Taxonomic Note

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Should names reflect the evolution of bacterial species?

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Systematics is the process of characterizing and arranging bacterial diversity in an orderly manner, recognizing groups of similar organisms in a hierarchical scheme whose basic entity is the species. To allow the exchange of scientific knowledge, taxa have to be named. Taxa are not static entities since they are subject to evolution, the direction of which can be inferred by using a wide range of techniques targeting specific traits. However, it is not clear how this dynamism should be reflected in taxonomic nomenclature. In the present report, several considerations are presented that deal with the relationship between the evolution of taxa and their nomenclature; an example is given which concerns the nomenclature of the species *Lactobacillus delbrueckii*.

Species definition is the aim of systematics. It is now widely recognized that the underlying basis of systematics is evolution (Stackebrandt *et al.*, 2002), accepting Darwin's foresight that '*our classification will come to be...genealogies*'. The central role of evolution in taxonomy in the past century has been outlined by Hennig (1966) and Mayr (1998) and re-proposed by several contemporary authors, including the bacterial species concept (de Quieroz & Gauthier, 1992; Cantino *et al.*, 1999; Cohan, 2002), reflecting the fact that '*the process of doing systematics requires periodic adjustment to scientific advances*' (Stackebrandt *et al.*, 2002).

The ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt *et al.*, 2002) underlined the need for elucidating interspecific and especially intraspecific population structure by different techniques, which is a clear attempt to investigate the evolutionary processes of differentiation and speciation.

Therefore, an evolutionary approach to systematics is a demand felt by a broad range of scientists, which has its salient reflections in the proposal of a phylogenetic taxonomy with its own nomenclature (de Quieroz & Gauthier, 1992; Cantino *et al.*, 1999). It is also underlined by the change in the name of the *International Journal of Systematic Bacteriology* to the *International Journal of Systematic and Evolutionary Microbiology*.

Even if all these issues reflect differing views of biological classification (Woese, 1998), their common denominator is the focus on the central role of evolution.

The present article aims to open a debate on the relationship occurring between evolution in classification and nomenclature, since, in our opinion, several contradictions arise between the *Bacteriological Code (1990 Revision)* and the positions of authoritative people. In other words, if an evolutionary flow is highlighted and a speciation event is detected, a new species will be described and named, but should evolutionary perspective reflect on nomenclatural designation?

According to Sneath (1989), '*nomenclature has been called the handmaid of taxonomy*' and it is determined by classification, so that '*progress in classification must reflect progress in knowledge...and changes in name must reflect progress in classification*'. He stated also that '*classification determines nomenclature, not nomenclature classification*' (Sneath, 1989) and, accordingly, General Consideration 4 of the *Bacteriological Code* (Lapage *et al.*, 1992) states that '*rules of nomenclature do not govern the delimitation of taxa nor determine their relations*'. On the contrary, the reverse is true, i.e. changes in classification determine changes in name, '*some changes in name are thus inevitable*' (Sneath, 1989), which can be inferred from Principle 9 of the *Bacteriological Code* (Lapage *et al.*, 1992), according to which '*the name of a taxon should not be changed without sufficient reason based...on further taxonomic studies*'.

The main consequence of these considerations is that names convey information, as strongly affirmed by the phylogenetic system of nomenclature (Cantino *et al.*, 1999). Nevertheless, Principle 4 of the *Bacteriological Code* (Lapage *et al.*, 1992) states that '*the primary purpose of giving a name to a taxon is to supply a means of referring to it*'.

It could be argued that the principal aim of names is stability

(Principle 1 of the *Bacteriological Code*), and different phylogenetic studies could lead to confusion and unnecessary changes of names if contrasting results are obtained.

This problem can be split into two parts, considering nomenclatural designation at the species or the subspecies level. Concerning the species, if a taxon has stemmed from another reaching a great extent of diversity at the DNA–DNA hybridization percentage, this will determine the change of the status of the isolate, implying the acquisition of a new name. Considering subspecies, if the population structure is revealed by several techniques (Stackebrandt *et al.*, 2002) and the evolutionary descent is clearly determined, evolution-based names will be forced to be stable afterwards, since the subspecies, as the species, are irreversibly separate (Cohan, 2002). The only further possible change could be the birth of a new species when a sufficient degree of whole-genome diversity is reached.

Genome sequencing will probably be the way to provide a definitive classification system for organisms, even if the great amount of data produced could also lead to some confusion (Pennisi, 1998; Lake *et al.*, 1999).

The case of the species *Lactobacillus delbrueckii* will be briefly explained as an example of the contradictions between the Rules of nomenclature and the recent perspective in bacterial systematics.

At the end of the nineteenth century, Beijerinck described *L. delbrueckii* (Beijerinck, 1901) as a simplified version of *Lactobacillus fermentum* var. *delbrückii*, described by Leichmann (1896). Rogosa & Hansen (1971), on the contrary, recognized that *L. delbrueckii* (type strain isolated from sour grain mash) and *L. fermentum* (type strain isolated from fermented beets) were separate species. At the beginning of the twentieth century, two closely related species, *Lactobacillus lactis* and *Lactobacillus bulgaricus*, were isolated from dairy products. The species *L. delbrueckii*, *L. lactis* and *L. bulgaricus* were at first considered as separated species (Rogosa & Hansen, 1971), but later, using the molecular methods available, they were found to exhibit DNA–DNA homologies of 90–100% with each other and were grouped into one species (Weiss *et al.*, 1983). As rule of priority (Rules 23a and 23b of the *Bacteriological Code*), *L. delbrueckii* was designated type species and the three type strains were described and validated as subspecies, namely, *L. delbrueckii* subsp. *lactis* with its type strain ATCC 12315^T, *L. delbrueckii* subsp. *bulgaricus* with its type strain ATCC 11842^T and *L. delbrueckii* subsp. *delbrueckii* with its type strain ATCC 9649^T.

In a recent study (Germond *et al.*, 2003), the phenotypic and genotypic characteristics of the metabolic pathways essential for growth in milk of 64 selected *L. delbrueckii* strains, out of a collection of over 120 strains, were analysed. These included 16S rDNA sequence mutations, expression of β -galactosidase and of the cell-wall-anchored protease, the characterization of the lactose operon locus and of its

repressor gene in particular, galactose metabolism, and the distribution of insertion sequences, as suggested by Gurtler & Mayall (2001) to elucidate the taxonomy and evolution of bacterial isolates.

The three subspecies of *L. delbrueckii* presented markedly different traits related to distinct ecological adaptation, and from those evidences it was concluded that *L. delbrueckii* subsp. *lactis* is the taxon closer to the ancestor, while *L. delbrueckii* subsp. *bulgaricus* acquired, by a series of mutations, a metabolism principally devoted to lactose utilization and lactic acid production. It also lost several sugar metabolic pathways during its speciation compared to *L. delbrueckii* subsp. *lactis*. This differentiation probably occurred under the evolutionary pressure of the use of these strains for the rapid fermentation of milk in yoghurt production. *L. delbrueckii* subsp. *lactis* was less subjected to selective pressure as it was used for cheese manufacture, where rapid fermentation is less essential, and it kept a wide range of sugar metabolic pathways. *L. delbrueckii* subsp. *delbrueckii* was found to be close to *L. delbrueckii* subsp. *lactis* by the same sugar metabolic pathways, but it was not able to grow in milk, since the lactose operon locus was completely deleted and an inactive casein-specific cell-wall-anchored protease was detected. Moreover, this subspecies is represented by only two strains in the major culture collections, which suggests that the deletion of the lactose operon was a limited phenomenon, involving a small number of strains which were then forced to evolve outside their native habitat, milk. The evolutionary relationships between the three subspecies of *L. delbrueckii* were defined by the analysis of the 16S rDNA sequences of the three type strains observing that all of the base differences between the 16S rDNA sequences of the three subspecies were C or G in *L. delbrueckii* subsp. *lactis* and T or A in *L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *delbrueckii*. It is known that the deamination of a cytosine when methylated can lead to a thymidine. The mutations are then oriented and seem to affirm that *L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *delbrueckii* accumulated six and four mutations, respectively, evolving from a strain very close to *L. delbrueckii* subsp. *lactis*, which seems to play the role of reservoir for speciation.

Considering the present Rules of nomenclature, particularly Rules 15, 23 and 55 (Lapage *et al.*, 1992), the three subspecies of *L. delbrueckii* cannot be renamed according to the reconstructed evolutionary history, which seems a contradiction if ‘taxonomy should be hierarchical in the sense that it reflects natural relationships, therefore a broad range of phylogenetic parameters should be considered, so that a practical system for classifying bacteria will be absorbed by the phylogenetic system’ (Stackebrandt, 1988).

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