Facts and Myths about Authentic Bacteria/ Lessons from Pioneers of Microbiology

A commentary on sundry aspects of bacteria in the natural world and in the laboratory, touching on: diversity, nutrition, the species concept, classification and taxonomy, "computer bacteroids," the "unculturability" myth, "astrobiology." extremophiles, and Gest's Postulates

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Gest's Postulates were promulgated in 1999 to address serious questions that developed in understanding the various relationships of extant bacteria and how their species should be classified and named (Gest: Amer. Soc. Microbiol. News **65:** 123). Aspects of the Postulates are reviewed here to remind scientists of possible penalties for contravening their provisions.

The Postulates addressed issues in continuing debates as to (a) whether 16S rRNA sequences found in natural sources actually signify extant bacterial species, (b) the legitimacy of using only 16S rRNA sequences as the basis for changing the names of established bacterial genera and species, and (c) the need for altering schemes for classifications of bacteria. Gest's Postulates relate to *actual* bacteria as contrasted with "virtual" bacteria reported to exist in natural sources as indicated by 16S RNA sequences ("virtuals" are sometimes referred to by the cognoscenti as "computer bacteroids"). The Postulates were designed to forestall premature corruption of *Bergey's Ultimate Manual of Actual Bacteria.* Accordingly, to register a 16S sequence with a generic or species name, the author must provide evidence that the sequence is (a) not a chimeric molecule due to PCR coamplification of 16S rRNA genes from mixed bacterial genomes (see Wang and Wang, Appl. Environ. Microbiol. **63**: 4645, 1997), (b) not due to common bacterial contaminants in reagents use to prepare genomic DNA (Tanner et. al., Appl. Environ. Microbiol. **64**: 3110, 1998), (c) not due to human commensals from saliva, exhalations, perspiration, or fingerprints, and (d) not due to laboratory variants of well-characterized species.

In addition, designation of a "virtual" as "unculturable" on the basis of observing no growth when yeast extract or similar complex supplements are added to a so-called "standard medium" will not be accepted by the Commission on Taxonomic Justice. Of particular importance is the "3/5" rule. Namely, failure to provide evidence for existence of a living cellular entity corresponding to “virtual” within three years will, in each instance, oblige an author to rescind the last five “16S virtuals” that he /she has designated in publications. Three such episodes will be declared a *taxonomic felony*, and after a grace period of one year will result in prohibition from obtaining cell cultures from the American Type Culture Collection and cooperating culture collections.

It is possible, however, to erase a taxonomic felony. To do so, the author must satisfactorily pass an examination (true/false) administered by the Judge Advocates Office of Nomenclature Reform. The examination is based on information in several classics of microbiology, including *Bacterial Nutrition* by B.C.J. G. Knight (1938), *The Collected Papers (i.e., Verzamelde Geschriften)* of M.W Beijerinck (1940), and *Microbiologie du Sol* by S. Winogradsky (1949) [for more details of these references see Gest 1999]. Erasure of a taxonomic felony also requires authors to state in succeeding papers dealing with “virtuals” the following observation by N. Palleroni (ASM News, October 1994, p. 537): “No shortcuts have ever been invented to bypass the integral study of the whole cell, since diversity is, of course, something more than a set of variations on the theme of a macromolecule.”

The “unculturability’ myth

For more than a decade, reports have continued to appear which state that more than 99% of naturally occurring bacteria cannot be cultured in the laboratory. This myth is repeated so often that it is now becoming routine in “popular” science articles.

An early claim of “unculturability” disproven by classic research in 1911

In ca. 1910, Frederick W. Twort (the discoverer of bacteriophage) undertook to isolate the bacterium responsible for tuberculosis of cattle. At the time, the disease was causing great losses of cattle in Britain and Europe. In the introduction of the classic 1911 paper of Twort and G. Ingram [Proc. Roy. Soc. LXXXIV, pp. 517-542], the authors noted: “All writers on this disease state that the causative agent cannot be cultivated outside the animal body.” Twort and Ingram proceeded to demonstrate that the agent, *Mycobacterium pseudotuberculosis*, can be grown in pure culture simply by adding extracts of dead cells of *Mycobacterium phlei*. This was one of the earliest researches showing requirements of many bacteria for “essential” growth factors. Later investigations showed that in this instance, growth of the organism requires a form of vitamin K. The Twort/Ingram paper is a model of arduous work, persistence, and deep thought. I think no reasonable scientist could read their paper and then make the statement that a bacterium is “unculturable” because it did not grow on “standard lab media.”

I have written a number of articles on the fallacious notion of "unculturability." The most detailed is readily available on the Internet, and can be accessed at http://hdl.handle.net/2022/3149 ["The modern myth of "unculturable" bacteria/scotoma of contemporary microbiology"]. It appears that the "unculturability" myth is now used mainly as a "sciencemanship" crutch to justify molecular metagenomics research grants as the panacea for unraveling the great complexities of bacterial ecology.

The power of misrepresentation. Charles Darwin's comment on this phenomenon: "Great is the power of steady misrepresentation; but the history of science shows how, fortunately, this power does not long endure" [*Origin of Species*].

What is a bacterial species?

The 1946 essay by C.B. van Niel (see reference at end) remains one of the most thoughtful analyses of the complex problems encountered in defining the concept of a bacterial species and how the bacterial species isolated in pure culture (now, ca. 7000) should, or could, be classified and named. It is remarkable that in 2009, there is still no generally accepted definition of a bacterial species. In 1946, van Niel asked a basic question and answered it as follows: " What. then, are species, i.e., the lowest of the taxonomic units? The answer is: that which the investigator who proposes the species wishes to include in accordance with his scientific tact. Obviously it is impossible to answer the question in any other way because Nature herself does not create species, but only individuals with their descendants, so-called ‘clones.’ And the systematist collects such clones into groups or bundles which he calls ‘species.’ How big or little he wants to make his packets---that depends upon his scientific attitude, which may vary for different individuals. He would free himself of this subjective delineation of species only in case he would base his deliberations on yet smaller units; *viz*., upon these clones themselves. But in that event he would have to culture all bacteria as single cell cultures, and that under every conceivable set of conditions under which they can possibly live; would have to note all their shapes and properties in dependence of these conditions, and incorporate them in the diagnosis of the respective clone. All clones which under these circumstances fail to reveal differences would have to be united into systematic entities, or ‘elementary species.’ And then he would proceed to group these entities, now more or less free from arbitrariness, into larger ones according to his precepts. In actual practice this is not possible; this need not be documented further because such a task would have no end…..” It is sobering to remember that van Niel wrote this decades before rampant lateral gene transfer among bacterial clones was discovered!

Diversity and ecology

The extraordinary biochemical diversity of bacterial life styles has been apparent since ca. 1910. A.J. Kluyver remarked on this as follows (in Kluyver and van Niel 1956): “It seems likely that a ‘macrobiologist’ who entered the microbiological scene around 1910 would have been most impressed by the great diversity in properties of the microbial species to which he was introduced by the microbiologist….Winogradsky, Beijerinck and those who followed them have made a thorough exploration of the world. Besides the fact that these investigations have proved the practically ubiquitous occurrence of many microorganisms on earth. they have thrown a clear light on the surprisingly large diversity in nutritional requirements of the various microbial types….I think we may expect that our ‘macrobiologist’ on being confronted with a nearly endless diversity of such monstrosities would find the microbiological scenery bewildering.” One class of “monstrosities,” the so-call “extremophilic” bacteria were discovered long before NASA began to learn about the existence of such terrestrial organisms. Extremophiles became popular in NASA news releases apparently because they give a slight glimmer of hope that such organisms might someday be found in the very harsh environments on Mars. For the public, NASA hype has quietly adopted all of terrestrial microbiology under the more glamorous title “astrobiology.” But there is still no evidence of any kind for the existence of life, past or present, beyond Earth.

So, what is new? For one thing, a *refocus* on how the myriads of bacteria interact with one another and with other kinds of organisms, micro- and macro-in nature. The latter is a grandiose prospect, likely to keep armies of scientists busy for a long time. There is little doubt that the study of pure cultures will remain the most reliable source of basic information for understanding the properties and evolutionary relationships of the vast majority of bacteria, as well as the dynamics of changes they catalyze in the biosphere.

As in other sciences, the great progress in microbiology and biochemistry over the past 50 years was accompanied by revelation of more and more layers of complexity in cell metabolism and growth. We can confidently expect this to continue. For prognostications on the status of biology in the year 2030, see the fascinating article by microbiologist John Postgate: “Biology forty years on,” in: *Biologist* **37**: 106-108 (1990). His view of the future: “I wish I could foresee a return to enlightenment in the boardrooms and corridors of power, but on present trends scientific research in 2030 will be largely in the hands of giant international corporations, mostly conducted in-house (for reasons of confidentiality), though some of it will be sponsored in compliant institutes of higher education and public service laboratories. That chimaera of the biotechnology era, the entrepreneurial savant will replace today's leader of research. I may be wrong; perhaps the environmental and human costs incidental to market-directed science and technology will have become so obvious as to swing the pendulum back again so that state agencies will be substantially in charge—as they were in the 1950s and 1960s. But in either case, both teaching and research will be on an emergency footing compared with today, compelled to cope with local, international, and global exigencies of overpopulation. Basic research will be as badly needed as ever, but it will have to be predicated increasingly on practical problems."

Additional Reading

Please note: Except for citation of bogus *"Bergey's Manuals,"* all references cited in this article are authentic. It is conceivable that the organizations mentioned in the text may be formed in the future.

Gest, H. 1993. Bacterial growth and reproduction in nature and in the laboratory. American Society for Microbiology (ASM) News **59**: 542-543.

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Gest, H. 1999. Gest's postulates. ASM News **65**:123.
Gest, H. 1999. Bacterial classification and taxonomy: a "primer" for the new millennium. Microbiology Today **26**: 70-72.
Gest, H, 2000. Report from the year 2025 meeting of the American Microbiological Society: Discovery of the bacterial "taxonomy gene." Microbiology Today **27**: 28-30. This paper details a suggestion made by Francis Crick in his book *Life Itself –Its Origin and Nature* [Simon & Schuster, 1981]. Namely, that an extraterrestrial "supercivilization" far more advanced than ours may have sent microbes to early Earth to test colonization possibilities. The 2025 report notes that the supercivilization could have deliberately designed bacterial genes containing messages of importance for edification of the higher forms of life that would eventually evolve on Earth. The very remarkable "bacterial taxonomy gene" was encoded in an obscure Chinese dialect that was finally decoded by a team of NASA's PABLUM PROJECT (Planetary AstroBiological Launch for Unidentified Microbes). Thus, the authentic names of terrestrial bacteria were revealed. Accordingly, most species names were changed for *Bergey's Ultimate Manual of Definitive Bacteriology*. One example: *Escherichia coli*, so named to honor Dr. Theodor Escherich (who first isolated the organism from the intestinal contents of human infants) will be renamed *Proteofermentoformicus lipocylindricus*.
Gest, H. and Favinger, J. 2001. Taxonomic ambiguities: a case history. Int. J. System. Evol. Microbiol. **51**: 707-710.
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diversity, “unculturability,” and fanciful speculations on the number of species of terrestrial bacteria.

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Gest, H. 2006. The 2006 astrobiology follies. Return of the phantom Martian microbes. http://www.bio.indiana.edu/~gest/

Gest, H. 2008. The modern myth of “unculturable” bacteria/scotoma of contemporary microbiology. http://hdl.handle.net/2022/3149
This article is dedicated to the pioneering microbiologists who isolated pure cultures of microbes responsible for (a) infectious diseases of animals and plants, and (b) the the cyclic transformations of major chemical elements on Earth. Their characterization of the biological, physiological, and genetic properties of these organisms paved the way for current research. The careers and contributions of more than 300 of the early pioneers are profiled in the classic book by William Bulloch: *The History of Bacteriology* (Oxford University Press 1938)

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van Niel, C.B.: 1946. The classification and natural relationships of bacteria. In: Cold Spring Harbor Symposia on Quantitative Biology, vol. 11: Heredity and variation in Microorganisms; pp. 285-301. [The Biological Laboratory, Cold Spring Harbor, L.I., N.Y.]