ON THE SPECIES-DEFINITION

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ABSTRACT. In modern classification the species are defined as groups of related sero-fermentative phage-types and are the fundamental units, while in orthodox classification, which is beginning with the higher groups, the species are defined only by biochemical (mostly fermentative) methods.

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We have two different classifications which we can call the orthodox and the modern classification. The orthodox classification is represented by Bergey's Manual and the modern classification by the 1st Report of the Salmonella Subcommittee in 1934.

In the orthodox classification the species are defined only by biochemical methods, whereas in the modern classification the species are defined biochemically as well as serologically and are subdivided into serofermentative phage types. In contrast to genera and subgenera which are defined biochemically, the definition of species is based mostly on serological methods.

The first 36 Salmonella species were published by the Salmonella Subcommittee in 1934. Further species were published by the Subcommittee in the Reports to the International Congresses in New York 1939 and in Copenhagen 1947. At the end of 1947, 140 Salmonella species were recognized and validly published in the Proceedings of these Congresses.

In the following years a change occurred, since the Enterobacteriaceae Subcommittee used only common names like "group" and "type." I regret that I have followed these suggestions for a long time, but finally I realize that it is wrong for members of a Subcommittee of the Nomenclature Committee to use only common names. We have to propose

scientific names and to use the accepted designations genera and species according to the Bacteriological Code.

Therefore, I returned to the 1st Report of the Salmonella Subcommittee and have published in 1961 all known "types" as species. I have defined the species as follows: "A species is a group of related serofermentative phage types."

To give an example, let us consider the species S. typhi-murium, one of the largest species consisting of many serofermentative phage types which are the smallest units. All cultures of S. typhi-murium belong to the serotype 1,4,5,12:i:1,2 but occur in 4 different serological subtypes:

- 1, 4, 5, 12:i:1, 2
- 4, 5, 12:i:1, 2
- 1, 4, 12:i:1, 2
- 4, 12:i:1, 2.

These 4 subtypes are combined in the Kauffmann-White-Scheme to 1,4,5,12:i:1,2, only for simplification.

Each of these serological subtypes can be divided into different fermentative types, for example, into inositol-positive or negative types or into d-tartrate-positive or negative types, etc. Further, each of these serofermentative types can be divided into several phage types. Therefore, the fundamental elements of the species are the exactly defined serofermentative phage types, for example: serotype 4,5,12:i:1,2 - fermentative type 20 - phage type 1.

Such a culture is the smallest entity which we are determining in our routine diagnosis, this being a serofermentative phage type. If we combine 20 or 40 of such fundamental elements all belonging to the serotype 1,4,5,12:i:1,2 to one group and call this group a species, we are right to do this. Since the officially accepted type species of the genus Salmonella is S. cholerae-suis, it is correct and logical to call S. typhi-murium a species as done in the 1st Report of the Salmonella Subcommittee.

As the same fermentative types can occur also in other serotypes, and as the phage types are not always determined, the diagnosis of the serotype is the most important procedure and the basis of the whole classification.

To avoid misunderstandings, it should be stressed that the orthodox definition of the species which is biochemically defined, is abandoned in the modern classification. The biochemical subdivisions of a genus are now called subgenera. In the genus Salmonella I have established 3 biochemically

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defined subgenera I, II and III, but I cannot agree to regard these 3 subgenera as species since they are defined only biochemically. We have to take those elements as species which can be defined both biochemically and serologically. Only in this way is an exact diagnosis possible (see Table).

Table

Genus	Salmonella
Subgenus	I II III
Species	<u>S. paratyphi-A</u>) <u>S. paratyphi-B</u>) I <u>S. typhi-murium</u>) etc.
Serofermentative phage type	<u>S. typhi-murium</u> serotype 4, 5, 12:i:1, 2) fermentative type 20) phage type 1) etc.

With regard to biochemical tests, especially fermentative reactions, the value of such a test is not the same in different genera or families. Also inside the same genus the value of different tests is not the same. The organic acids, like d-tartrate, sodium-citrate and malonate, are much more important than other tests like maltose fermentation. Therefore, it is not possible to regard all these tests as equal and to treat the results uniformly.

I have to mention this as it is not possible to discuss the problem of species definition without a general discussion on classification. Therefore, I have to touch also the problem of the so-called natural classification.

As known it is possible to make transductions or recombinations in the laboratory, to create new species. We suppose that such recombinations etc. occur also in nature, but we don't know the parents of these cultures and cannot establish a family tree. Therefore, I am regarding a natural classification as a utopia.

We have to classify our cultures by the determination of their reactions, in other words we have to diagnose the phenotype. In the diagnosis of Enterobacteriaceae we are determining the biochemical, the serological and the phage reactions. In this way we have established a solid classification from below, just as a house is built from the basement and not from the roof. The basic elements are the species which are composed of many serofermentative phage types. Therefore, we have to start our classification with these species, and not with higher units like genera, families etc.

While the orthodox classification starts with the higher units, the modern classification begins from below. The higher groups are only biochemically defined and therefore badly defined. There are not sharp frontiers between these higher groups, they cannot be defined exactly. However, a species, like S. typhi-murium, can be defined exactly by 40-50 different reactions. A culture which has the antigens 4, 5, 12:i:1, 2 and the typical fermentative behaviour, can be diagnosed quickly and precisely. Every day hundreds of such cultures are diagnosed in many laboratories all over the world. Such a diagnosis gives all the necessary data for an epidemiological investigation, not only for practical purposes, but also for scientific studies. If we know that the O antigens are 4, 5, 12, we know simultaneously the chemical constitution of the sugar-components. We know that this culture contains glucosamine, heptose, galactose, glucose, mannose and abequose which constitute the determinative component for the O antigen 4 of the Kauffmann-White Scheme. In determining serological properties, we are determining in reality part of the chemical constitution of the bacterium. These results in combination with the determination of the enzymes, or the fermentative properties, give such a complete and reliable picture that it is the solid basis for our classification.

I am convinced that we cannot establish a better or more expedient classification.

In summarizing, I should like to stress the following points:

We have to start our classification from below, beginning with the species which are groups of related serofermentative phage types. This is the decisive point in modern classification, in contrast to orthodox classification in which only

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biochemical methods are used for the determination of higher groups.

The modern classification was started by the 1st Report of the Salmonella Subcommittee, and is characterized by the combination of biochemical and serological methods in the diagnosis of species. During thirty years it has been clearly demonstrated that this system is effective and useful both for practical and scientific investigations. This is valid not only for the genus Salmonella, but also for other genera of the family Enterobacteriaceae and for other families. It is therefore a general principle.

In routine diagnosis the most important investigation is the determination of the serofermentative phage types, that means the diagnosis of the species, but not the definition of higher groups, like families, orders etc., since only the species can be sharply defined.

Therefore, the International Nomenclature Committee is requested to direct the attention to the species level and to start classification from below and not from above. We have to start with the definition of the species and to analyse the organisms both by biochemical and serological methods.

In contrast to orthodox classification the definition of the species as a group of related serofermentative phage types is the fundamental point and the solid basis of modern classification.

If we want to classify, we have to know exactly the whole material which we want to classify, but in bacteriology this is not the case today, with the exception of the genus Salmonella and some other genera.

Therefore, it has not been possible to establish a general, useful classification. To do this we have to start with the serofermentative phage types which are the fundamental elements forming the species. The 1st Report of the Salmonella Subcommittee is based on such an investigation, but Bergey's Manual has not such a basis. The necessary knowledge of the whole material which should be classified is lacking. This fact explains clearly the good results of modern classification and the failure of orthodox classification or of Bergey's Manual. There is "chaos" in classification because we have not the necessary knowledge today. I can only advise the abandonment of every classification of a new field until it has been investigated in the same way as the genus Salmonella. We can talk on this problem 30 years

from now since the teamwork of many investigators for a period of about 30 years is necessary to analyse a large group of bacteria before a useful classification can be started.

REFERENCES

- Kauffmann, F. 1961. Die Bakteriologie der Salmonella-Species. Munksgaard, Copenhagen.
- _____. 1961. The species-definition in the family Enterobacteriaceae. Intern. Bull. Bact. Nomen. Taxon. 11:5.
- _____. 1960. Two biochemical subdivisions of the genus Salmonella. Acta path. et microbiol. scandinav. 49:393.
- _____. 1963. Zur Differential Diagnose der Salmonella Subgenera I, II and III. Acta path. et microbiol. scandinav. (in press)
- Salmonella Subcommittee. 1934. The genus Salmonella
- Lignières, 1900. Jour. Hyg. 34:333.