cyanobacteria, which can be identified, for the most part, by their morphology, was represented predominantly, if not exclusively, by bacteria [18]. Another thing is important. The biosphere of the present type was formed about 2.4 billion years ago by bacteria that had existed much earlier. The system of cycles, which Winogradsky described when analyzing the life cycle, is vital for the creation and sustenance of the biosphere. This system of cycles is the result of interactions among specific groups of microorganisms. The subsequent evolution, the order of which is an empirical fact, fits well into the system created by bacteria, the "single giant organism." The individual parts of the system evolved separately. For instance, the redistribution of dominant groups of primary producers occurred; in addition to osmotrophy, the phagotrophic and zootrophic pathways, allowing nutrition by food particles, evolved. Nevertheless, every successive step correlated with the state of the biosphere; therefore, the whole multitude of biological objects fits eventually into the initial system created by bacteria. Some of the functions, first of all, those discovered by Winogradsky, e.g., nitrogen fixation and the sulfur cycle, remained the prerogative of bacteria. In this regard, "we all evolved from the cyanobacterial mat" as a complete and expedient system.

Let me briefly mention Winogradsky's attitude to medical microbiology. He was a botanist and had a different system of values. His purpose was to gain insight into natural processes. Thus, *Soil Microbiology* is filled with harsh statements concerning the incompatibility between his goals and the goals of medical microbiologists, personified in Koch. In the chapter *The Basics of Ecological Microbiology*, Winogradsky emphasized repeatedly that the objectives pursued by explorers of the nature differ from those pursued by investigators of human diseases. The axiologies (if I may use this word again) of biologists and physicians are different. Their understandings of nature overlap in some places, such as universal properties of bacteria as living organisms, but their purposes are dissimilar. They differ as Metchnikoff's immunology and Winogradsky's autochthonous microflora of the soil without plant cover do. A hundred years of experience has shown that medical and general ("natural-history-based," however awkward it sounds) microbiologies are sisters rather than spouses. The coupling of medicine and biology is unnatural in view of the difference between the systems of immanent values.

It is possible that this fact was the chief cause of the botanist Winogradsky's failure during the plague episode, the failure from which he struggled to recover for a long time, as well as of his resignment from the Institute of Experimental Medicine, where the Department of General Microbiology remained, despite its famous directors, a foreign body, as Winogradsky described in *The Record of Our Life.* One of the students of Metchnikoff, D.K. Zabolotny, whom Winogradsky persistently called "old friend," succeeded in that plague epic.

WINOGRADSKY AS THE FOUNDER OF A SCHOOL

This statement sounds bizarre. Winogradsky had no students, except Omelyanskii. He had panic attacks when he heard about teaching and refused to teach even when V.V. Dokuchaev insisted on the introduction of the course of agricultural microbiology at the Department of Agriculture in the Ministry of Inner Affairs and the foundation of the respective division, which Winogradsky might head; Winogradsky refused to teach even when teaching was a matter of survival. However, Winogradsky became a founder of a school, the Russian school of general microbiology. The authors of the very successful book about Winogradsky [19], which, despite some funny mistakes (for instance, professor Beijerinck was a confirmed bachelor because of a big disappointment experienced early in life, rather than a Bachelor of Science), each microbiologist is advised to read, point out, in the footnote on page 38, that Winogradsky, according to all formal appearances, was a true founder of a scientific school, although the "Winogradsky School" is not so famous as the "Beijerinck School."

Actually, this is not quite true. The Winogradsky School in Russian (Soviet) microbiology was founded thanks to Omelyanskii and his textbook, where Winogradsky's ideas are predominant and extend over the whole domain of microbiology. The fact that Omelyanskii wrote a textbook has not been qualified as an achievement, although he devoted all of the 1920s to its writing and worked on the book until his death. The textbook included the contemporary knowledge and task priorities; it described the time rather than remarkable experiments. Being a trained chemist, Omelyanskii realized the incompleteness of his microbiology education. Thus, he wrote his *General Microbiology* [20, 21] (which has been republished many times) with great care and completeness. His next textbook, *A Practical Guide to Microbiology* [22, 23], is distinguished by being a collection of methods which Omelyanskii applied himself. This textbook was written according to the pattern adopted by all modern textbooks on microbiology. Due to the isolation of Soviet microbiology, Winogradsky's ideas, outlined by Omelyanskii in his textbook, became the standard way of thinking until they gave way to the biochemically-oriented ideas, as in Schlegel's textbook [24], which reflected a new era of microbiology. The era of metabolic pathways was followed by the era of genomics, the methods of which, at a certain stage, have been applied to the problems of microbial life in nature, posed by Winogradsky. This era is still in the process of development.

When we consider the connection of Winogradsky's ideas to the present, we should realize that, when we investigate microbial activity in nature, we are guided by the same Weltanschauung that Winogradsky formulated in *The Cycle of Life* [14]. This Weltanschauung includes the understanding of nature as a whole thing and expediency, understood as conformity with the large system of habitats of microorganisms, creators of the biosphere. Winogradsky is by right considered the founder of the contemporary ecological microbiology. In light of the fact that microorganisms catalyze the entire system of biogeochemical processes, Winogradsky is not only a great bacteriologist, but also a great naturalist.

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