

Forces Shaping Bacterial Systematics

Taxonomists continue to wrestle with fundamental questions about how to define and authoritatively classify bacterial species

Erko Stackebrandt

Microbial taxonomists are cautious about adopting novel approaches for classifying organisms. Not that I was ever against progress, but I often struck a wait-and-see attitude during more than 30 years of exposure to the latest scientific means for identifying, naming, and classifying microbial species and genera.

This caution comes for two main reasons. First, taxonomists have been struggling for at least 100 years to determine phylogenetic relationships among microorganisms. Second, on virtually a daily basis since the 1970s, species definitions have proved arbitrary because of the amazing genomic and phenotypic diversity of these organisms. Even with large data sets, the rank and category of microbial taxons remain far from obvious, prompting taxonomists to

continue searching for novel demarcating properties.

Yet, taxonomists also try to balance the benefits from new ways of classifying microorganisms against anything that could weaken the system in use. Indeed, after an extended period of dealing with an unsatisfactory system, microbial taxonomists began to adopt new classification methods that other specialists, including botanists, chemists, biophysicists, and geneticists, had developed for other purposes. However, before microbial taxonomists accepted those new methods, they first were evaluated by reexamining a diverse range of microorganisms. Notably, sequence-based methods were not accepted into microbial taxonomy until the early 1980s. Moreover, many taxonomists continue to resist accepting genome sequences as a means for defining microbial species.

Summary

- The amazing diversity of microorganisms makes it challenging for taxonomists to determine phylogenetic relationships among bacteria.
- By the end of the 19th century, leading biologists were calling for unified and systematic reforms to guide the naming and classifying of microorganisms.
- During the middle of the 20th century, the International Committee for Bacteriological Nomenclature developed criteria for classifying microbial species and encouraged reliance on culture collections as repositories of type and reference materials.
- By the late 1970s, taxonomists began to adjust to new molecular-based methods for classifying bacterial taxa above the rank of species—eventually agreeing that modern systematics should be polyphasic.

Nomenclature Practices Were Hazy the First Century of Microbiology

Some long-lasting problems in microbial systematics trace to the first description of a bacterial species in 1872. Because the early microbiologists were botanists, they adopted its binomial nomenclature, and thus its superficial phenomenological descriptions were immediately applied to microorganisms.

Between 1880 and 1920, the scientific community came to recognize the importance of microorganisms as etiological agents and as crucial components in food processing, agriculture, and ecology. As these efforts gained momentum, microbiologists bestowed vast numbers of novel names onto the organisms that they studied. Soon, this practice raised qualms among

Erko Stackebrandt is Director of the DSMZ-German Collection of Microorganisms and Cell Cultures GmbH in Braunschweig, Germany.



leading scientists. “We believe that the expansion of bacteriology, above all questions about variability, relationships, the distribution of inside and outside living organisms et cetera, should be handled by an orderly national or, better, international association of scientists,” noted Karl Bernhard Lehman (1858–1940) from Berlin, Germany, near the end of the 19th century. “One task of this collaboration should concentrate on the improvement of the mainly haphazard and non-scientific nomenclature of the Schizomycetes that provokes the sarcasm of any natural scientist.”

At first, there were no generally accepted rules for describing taxa, leaving taxonomists to classify organisms on a subjective basis. However, the Society of American Bacteriologists, which later became ASM, presented a major report on characterizing and classifying bacterial types that became the basis for the first edition of *Bergey's Manual* in 1923.

Although successive editions of *Bergey's Manual of Determinative Bacteriology* provided comprehensive descriptions of microbial species for more than 60 years, the editors were not recognized as the international authority for defining taxa. Hence, other systems were developed in parallel, and some of them changed rapidly to reflect new insights into the biology of strains. Some of those schemes were disputed, while others were temporarily discarded and then revived. By the end of 1970, about 30,000 bacterial names were compiled, and those listings included an enormous number of synonyms.

Despite those problems, however, the basis for later progress was laid between 1930 and 1980. For example, the Commission on Nomenclature and Taxonomy, which took shape in Paris in 1930, recommended that bacterial nomenclature standards be determined by a single international committee—at first called the Nomenclature Committee for the International Society for Microbiology and later renamed the International Committee for Bacteriological Nomenclature. This committee established a forum for disseminating its activities and conclusions. It also developed criteria for classifying microbial species and prepared lists of type species as well as names for species and genera *conservanda*. During this period, culture collections came to be more broadly appreciated as repositories of type and reference materials.

Stability of Nomenclature

Several initiatives from that era proved influential. During the 2nd International Congress for Microbiology, held in London in 1936, committee members began to draft a Code of Bacteriological Nomenclature that was presented at the 3rd International Congress in New York three years later. Although delayed because of the war, the code was approved at the fourth such congress, held in Copenhagen in 1947. Its name was later changed to the International Code of Nomenclature of Bacteria, and its fifth version was published in 1992. Its name changed again to International Code of Nomenclature of Prokaryotes in 1999, and it is available electronically (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=icnb.TOC&depth=2>).

In 1939, the members of the international committee, which became the International Committee on Systematics of Prokaryotes (ICSP) within the International Union of Microbiological Societies (<http://www.iums.org/>), also established a Judicial Commission (JC). Today, the JC oversees the nomenclature of prokaryotes, determines the rules by which prokaryotes are named, issues opinions concerning taxonomic matters, and revises the code.

That commission also was asked to review the basis of bacterial names, which traced to the 1753 publication of *Species Plantarum* by Linnaeus. During more than a decade, experts searched through lists of published names, reevaluating which of them should remain. The commission's *Approved Lists of Bacterial Names*, published in January 1980, abruptly reduced the number of valid bacterial species to about 2,500. This list is available online (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=bacname.TOC&depth=2>).

During the 5th Congress in Rio de Janeiro, Brazil, participants established a board to publish the International Bulletin of Bacteriological Nomenclature and Taxonomy. It was first released in 1951 by Iowa State College Press and has appeared quarterly since then. In 1966 the ICSB journal became the *International Journal of Systematic Bacteriology*, which ASM published until 1997. Subsequently renamed, the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) is now being published by the Society for General Microbiology in the United Kingdom.

Stackebrandt Calls Microbial Systematics Both His Hobby and His Work

Erko Stackebrandt came to science in part because of his inability to ski. When preparing to enter Ludwig-Maximilians University of Munich, he ranked biology only third after architecture and sports on his list of major interests. However, he failed the entrance exam in architecture, and the sports exam included a test in Alpine skiing, a skill that he had not acquired because of “being born and raised in the north German flatlands,” he recalls.

“I decided to study biology at the same university,” he says. “What I did not know at the time— because I had not prepared for a career in biology— was the fact that I could not have found a better place for studying this discipline.”

He showed no inclination for science before college, despite having access to his father’s microscope. “My preoccupation with this instrument, which was restricted to the observation of a few preparations of pond water and hay extracts, was probably less than that shown by most children who had access to this magnificent toy,” he says.

During Stackebrandt’s first two years at the university, he developed a deepening interest in zoology and botany, and made up for earlier lost time. “I caught up with activities that most of my classmates had begun a decade earlier, such as collecting insects and plants,” he says. “Within a few seasons, I had a nice collection of more than 2,000 specimens of beetles, mainly from Bavaria, Austria, and Southern Tirol.”

Although he could have pursued entomology, he says, “my future took an unexpected twist when I was confronted with the newly introduced discipline of microbiology

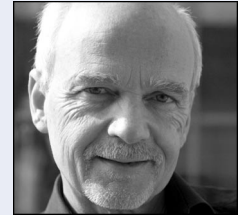
at the university and the professor who represented it from the end of the 1960s.” Otto Kandler, a botanist and microbiologist whose scientific focus in microbiology was on the physiology and taxonomy of gram-positive bacteria, became his mentor.

“The department was the world center for the analysis of the chemical composition of the [bacterial] cell wall,” Stackebrandt says. “The scientific atmosphere provided by Otto Kandler and his collaborators was fascinating and impressive for students because they could reconstruct the search for natural entities in bacteriology, and, equally important, the names of the supervisors could be found on the many publications that originated from this laboratory. “We were raised in the spirit that hard work and serious dedication to the scientific task provide excellent cornerstones for one’s career.”

Now Stackebrandt, 63, directs the German Collection of Microorganisms and Cell Cultures and is professor of microbiology at the Technical University, Braunschweig, Germany. He is a microbiologist who is deeply involved in studying the systematics, evolution, and molecular phylogeny of archaea and bacteria. His main interests are in the development and application of novel molecular tools for characterizing and more precisely identifying bacteria.

“My interests have not changed: covering bacterial systematics and molecular evolution, trying to order the enormous richness of prokaryote diversity,” he says. “The question about the meaning of the term ‘species’ in bacteriology, their delineation from each other, and the application of novel rapid

methods for authentication and rapid assessment of their phylogenetic position go hand-in-hand.”



He has worked on numerous projects supported by the German Science Foundation of the Ministry for Science and Technology. These include research on German soil and peat, Mediterranean coastal waters, the North Sea and Baltic Sea, Antarctic lakes, Australian soil and artesian wells, and the formation of stromatolites, as well as on giant ants, holothurians, the rumen of cows, and the digestive tract of koalas.

After completing his undergraduate and graduate studies, Stackebrandt served as a postdoctoral fellow with Carl Woese at the University of Illinois, Urbana-Champaign, and then worked with Karl Schleifer at the Technical University in Munich from 1979–1983. He was professor of microbiology at the University of Kiel from 1984–1990, and then spent three years in Australia, starting in 1990 as professor of microbiology at the University of Queensland in Brisbane.

Stackebrandt has two grown sons, one studying philosophy and politics, and the other soon to receive his university entrance diploma, “with a major interest in golf and science,” he says. He claims to have no outside interests. “My hobby is my work, still, after 40 years working in the field of microbial systems,” he says.

Marlene Cimons

Marlene Cimons is a freelance writer in Bethesda, Md.



IJSEM is now the 7th-highest-ranked non-review-type journal in microbiology, the premier journal for issues relating to nomenclature and taxonomic methods, and remains the publishing forum for the Judicial Commission. Validated species names are compiled monthly in the IJSEM Notification List, while those first published outside this journal may subsequently be validated, provided that type strains are deposited in at least two public collections in different countries. To date, IJSEM has validated about 6,500 species and 1,500 genera (www.bacterio.cict.fr).

Platform for a Pragmatic Classification System

Although bacteriologists carefully safeguard the *Code of Nomenclature*, these efforts are narrowly restricted to procedures for protecting validly named type taxa. However, the *Code* is not being used to influence several fundamental scientific questions, including current debates about species concepts and definitions. Despite a good deal of input from scientists in fields such as population genetics, microbial ecology, and system biology, the *Code* continues to rely on a pragmatic, somewhat arbitrary, but sound working definition of ranks between class and subspecies.

Despite significant differences in the types of scientific information being evaluated, the classification process from 130 years ago was not less laborious than it is today. Then as now, taxonomists included as much available information as they could—carefully detailing the cultural, physiological, and (later) serological properties into their taxon descriptions.

However, after 1960, bacterial taxonomists came to recognize more about the natural relationships among prokaryotes and began to elucidate those relationships more systematically. For instance, new guidelines that included minimal standards for describing microbial species and genera were published by the ICSP. Those guidelines include sections devoted to individual genera such as *Brucella*, *Bacillus*, and *Flavobacterium*, or groups of genera such as clostridia and *Clostridium*-like organisms, methanogenic bacteria, and the *Enterobacteriaceae*. These minimal standards are not inflexible rules, and are updated regularly whenever new methods or

information becomes available to help to delineate taxa.

The past 30 years of prokaryotic systematics has benefited tremendously from scientific progress. Various methods, including phenetic analysis, chemotaxonomy, DNA-DNA reassociation, and base composition analysis of DNA, have helped to reassess the degree of natural relatedness among similar bacterial strains. However, these methods cannot be used for relating higher taxa, even at the rank of genera.

This problem was overcome in the late 1970s when Carl Woese at the University of Illinois and his collaborators provided a system for classifying taxa above the rank of species. Based on comparisons of genes encoding ribosomal RNA (rRNA) molecules, this platform provides a very different view of this hierarchy from what Bergey's *Manual of Determinative Bacteriology* depicts. Indeed, this newer view presented such a break from the past that many traditional taxonomists at first rejected its validity. The molecular-based information, showing impressively the inability of epigenetic methods to depict phylogeny among prokaryotes, totally revised the traditional hierarchic structure at the levels of families, orders, and classes.

This lack of congruency almost separated bacterial systematists into two camps—one, whose members held onto traditional taxonomy, and the other, whose disciples embraced the newer molecular-derived approach to taxonomy. Matters came to a head in 1980. Larry Wayne of the Veterans Administration Medical Center in Long Beach, Calif., was asked to chair an Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Following a meeting in Paris, the committee members later concluded that “an ideal taxonomy would involve one system” that was to incorporate elements, including phylogenetic, descriptive, diagnostic, and associative, from many different systems.

Thus, modern systematics should be “polyphasic,” and no longer driven by single techniques, according to the 1987 report from the committee. Noting the value of recent DNA-DNA reassociation studies and 16S rRNA gene sequencing results, the ad hoc committee members further advised that “complete deoxyribonucleic acid (DNA) sequences would be the reference standard to determine phylogeny and phylogeny should determine taxonomy.” They

also urged caution about inferring hierarchies among microbial species based on any one class of conserved molecules, such as rRNA, and recommended the active search for alternatives.

New Horizons

Indeed, by the early 1990s, researchers were determining the sequences of alternative genes encoding enzymes that serve housekeeping functions. Although phylogenies that are based on some sets of genes align closely with those that are based on 16S rRNA sequences, phylogenies that are based on yet other genes lead to a very different picture of evolution. Many scientists now attribute many of these discrepancies to the large numbers of horizontal gene transfers that occur among bacteria.

At the strain level, key recombination events may be analyzed using multilocus sequence typing. This approach, which was developed to track variants among pathogenic bacterial species, has had a wider impact because it provides insights into forces that drive speciation. In turn, taxonomists began to use this approach because it may provide a genome-based means for circumscribing a taxon.

The value of DNA sequence data, especially 16S rDNA genes and protein-coding genes, for classifying bacterial species was reinforced in 2002 by an Ad Hoc Committee for the Re-evaluation of the Species Definition in Bacteriology. Nonetheless, the committee encouraged taxonomists to propose new species based on other genomic methods or techniques provided that, within the taxon studied, there is a sufficient degree of congruence between the technique used and DNA-DNA reassociation. Although laborious, the reassociation method

remains the gold standard for delineating species.

The view that new means are needed for identifying microbial species continues to gain momentum. In the near future, taxonomists are likely to encounter plenty of difficulties if they insist on using taxon “species” as defined today, according to Dirk Gevers of Massachusetts Institute of Technology in Cambridge, Mass., Frederick Cohan of Wesleyan University in Middletown, Conn., and Jeffrey Lawrence of the University of Pittsburgh in Pittsburgh, Pa., and their collaborators.

That definition was left arbitrary to facilitate communication among scientists. However, novel approaches will require taxonomists with open minds and new skills in phylogenetics, diagnostics, and bioinformatics. They will also need to display a higher degree of tolerance to change than that shown by systematists in the 1980s.

Progress accelerates. Participants at a 2006 American Academy of Microbiology colloquium recommended that the genome sequences of all validly described bacterial type strains be analyzed. Meanwhile, the colossal body of annotated genome sequence data along with multilocus sequence analysis from selected species with different degrees of recombination will drive a renaissance in microbial physiology research.

Systems biology will soon lead us to what 19th-century pioneers in microbiology could only dream about: a comprehensive effort to study the microbiota and to understand how they function and interact in their natural habitats. Whether newly emerging groups of genomically related strains will be treated as “species” remains to be decided.

SUGGESTED READING

- Baptiste, E., Y. Boucher, J. Leigh, and W. F. Doolittle. 2004. Phylogenetic reconstruction and lateral gene transfer. *Trends Microbiol.* 12:406–411.
- Cohan, F. M. 2006. Towards conceptual and operational union of bacterial systematics, ecology, and evolution. *Phil. Trans. Royal. Soc.* 361:1985–1996.
- Gevers, D., F. M. Cohan, J. G. Lawrence, B. G. Spratt, T. Coenye, E. J. Feil, E. Stackebrandt, Y. Van de Peer, P. Vandamme, F. L. Thompson, and J. Swings. 2005. Re-evaluating prokaryotic species. *Nature Rev. Microbiol.* 3:733–739.
- Lapage, S. P., P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seeliger, and W. A. Clark. 1992. *International Code of Nomenclature of Bacteria*. Washington, D.C., American Society for Microbiology.
- Lehmann, K. B. 1896. *In* K. B. Lehmann and R. Neumann (eds.) *Preamble* 1st ed. *Bakteriologie und Bakteriologische Diagnostik*. München: Verlag JF Lehmann.
- Maiden, M. C. J., J. A. Bygraves, E. J. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, K. Zurth, D. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt. 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic organisms. *Proc. Natl. Acad. Sci. USA.* 95:3140–3145.



- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath. 1980. Approved Lists of Bacterial Names. *Int. J. Syst. Bacteriol.* 30:225–420.
- Stackebrandt, E., W. Frederiksen, G. M. Garrity, P. A. D. Grimont, P. Kämpfer, M. C. J. Maiden, X. Nesme, R. Rossello-Mora, J. Swings, H. G. Trüper, L. Vauterin, A. C. Ward, and W. B. Whitman. 2002. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int. J. Syst. Evol. Microbiol.* 52:1043–1047.
- Tindall, B. J., P. Kämpfer, J. P. Euzéby, and O. Aharon. 2006. Valid publication of names of prokaryotes according to the rules of nomenclature: past history and current practice. *Int. J. Syst. Evol. Microbiol.* 56:2715–2720.
- Wayne, L., D. J. Brenner, R. R. Colwell, et al. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37:463–464.



What Sets Apart ASM Member Authors & Subscribers From All The Rest?

Advantages for ASM Member Online Subscribers

- Subscribe to the Journals Individually, or to All 11 Online for Only \$190.
- Free Access to the ASM Minireview Collection:
- Access to the Online Journals from any computer—anywhere
- Table/Figure Searching:
- Conduct keyword searches of table titles/ footnotes and figure legends.

Advantage for ASM Member Authors

- Discounted Page Charges
- Discounted Reprint Fees
- Discounted Color Figure Charges

www.journals.asm.org

NEW for 2007, your subscription

will also allow access to newly accepted primary-research manuscripts weeks before the final, typeset versions are published.

Click on the desired journal link at:

<http://journals.asm.org/subscriptions/papumbrellapre.shtml> and then click on "Accepts"



American Society for Microbiology

Journals Department
1752 N Street, NW, Wash., DC 20036-2904
Ph: 202-737-3600, Fax: 202-942-9355
E-mail: service@asmusa.org