
REVIEWS

Ray Fungi and Related Organisms (*Actinomycetales*)

L. V. Kalakoutskii

*Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences,
pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia
Pushchino State University, Ministry of Education of the Russian Federation, Pushchino,
Moscow oblast, Russia
e-mail: vkm@ibpm.pushchino.ru*

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The first presentation of a contemporary version of a unified phylogenetic tree of the life on Earth [1, 2] is bound to cause an interested discussion in which representatives of all the biological disciplines will be involved. Comparing this version with the ones suggested by Ernst Haeckel in the 1860s and 1870s and subsequently by other authors, the scale of the road travelled, in less than a century and a half, by generations of professionals who shared the interest in the attempts to discover the possible phylogenetic (Haeckel's term) relations between all the extinct and extant organisms becomes evident. To a modern microbiologist, who is inclined to consider journal editors' request to provide phylogenetic evidence to support the taxonomic conclusions as routine, the scale and concomitant difficulties of this road may well be unknown. In retrospect, this chain of events causes one to recollect the words of the Russian poet N.A. Nekrasov, "It was a work of frightening dimensions, far beyond the power of a single man."

The phylogenetically-oriented taxonomy of bacteria initially lagged behind the taxonomy of higher organisms (in the shadow of botany). Now, however, it has moved into the leading position [1, 3], thus causing the reconsideration both of its place in the system of biological sciences and of the natural system of microscopic organisms. This process was not limited to the radical revision of the concepts of genealogy and relations between all the accepted taxonomic units [4]. The echo of these newly acquired phylogenetic concepts is discernible in the progress of the methods and methodology used for biodiversity assessment [5] and identification [6], in biotechnology [7, 8], and in other spheres related to the study and/or application of bacteria.

The difficult progress of the pioneers, who used all the means available to them in order to provide the content for the concept of relatedness between microorganisms, deserves our grateful interest. The title of this article purposefully reproduces the title of N.A. Krassilnikov's monograph [9] published in 1938 by the Academy of Sciences Publishing House (presented by the Institute of Microbiology and edited by B.L. Issatchenko). This work, as well as certain previous and subsequent publi-

cations by Krassilnikov, his close colleagues, and his pupils (see the complete bibliography in [10]), gives a clear idea of the main landmarks in the study of actinomycetes at the Institute of Microbiology during the 1930s. A number of publications were concerned with the biography of Krassilnikov as a scientist [10, 11]. For these two reasons, I feel free not to discuss all the particulars of the mentioned works, however important some of them. Both the title and the contents of the 1938 book are attractive to us because they signify one of the first attempts in our science to establish a concept of a natural group of microorganisms and of the possible phylogenetic relations both inside this group and of the group as a whole with other segments of the microbial world. This attempt was made with the means available at the time and under very peculiar historical circumstances.

According to the modern concepts, the order *Actinomycetales* is included in the phylum and class *Actinobacteria* of the domain *Bacteria* and consists of dozens and dozens of microbial genera [4]. In retrospect, it is noticeable that both the composition of the "core" and the "outer borders" of the order, which were determined during the 1930s and 1940s, remained rather constant in spite of continuous addition of new members. A comparative study of the tables of contents of all the editions of *Bergey's Manual* can serve a good illustration of the thesis that *Actinomycetales* have been an "island of relative stability" in the troubled sea of evolving taxonomical concepts. The changes became especially drastic when a tendency evolved to correlate the taxonomical hierarchy with phylogenetic concepts [3], which were initially based mostly on the results of comparative analysis of conservative 16S rRNA sequences. Even then, the class *Actinobacteria* was possibly the first instance of the currently accepted consensus between the phylogenetic ideas and the taxonomic hierarchy at the class–order–family–genus levels [12]. It can be added that, by using genomic probing to reveal those actinomycetes that still resist traditional cultural techniques, vast diversity and broad distribution of new related organisms was and still is being shown [8, 13].

The activity of the Institute of Microbiology, Academy of Sciences of the USSR, is traditionally considered to have started in 1934, the year of the resolution establishing it in Moscow. B.L. Issatchenko [14, 15] considered that the Microbiological Laboratory of the Academy of Sciences, active in Leningrad since 1929, was the immediate precursor of the institute. The leader of that laboratory was G.A. Nadson, who then moved to Moscow together with his closest pupils (including Krassilnikov) to join the newly formed institute and was in charge of it till his arrest. Some specialists from Moscow organizations also joined this institute, including from the Institute of Microbiology of Moscow State University.

At least two aspects of the theoretical background that Nadson's group brought to the institute deserve special attention. These can be summarized as deep ties with the traditions of the botanical mode of thinking and keen interest in microbial variability.

The ties between microbiology and botany have deep roots and can be traced back to the time of F. Cohn and the other pioneers of systematic classification of bacteria; they considered fungi to be lower plants and nonphotosynthetic bacteria to be schizomycetes, i.e., fragmenting fungi. The term *microflora*, although deprived of its original content, is still widely used. By the 1920s and 1930s, the methods and traditions of microbial systematics in medical and general microbiology were already visibly diverging. In the first case, not the microorganism but rather the process it induced was the focal point. General microbiologists, on the other hand, gravitated to the organism-centered approach typical of botany with its comparative studies of natural biodiversity, including (but not limited to) potentially hazardous (henbane, hemlock) and useful (agricultural) organisms. The botanical concepts of a species seemed then more definite. Botanical analogies were common in the publications of the opponents of polymorphism, and later, of pleomorphism and cyclogeny; these analogies can be seen, for instance, in the brilliant papers of S.N. Winogradsky on these topics.

It may be further assumed with a measure of confidence that a certain harmony in the botanical concepts related to the correlation between phylogeny and taxonomy was one of main attractions of botany. To the overwhelming majority of the researchers of the period, the Linnaean system, based on grouping organisms according to morphological similarity (mainly that of the generative organs), seemed to be in perfect accord with the elements of genealogical concepts developed afterwards: it grouped organisms not by similarity alone but also by their relations and, consequently, by their origin. The revision of such concepts, which took into account, among other things, the somewhat painful experience acquired by bacteriology, has started only recently [1]. In the earlier period, it seemed quite logical to assume that, if a "natural system" based on comparative analyses of morphological similarities was

possible for higher plants, then the same approach to microbial taxonomy was worth testing.

Some formal factors were pushing in this direction. Binominal Latin nomenclature, and, later on, some elements of the taxonomic code were borrowed by microbiology from botany, together with the idea of hierarchical taxonomy. It is worth mentioning that trinominal names for some species survived for a long time in medical microbiology, which was not much interested in taxonomic hierarchies and used rather pragmatic groupings suitable for identification of pathogens. In *Bergey's Manual*, up to the ninth edition, pragmatic (and very variable) groupings were also preferred, although binominal nomenclature was maintained. Until the 1980s, various plans of bacterial grouping based on hypothetical phylogenetic relations were not taken seriously by systematists, partly because of their mutual contradictions.

The term "microbial variability" encircled a broad range of problems of ever-increasing importance; even now, its new aspects still attract specialists. The experimental work of Nadson and G.S. Filippov on radiation-induced variability in fungi is known to have provided the crucial impulse to all the subsequent work of the 1930s and 1940s on induced mutagenesis [16]. The arising of molecular biology in the 1940s and 1950s was directly related to the work of Avery *et al.* [17] revealing the key role of DNA in variation (transformation) of pneumococci. The idea of a possible link between the variability of plants and their systematic relations has been widely discussed, especially after the first publications of N.I. Vavilov on the "homologous series of inheritable variability" [18], and was to some extent related to Haeckel's then-popular "recapitulation law."

Heated discussions took place in systematic bacteriology. The widespread occurrence of relatively easily observable bacterial variability (including "natural") provided reasons to suspect that the ideas of the founders of monomorphism had transformed into a dogma pushing bacteriology into an evolutionary dead end. The publications of the followers of pleomorphism and cyclogeny in the 1910s–1930s were an extreme reaction to this situation (opening, in turn, a new field for discussions); they postulated either "unlimited variability" (and therefore the nonexistence of individual species) or the reproduction by bacterial species of certain partially specific, highly complicated cycles of development (sexual process, symplasm, filterable forms, etc.) As usually, in the course of the most heated discussions, little attention was paid to the adequacy of the methods applied to the nature of the problems. In the 1930s, optical microscopes were still researchers' principle tools. Electron microscopy did not yet exist, nor did microbial paleontology; the contacts between microbiology and genetics or biochemistry were as yet in their embryonic phase.

It is difficult for a contemporary reader to comprehend the atmosphere in which scientific activity developed in Moscow during the 1930s. For deeper understanding both of what was said and written and of the relationships within the scientific community it is, however, necessary to consider this atmosphere. Even nowadays an opinion exists that Soviet biology developed relatively normally until in August 1948 a catastrophe occurred caused by a certain crushing blow from "outside." This picture is very remote from the actual reality. In spite of the increasing number of professional institutions and their employees, malignant changes in the scientific atmosphere developed gradually but inevitably at least from the "great break" of 1929–1930. Their scope and character can be deduced from careful reading of newspapers and even of professional publications of the 1930s and 1940s. Soviet microbiology, even in the relatively peaceful years, had to respond to the slogan "building socialism in one separate country" and thus became more and more isolated from the international research process. The results were evident. While during the 1930s the average level of the articles published in *Mikrobiologiya* was comparable with that of the articles in *Zentralblatt* or *Journal of Bacteriology*, during the 1950s these levels were, with rare exceptions, not comparable within the same value system.

From the early 1930s, the number of books and articles published by Soviet authors abroad decreased gradually but steadily. The reference lists in the Russian publications became less complete and more selective, making it difficult to establish priority. The regularly published editorial papers urged to work along the lines of the new slogans and economic problems ("struggle for harvest," canal building, etc.). The theoretical reviews dealt more and more with the relations between microbiology and dialectics (or with the class standing of Darwin and Pasteur) and less and less with the parallel developments in genetics, cytology, chemistry of natural compounds, etc.

As early as in 1931, an interrogator addressing an arrested professor of bacteriology in order to make him confess to wrecking, used the following peculiar reasoning [19]: "Why don't you want to confess? It is known for certain that all bacteriologists are wreckers. Are you maybe the only saintly person among them?" Among other professionals, E.E. Uspenskii, D.M. Novogradskii, L.A. Zilber, G.A. Nadson, and finally N.I. Vavilov (whose pupils remembered, among other things, his motto: "to stand on the globe") perished in the gulag during the 1930s. After their arrest on unknown charges, their names were not to be mentioned and their widely known publications were not to be cited. Among the specialists retaining a degree of freedom, many were forced to limit themselves to highly specific issues, thus avoiding the topics "stained" by participation of their perished comrades and teachers. To continue research, especially bordering with genetics, and to discuss and publish their results became a very difficult task under these conditions; it took persistence, outstanding abili-

ties, and a plethora of other features. Of those who successfully overcame the obstacles, it can often be said that they rescued not what they intended to rescue but rather what they could.

On his moving to Moscow, Krassilnikov rearranged the topics of his research significantly. His early works were concerned mostly with yeast taxonomy, ecology, and variability, as well as with the life cycles of certain bacteria (*Azotobacter* and mycobacteria). Later on, he concentrated on actinomycetes. He was aware of the earlier works on the subject, both in Russia [20–22] and abroad [23–27], and widely cited them while discussing his own results and conclusions.

Creation of a vast collection, whose main body consisted of soil isolates obtained from the arid regions beyond the Volga River, was the first stage of his work. Various media, including chemically defined ones (which were first introduced to actinomycetology by Krainsky [22]), were used for the isolation. Gradually, type strains, including some obtained via exchange with other researchers, were also added to the collection. The general goal of the research was stated as follows: "In this work I do not aspire to elucidate all the aspects of the life of *Actinomycetales*. This would have required an unacceptable increase in volume and a venturing beyond the limits I determined in the beginning of experimental work. Moreover, being a morphologist, I'm far from the purely biochemical aspects and can dwell on them only with difficulty. My work is limited therefore to the *structure, development, and phylogeny* of some groups of *Actinomycetales* in order to use these data, as well as published data, first, to compare these organisms to each other and to the ones I've isolated from soil and, second, to reduce this diversity to certain *systematical units*, genera and species, and to try to construct a key for their determination..." (my italics, L.K.).

In another passage, Krassilnikov wrote that "... the lack of solid knowledge concerning the *phylogenetic relations* between groups and species of bacteria is the main reason for the imperfect character of bacterial taxonomy" (my italics, L.K.). Thus, the author conceived developing the taxonomic notions by introducing phylogenetic reasoning as one of his main goals. Or maybe even founding the taxonomic concepts on the phylogenetic ones? It is a difficult question, and nowadays experts on phenetics, cladistics, and evolution tend to give varied answers to it.

The phylogenetic relations, however, must be revealed before any use of them can be made. The possible phylogenetic relations of actinomycetes and between them and other organisms were discussed by Oerskov [26], Jensen [27], and Lieske [25] in some way or other. Krassilnikov was probably the first to attempt experimental revealing of such relations, using the means available to him.

To begin with, for the morphological comparisons (cell division, sporulation, and spore germination) the author rejected the "microscopy of stained smears"

popular in that period, especially among medical bacteriologists, in favor of vital microscopy of preparations (usually in “hanging drops”) and of untreated colonies. He wrote: “It is clear that, in order to discuss relations between microorganisms, i.e., to try to establish a natural classification revealing phylogeny, it is necessary to study not some single episode in the life of an organism but rather its whole life cycle, paying attention to all the stages of its development.” As a result of research in this direction, certain collective images were developed for the life cycles of the genera that the author suggested as the core of the order *Actinomycetales* (the genera *Actinomyces*, *Proactinomyces*, *Mycobacterium*, *Mycococcus*, and *Micromonospora*). Life cycles were reproducible under comparable conditions and as such were suggested for use as the leading systematic features to differentiate actinomycetes on the genus–family level within the order. The genus *Mycococcus* was established as a taxonomic unit mostly because of the characteristics of its life cycle (“budding cocci”). In the course of this work, some obscure points in the notion of the “normal” life cycle of these organisms that had been introduced by authors influenced by the concepts of pleomorphism and cyclogeny were elucidated.

The ideas that should play the key role in the formation of the concept of possible relations of the organisms within the order (and with other microbes) could, according to Krassilnikov, be derived from analysis of their variability. “It is self-evident that for microorganisms, especially for yeasts, bacteria, and *Actinomycetales*, due to their simplified structure, the *problem of species* can never be solved by morphological methods alone. *Production of new forms* is, together with other methods, important for *establishing their phylogeny*.”

“...Race production as a way to determine phylogenetic relations between microorganisms was first described by Filippov (1932) [28]. Subjecting the yeast *Torulopsis* to X rays, the author obtained a number of *stable new races* with morphophysiological properties some of which, according to the rules of modern taxonomy, should have been described as *Torulopsis*; some as *Eutorulopsis*; and some, as *Mycotorula*. The author believes that *similarity* of the races of these organisms he obtained indicates their *genetic relatedness*” (my italics, L.K.).

The cited data, together with some others, were the basis of the “experimental variability method,” which was widely used to analyze the proposed “genetic relatedness” of *Actinomycetales*, especially of the above-mentioned genera. The organisms were grown under a variety of laboratory conditions (medium composition, temperature, salinity, pH, etc.). Irradiation was, however, seldom used due to its uncertain role in nature. The deviant forms were isolated and compared with the original forms and with each other. A number of cultural and morphological features were used for comparison; changes in the life cycle and the frequency of production of stable and unstable transitory forms were,

however, the main criteria. No deep investigation of the possible causes and mechanisms of induced variations was performed, fully in accordance with the current level of genetic knowledge and with the “spirit of the epoch”; in particular, the relative roles of induction and selection in producing variant colonies were not considered. A great multitude of forms differing from the originals in all the studied characteristics were isolated and described. One of the conclusions was that variability of actinomycetes, though wide, was not unlimited. The extreme notions mentioned in [25] that they are all representatives of one species and that the observed variability is simply its expression under varying conditions were hardly realistic.

The representatives of *Actinomyces*, *Proactinomyces*, *Mycobacterium*, and *Mycococcus* produced variants which in their morphology and life cycles were often similar to their neighbors in rows (actinomycetes to proactinomycetes and mycobacteria; proactinomycetes to actinomycetes and mycobacteria, etc.). These data were considered important evidence of a close relationship between these organisms, a reason to combine them in one natural group. Concerning the spectrum of variant forms, Krassilnikov stated: “Thus, a picture of sequential morphological degradation among certain *Actinomycetales* was observed. On the one hand, we have actinomycetes as the most advanced forms, with a more complex structure and a more or less developed fruiting apparatus; on the other hand, mycococci, with a simple cell shape and a short life cycle. Between these, intermediate groups can be placed: proactinomycetes, which are closer to actinomycetes, and mycobacteria.”

Analysis of the frequencies of the registered variants led the author to the conclusion that data on variability can be used to form far-reaching hypotheses concerning sequential origin of the representatives of a group of genera from a common ancestor. The representatives of the groups with simple structure and a “short” life cycle were found to produce more, and not less complex forms of the above-mentioned row (e.g., proactinomycetes produce more actinomycete-like variants than mycobacteria-like ones, etc.). The author therefore considered it possible to treat this row as a sequence of degradations rather than as a sequence of progressive developments. “Under laboratory conditions, these forms tend to produce races *resembling the more highly developed progenitors from which they originated*” (my italics, L.K.). In another passage, discussing the emergence of an actinomycete-like race in the variability spectrum of one of the proactinomycetes, he stated: “... the actinomycete-like race indicates that this organism *originated from actinomycetes* in some remote past and *recalls this relationship* from time to time” (my italics, L.K.).

These are evidently not trivial conclusions for the time when evolution was thought of as “progressive

development"; in bacteriology, this concept usually meant the coccus → rod → mycelium formula [29–31].

It is widely known that the term *Proactinomyces* was suggested by Jensen [27], who considered the possibility of actinomycetes ascending from simple forms (mycobacteria, corynebacteria) to more complex ones. Krassilnikov remarks on this topic: "In my opinion, the term *Proactinomyces* is more fitting than the ones suggested earlier because it stresses the genetic relationship of these organisms with actinomycetes. However, on the basis of the phylogenetic concept (see below), the name metaactinomycetes seems more appropriate".

Let us try to consider the origin of this idea apart from the facts that were its foundation. It is possibly rooted in the discussions on whether actinomycetes are bacteria or fungi. No established opinion existed in the professional community in that period, and a number of original, rather contradictory suggestions coexisted. Considerations of nomenclature possibly contributed to persistent interest in this problem. The overwhelming majority of bacteriologists forgot rather quickly about the proposed affiliation of bacteria with "schizomycetes," and the roots of bacterial origin were discerned with uncertainty. Any mention of "fungi" in the widely used names of actinomycete species, genera, and families was, however, bound to cause relevant associations. This, in turn, promoted interest in the problem. There was certainly no way at that period to solve this problem experimentally. The concerns of the similar structural plan (mycelial organization, life cycles) and way of life, odd information on the behavior of nuclear elements, and the rudimentary knowledge about the metabolic pathways and cell wall chemical composition were then the main issues in this argument. The method of experimental variability continued to provide information on the hypothetical bacterial relatives of the ray fungi. However, this method is not known to have been used to test the possible relationship with fungi. The possibility of convergent evolution was usually not mentioned in the context of similarity of mycelial organisms.

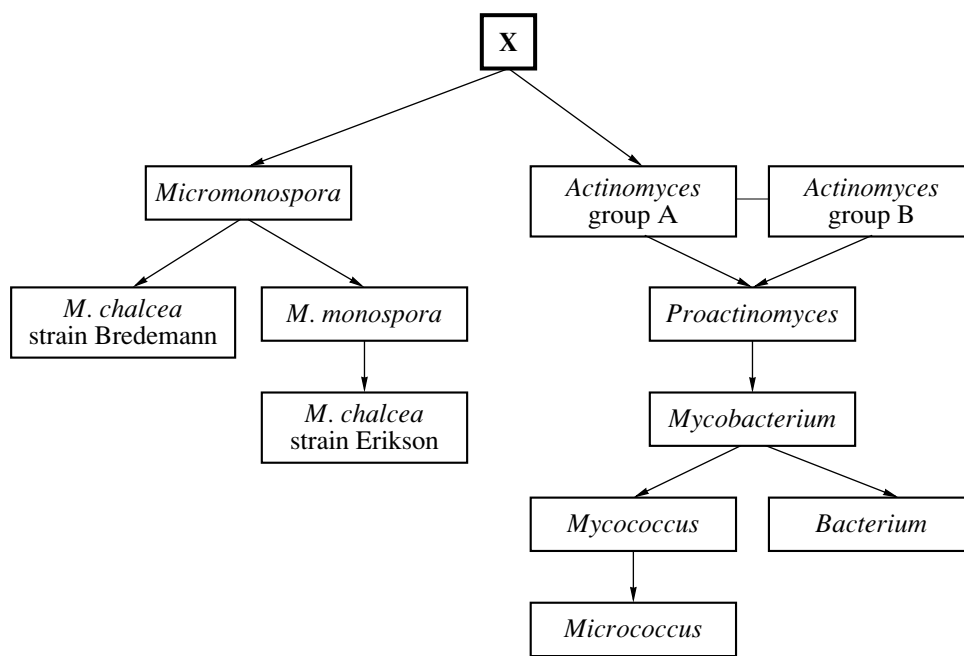
Krassilnikov maintained that actinomycetes (all the known representatives together with the yet undiscovered members of *Actinomycetales*) comprised "a special group of organisms." He believed that they are related to certain bacteria (gram-positive ones were mostly mentioned) but the ways of their evolution are closely intermingled with those of fungi. "Parallelism in the evolutionary development of these organisms can be noticed. Fungi are known to include representatives with varying degrees of morphological differentiation, from complex well-developed mycelial forms with complex fruiting to unicellular yeasts. All the intermediate forms of gradual degradation exist in between. This analogy is evidently no proof of any phylogenetic relationship between fungi and actinomycetes. As such, it illustrates the parallelism of morphological degradation in these two completely independent groups of

organisms. Such parallelism, however, as biological knowledge confirms, is the more pronounced the closer the organisms are to each other. Probably actinomycetes, though now a completely independent group, at a certain stage of their evolution had a common origin with fungi, or at least with some of the fungi, taking into consideration the fungal polymorphism ... Development under different conditions resulted in modern fungi for one of these branches. The other branch either remained in its primitive condition (not a likely suggestion) or developed in the direction of modern actinomycetes. Lieske (1921) considers actinomycetes to be the common progenitors of fungi and bacteria. This may be the case, but it must be remembered that, even if actinomycetes were the progenitors of fungi, they themselves were forms different from the modern ones almost beyond comparison."

The hypothesis about the origin of actinomycetes in the course of retrograde evolution from the original form common with fungi certainly lost its charm after subsequent research revealed the scale of differences between pro- and eukaryotes and, later on, between the three principal domains of life. It should be noticed, however, that the scale of secondary losses and acquisitions by the cells of fungi and related lower eukaryotes became evident only in recent years; this may promote a serious reconsideration of the possibilities and results of retrograde evolution. Research on the recently discovered picoeukaryotes, with a cell size close to bacteria, may produce interesting results on the topic [32]. Even nowadays, some authors [33] still place actinomycetes much closer to the hypothetical common ancestor than is usual for the generally accepted version of the phylogenetic tree of bacteria and archaea. In particular, the ability of actinomycetes to produce cholesterol is stressed as the feature uniting them with eukaryotes and separating them from the majority of known prokaryotes.

Treating the early workers in bacterial taxonomy as idealists or pragmatists on the basis of their support or denial of the use of the phylogenetic concept [3] is perhaps valid. The role the first ones played, however, was likely not limited to maintaining a certain degree of interest in this problem within professional circles. This intellectual training, however slow and contradictory, prepared the scientific community for the modern stage of development. Moreover, the early believers in enriching bacterial taxonomy with phylogenetically based concepts provided many points of view for microbiologists to evaluate the arrangements designed by pragmatic taxonomists. Their role was possibly analogous to the role played among the pragmatists by numeric taxonomists, who rejected any initial weighing of characteristics but contributed greatly to pragmatic taxonomy by critically reordering its "attic."

To combine the taxonomic and phylogenetic concepts, weighing of characteristics to establish their relevance was certainly inevitable. From the taxonomic



Presumed relationships between the representatives of microbial groups included in the families *Micromonosporaceae* and *Actinomycetaceae* of the order *Actinomycetales* [9].

point of view, the task was to include in the proposed natural group all the organisms related to actinomycetes and to exclude the unrelated ones. The characteristics to which Krassilnikov ascribed the highest priority have already been listed (morphology, life cycles, variability spectra). His opinion concerning other characteristics observable in the 1930s and proposed as taxonomic and/or differentiating is worth mentioning. All the morphological characteristics and those related to morphology (motility, etc.) received high weights. Among the cytological features, the results of Gram staining received high weight, and those of acid-resistance staining, low weight. After analyzing the variation of these characteristics, it was suggested, for instance, to combine the genera *Corynebacterium* and *Mycobacterium* and to exclude the genus *Mycoplana* from the order. The same notions lead to the suggestion that bacteria related to actinomycetes ought to be mostly gram-positive (excluding the endospore-forming ones) rather than gram-negative. Physiological characteristics (anaerobiosis, pigmentation, ability to utilize certain substrates) were considered suitable for differentiating between species or subspecies but not between genera. Ability to cause diseases of humans, animals, or plants was treated similarly. Therefore, it was recommended to include in the genus *Actinomyces* (later renamed to *Streptomyces*) only aerobic forms with nonfragmenting substrate mycelium and various sporophores (usually on the aerial mycelium). It was suggested to include the anaerobic forms with fragmenting mycelium in the genus *Proactinomyces* and to revise the genus *Actinomyces* appropriately.

The scheme of proposed phylogenetic relations of “ray fungi and related organisms” as published in 1938 is presented in the figure. The author’s subsequent works [34–36] provide data on its further development. The works [27, 31, 37] illustrate some other schemes for classification of actinomycetes suggested by other authors during that period.

Let us digress from the questions of a common root and of the sequence of origin of the groups included in the order *Actinomycetales*. It was already stated that the borders of its “core” were determined with exceptional precision, even considering the long term. The thesis that actinomycetes form a separate and independent clade was soundly substantiated after the method of semantid comparative analysis was developed. Unlike a number of other bacterial taxonomic groups that were pragmatically sound at the time of their establishment and which were established with a different set of characteristics as starting concepts, almost no organisms were to be excluded from the actinomycetes group. New forms (microbacteria, propionic acid bacteria, and others) were added to this order (later, class) not only as the result of the use of improved methods to reveal new forms in nature but also due to the transfer to the group of a number of gram-positive bacteria; in the course of development of the methods of phylogenetic analysis, their relationship with the “ray fungi” became more and more solidly proven. Subdivision of the order into the families *Micromonosporaceae* and *Actinomycetaceae* (based on peculiarities of the sporulation process) proved thoroughly reasonable. The suggestion to further subdivide the *Micromonospora* subgroup and the genus *Actinomyces* (*Streptomyces*) (see figure) can be

viewed as the origin of the modern concept of the heterogeneity of the related forms united within these groups; they were subsequently differentiated using a complex of morphological and chemotaxonomic criteria.

The success of the pioneers of the hierarchical taxonomy of actinomycetes is sometimes explained as the result of their relatively complex morphology and life cycles as compared, e.g., to proteobacteria. This complexity, however, is the feature of only some of the species, namely, those that some early authors termed as the "higher forms." Moreover, there are groups among prokaryotes that have no less complex structure, life cycles, and ecology, e.g., myxobacteria, cyanobacteria, etc. These groups attracted some very prominent researchers in the 1920s–1940s. However, revealing of related forms and development of a hierarchical taxonomy compatible with modern phylogenetic concepts progressed by ways that in retrospect turned out to be more difficult.

The emergence in the 1930s of the Moscow research center which for many years concentrated effort on the "organism-centered" comparative study of a number of actinomycete cultures, can be confidently assumed to have been a factor promoting interest in investigating this group for numerous and varied purposes. The era of antibiotics was to begin in the near future, making actinomycetes the group of the best studied organisms instead of "a poorly studied group." This new epoch, together with its outstanding achievements, brought up numerous problems, including the need to reexamine the whole of actinomycetology for its maturity in every respect. The Moscow microbiologists of the 1930s passed this creative examination excellently. Their publications on microbial antagonism and production of bactericidal compounds [38–44] were pioneering ones and gained justified acclaim among the generations of researchers to follow.

REFERENCES

1. Sugden, A.M., Jasny, B.R., Culotta, E., and Penisi, E., Charting the Evolutionary History of Life, *Science*, 2003, vol. 300, p. 1691.
2. Daublin, V., Moran, N.A., and Ochman, H., Phylogenetic and the Cohesion of Bacterial Genomes, *Science*, 2003, vol. 301, pp. 145–146.
3. Woese, C.R., How We Do, Don't and Should Look at Bacteria and Bacteriology, *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, 3rd ed, Dworkin, M. et al., Eds., New York: Springer, 2003. <http://link.springer-ny.com/link/service/books/10125>.
4. Lilburn, T.G. and Garrity, G.M., Exploring Prokaryotic Diversity, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 7–13.
5. Venter, C.J., Remington, K., Heidelberg, J.F., et al., Environmental Genome Shotgun Sequencing of the Sargasso Sea, *Science*, 2004, vol. 204, pp. 66–74.
6. Schleifer, K.-H., Microbial Diversity: Facts, Problems and Prospects, *Syst. Appl. Microbiol.*, 2004, vol. 27, no. 1, pp. 3–9.
7. Keller, M. and Zengler, K., Tapping into Microbial Diversity, *Nature Rev. Microbiol.*, 2004, vol. 2, pp. 141–150.
8. Bull, A.T., Ward, A.C., and Goodfellow, M., Search and Discovery Strategies for Biotechnology: the Paradigm Shift, *Microbiol. Mol. Biol. Rev.*, 2000, vol. 64, pp. 573–606.
9. Krassilnikov, N.A., *Luchistye gribki i rodstvennye im organizmy (Actinomycetales)* (Ray Fungi and Related Organisms (*Actinomycetales*)), Moscow: Akad. Nauk SSSR, 1938.
10. Gutina, V.N., *Nikolai Aleksandrovich Krassilnikov* (Nikolai Aleksandrovich Krassilnikov), Moscow: Nauka, 1982.
11. Kalakoutsii, L.V., Nikolai Aleksandrovich Krassilnikov, *Int. J. Syst. Bacteriol.*, 1975, vol. 25, pp. 393–395.
12. Stackebrandt, E., Rainey, F.A., and Ward-Rainey, N.L., Proposal for a New Hierarchic Classification System, *Actinobacteria Classis nov.*, *Int. J. Syst. Bacteriol.*, 1997, vol. 47, pp. 473–491.
13. Joseph, S.L., Hugenholtz, P., Sangwa, P., Osborn, C.A., and Janssen, P.H., Laboratory Cultivation of Widespread and Previously Uncultured Soil Bacteria, *Appl. Env. Microbiol.*, 2003, vol. 69, pp. 7210–7215.
14. Issatchenko, B.L., Institute of Microbiology, 220 let Akademii Nauk SSSR. *Spravochnaya kniga* (220 Years of the Academy of Sciences of USSR), Moscow: Akad. Nauk SSSR, 1945, pp. 201–202.
15. Issatchenko, B.L., Microbiology in the Academy of Sciences of USSR), *Mikrobiologiya*, 1945, vol. 14, pp. 137–145.
16. Nadson, G.A. and Filippov, G.S., On the Effect of X-Rays on the Sexual Process and Mutant Induction in Lower Fungi (*Mucoraceae*), *Vestn. Rentgenol. Radiol.*, 1925, vol. 3, pp. 305–310.
17. Avery, O.T., McLeod, C.M., and McCarty, M., Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types, *J. Exp. Med.*, 1944, vol. 79, pp. 137–158.
18. Vavilov, N.I., The Law of Homologous Series of Inheritable] Variability, *Sel'skoe Lesn. Khozyaistvo*, 1921, no. 1/3, pp. 84–99.
19. Aristovskaya, T.V., *Vospominaniya (Recollections)*, St. Petersburg: ZAO Kopi Servis, 2003.
20. Berestnev, V., Zur Frage der Klassifikation und systematische Stellung der Strahlenpilze, *Zentralbl. Bakteriol. I Orig.*, 1899, vol. 26, pp. 390–397.
21. Tsiklinsky, P., Sur les mucedinees thermophiles, *Ann. Inst. Pasteur*, 1899, vol. 13, pp. 500–505.
22. Krainsky, A., Die Actinomyceten und ihre Bedeutung in der Natur, *Zentralbl. Bakteriol. II*, 1914, vol. 41, pp. 649–658.
23. Breed, R.S. and Conn, H.J., The Nomenclature of the Actinomycetaceae, *J. Bacteriol.*, 1919, vol. 4, pp. 585–602.
24. Waksman, S.A. and Curtiss, R.E., The Actinomycetes of the Soil, *Soil Sci.*, 1916, vol. 1, pp. 99–134.
25. Lieske, R., *Morphologie und Biologie der Strahlenpilze*, Leipzig, 1921.

26. Oerskov, J., *Investigation into Morphology of the Ray Fungi*, Copenhagen, 1923.
27. Jensen, H., Contribution to Our Knowledge of the Actinomycetales, *Proc. Linn. Soc. N.S.W.*, 1932, vol. 57, pp. 364–372.
28. Filippov, G.S., Variability of *Torulopsis glutinis* (*Torula glutinis*) upon Irradiation with X-Rays, *Vestn. Rentgenol. Radiol.*, 1932, vol. 10, pp. 512–517.
29. Orla-Jensen, S., Die Hauptlinien der natürlichen Bacteriensystem, *Zentralbl. Bakteriol. II*, 1909, vol. 22, pp. 305–317.
30. Kluyver, A.J. and Van Niel, C.B., Prospects for a Natural System of Classification of Bacteria, *Zentralbl. Bakteriol. II*, 1936, vol. 94, pp. 369–403.
31. Stanier, R. and Van Niel, C.B., The Main Outlines of Bacterial Classification, *J. Bacteriol.*, 1941, vol. 42, pp. 437–466.
32. Baldauf, S.L., The Deep Root of Eukaryotes, *Science*, 2003, vol. 300, pp. 1703–1706.
33. Cavalier-Smith, T., The Neomuran Origin of Archaeobacteria, the Negibacterial Root of Universal Tree and Bacterial Megaclassification, *Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 1–76.
34. Krassilnikov, N.A., *Oprelitel' luchistykh gribkov* (Determinative Manual of Ray Fungi), Moscow: Akad. Nauk SSSR, 1941.
35. Krassilnikov, N.A., Classification of Ray Fungi (*Actinomycetales*), *Mikrobiologiya*, 1945, vol. 14, pp. 164–171.
36. Krassilnikov, N.A., *Oprelitel' bakterii i aktinomitsetov* (Determination Manual of Bacteria and Actinomycetes), Moscow: Akad. Nauk SSSR, 1949.
37. Waksman, S.A. and Henrici, A.T., The Nomenclature and Classification of the Actinomycetes, *J. Bacteriol.*, 1943, vol. 46, pp. 337–341.
38. Khudyakov, Ya.P., Lytic Effect of Soil Bacteria on Parasitic Fungi, *Mikrobiologiya*, 1935, vol. 4, pp. 193–199.
39. Borodulina, Yu.K., Relations between Soil Actinomycetes and *Bacillus mycoides*, *Mikrobiologiya*, 1935, vol. 4, pp. 561–586.
40. Nakhimovskaya, M.I., Antagonism between Actinomycetes and Soil Bacteria, *Mikrobiologiya*, 1937, vol. 6, pp. 131–157.
41. Novogradskii, D.M., Antagonistic Relations among Microbes and a Biological Method to Control Fungal Diseases of Plants, *Usp. Sovrem. Biol.*, 1936, vol. 5, pp. 509–517.
42. Nakhimovskaya, M.I., A Review of Antagonism among Bacteria, *Mikrobiologiya*, 1938, vol. 7, pp. 238–264.
43. Krassilnikov, N.A. and Korenyako, A.I., Bactericidal Substance Produced by Actinomycetes, *Mikrobiologiya*, 1939, vol. 8, pp. 673–684.
44. Kriss, A.E., On the Lysozyme of Actinomycetes, *Mikrobiologiya*, 1940, vol. 9, pp. 32–38.