

The concept of bacterial species has become ever more fuzzy recently

Microbial systematics

“Species Don’t Really Mean Anything in the Bacterial World”

For more than a hundred years microbiologists have been trying to sort bacteria into species. Though how to define a bacterial species is still ambiguous, despite new technologies like genome sequencing that have added more information than ever before. Perhaps the idea of bacterial species ought to be buried completely.

What is a bacterial species? *Mycobacterium tuberculosis* causing tuberculosis, *Clostridium tetani* and *Neisseria gonorrhoeae* causing tetanus and gonorrhoea respectively are bacterial species. They were named by Robert Koch, Arthur Nicolaier and Albert Neisser in the late 19th century. At that time, many new bacteria were discovered and named. Their pathogenicity made it easy to name etiologic agents. However, the cases with an extremely narrow species definition are the exception. The vast majority of bacteria are still waiting to be classified because there’s no robust definition of what a bacterial species is.

Morphology is not enough

The discussion about definition and interpretation of the term species in general goes back to the 18th century, when Carolus Linnaeus formalised the taxonomic ranking of species. At first glance the definition of a species seemed to be so simple; “I look at the term species as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other”, wrote Charles Darwin. Appearance – or in scientific words: morphology – was the main distinctive feature used to categorize species. Indeed, humans intuitively use a concept of species in every day life. If the one big animal with a yellow coat and black stripes is a great hunter the others looking alike will have the same attitude. Let’s stay away from all tigers. One can eat little red fruits from plants with leaves similar to clover leaves – strawberries aren’t poi-

sonous. However, one should keep one’s hands off mushrooms with red and white spotted caps. In everyday life sorting species according to obvious, visible morphological traits makes living easier. In a similar way describing pathogenicity of bacteria is a very helpful and practical means to sort out etiologic agents. However, classifying all known bacteria into species needs more than that – namely a definition of meaningful biological and ecological criteria.

In 1872 the German botanist Ferdinand Cohn demonstrated the first system for classifying bacteria proposing bacterial genera and species according to their morphology. Until then many scientists believed that all bacteria were variations of one and the same organism. Later, when culturing of bacteria advanced, pathogenic, physiological and biochemical features were added for classification of the tiny organisms. *Bergey’s Manual* became the primary reference for bacterial taxonomy. By 1955, the field had

adopted a pragmatic but largely arbitrary – or should one say pretty spongy? – definition of bacterial species, namely “the type culture together with such other cultures or strains of bacteria that are accepted by bacteriologists as sufficiently closely related”.

Conflicting criteria

Since then bacterial taxonomists have added new analysis techniques – but all innovations were subjected to back-and-forth debates for some 30 years before finally being recognised as necessary for classification purposes. Well, it seems that taxonomists don’t break new ground very fast. First, chemical compounds like fatty acids and sugars were adopted to differentiate bacteria – chemotaxonomy was born. In the 60s DNA-DNA-hybridization (DDH) data as an indication for genetic similarity were integrated into the means for describing bacterial species. In the 70s microbiologists experienced an earthquake-like change in bacterial systematics when Carl Woese from the University in Urbana/Champaign (Illinois) separated the Archaeobacteria from all other bacteria and defined them as an independent kingdom of life on the basis of 16S rRNA gene sequences. In the following years protein sequencing and methods for culturing anaerobic bacteria were developed.

Using the rich menu of tests available avid scientists by and by named some 40,000 bacterial species. However, many of them were duplicates or clones of already named species. In 1980 a group of dedicated scientists went through this smor-



Species or not species – that’s the question.

gasbord with a fine-tooth comb and designated some 2,500 names as “validly published species”. Today almost 7,000 species have been named, most of them listed in the last edition of *Bergey’s Manual*. This bible of bacterial systematics assigns those species to 26 phyla, 41 classes, 88 orders, 240 families and 1194 genera.

The gold standard for classifying bacteria reads as follows. Two strains belong to one species if:

- ▶ Their genomes show more than 70% DDH,
- ▶ The 16S rRNA sequences show 97% or more identity, or
- ▶ Phenotypic data show a high degree of similarity.

Don’t be fooled, these criteria are far from being well-founded.

The degree of DDH was by no means based on a theoretical species concept but was calibrated empirically to harmonise data with the already recognised phenotype-based species. Soon, conflicting data appeared. There were quite a few cases where strains of bacteria did not fulfil the 70% DDH criteria but should have been

grouped as one species according to their rRNA sequences. These intriguing data come from lateral gene transfer (LGT). The sheer enormity of bacteria exchange and recombinant genetic material has been drastically underestimated by scientists for a long time.

A veritable gene hotchpotch

That gain or loss of bits and pieces of genes may entail dramatic change in the phenotype. Natural transformation, transduction and conjugation are three mechanisms for transporting foreign DNA into the cell. The relative contribution of each mechanism varies from species to species. *Neisseria meningitidis* and *Helicobacter pylori* for example can take up naked DNA. In the pre-genomic era, only a handful of examples of LGT were detected as discordant between phylogenetic reconstructions with different housekeeping genes. Recent large scale genomic sequencing revealed that recombination of DNA leads to a veritable gene hotchpotch from which even the most advanced and dedicated genome analysis programmes cannot deduce which genes dis-

criminate one species from another, not to mention building phylogenetic trees.

30% variation within “species”

In the study of 70 closely related and fully sequenced bacterial genomes Konstantinos Konstantinidis and James Tiedje (Michigan State University, USA) found that ANI (average of nucleotide identity) was around 94% corresponding to the traditional 70% DNA-DNA reassociation standard of the current species’ definition. However, individual strains of the same species could vary up to 30% in gene content, raising the question as to whether they should belong to the same species or whether one should define bacterial species on a more ecological basis as ecotypes, provided that this variation is driven by differences in the ecology of strains.

Given the fact that we need to know more about bacterial genomics the Joint Genome Institute in collaboration with the German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig (Germany) started to sequence full genomes of all so far named bacterial species. The

In this dispute Frederick Cohan from Middletown, USA, came up with the idea to avoid the term species and instead use ecotypes for describing populations of sufficiently similar bacteria living in certain habitats. One main critique, however, is that ecotypes only describe the evolution of a species allowing for ecological issues. "Ecotypes cannot substitute for the term species," says William Hanage. He argues that using the term bacteria with the same connotation as animal or plant species is "simply silly". "In fact, species don't really mean anything in the bacterial world," says Edward Feil. "Species means different things to different people and we simply have to live with that." Carl Woese and Nigel Goldenfeld (University of Urbana-Champaign, USA) wrote in *Nature*: "... the emerging picture of microbes as gene-swapping collectives demands a revision of such concepts as organisms, species and evolution. rather than discrete genomes, we see a continuum of genomic possibilities, which casts doubt on the validity of the concept of a 'species' when extended into the microbial realm."

Now you may feel stranded by the fact that nobody can tell you whether bacterial species is a matter of fact or fantasy. Hold on, it becomes worse. The author of this article was perplexed and had to learn that scientists are earnestly discussing in one of the most prestigious journals the fact that the term "Prokaryote" has been put up for discussion because it is defined via a negative feature, namely, the missing nucleus.

Omitting the term "prokaryote"?

Norman Pace from the University in Boulder (Colorado, USA) argued in *Nature* for omitting the term "prokaryote" completely from the scientific word pool and using the term "microbes", if one likes to pool Bacteria and Archaeobacteria. This view provoked strong opposition from William Martin (University of Düsseldorf, Germany) and Eugene Koonin (National Center for Biotechnology Information, Bethesda, USA), because many microbes are eukaryotes. They see the term "prokaryote" as being a valid definition for organisms because they are the only ones that translate nas-

cent RNA transcripts on their main chromosomes.

Michael Doland and Lynn Margulis (University of Massachusetts-Amherst, USA) joined this debate with the argument that another important feature of prokaryotes is the uni-directional exchange of genetic material while eukaryotes conduct this genetic trade by cell fusion. Thomas Cavalier-Smith (University of Oxford, UK) quibbled that there is indeed a need for a "concept of a bacterium" but one cannot term bacteria as microbes. Instead he embraces the terms Archaeobacteria and Eubacteria to name them.

After the author received notice of this discussion she rigorously deleted the term "microbes" from this article and minimised the use of the term "prokaryotes" as far as possible. She hopes that irrespective of whether one sees this discussion as a simply linguistic or as a conceptional problem, it documents that microbiologists and evolutionary biologists really should sit down together to at least finalise this issue.

KARIN HOLLRICHER