International Committee on Systematic Bacteriology

Subcommittee on the Taxonomy of *Mycoplasmatales*

Minutes of Interim Meeting, 22 September 1976

London, United Kingdom

Minute 1. Call to order. The meeting was called to order at 9:30 a.m. on Wednesday, 22 September 1976, by the Acting Chairman, E. A. Freundt, at the Ciba Foundation.

Minute 2. Record of attendance. Members present were: E. A. Freundt, Acting Chairman, D. G. ff. Edward, S. Razin, R. F. Whitcomb, G. S. Cottew, R. M. Lemcke, D. Taylor-Robinson, J. Fabricant, J. G. Tully, and M. F. Barile. The Chairman-Elect of the Board for the WHO/FAO Programme on Comparative Mycoplasmaology, P. Whittlestone, attended the meeting as an observer.

Minute 3. Report of the Chairman. The Chairman of the Subcommittee since its establishment in 1966, Dr. D. G. ff. Edward, had resigned from office in December 1975. The Secretary, in his capacity of Acting Chairman, expressed the Subcommittee's deep appreciation of the effort Dr. Edward had devoted to the work of the Subcommittee and for his outstanding contributions to the development of mycoplasma taxonomy during its transition from a very primitive state to its present level.

With the purpose of informing the ICSB of the close liaison between the Subcommittee and the Board for the WHO/FAO Programme on Comparative Mycoplasmaology on various international aspects of mycoplasma taxonomy, several working documents and publications of the Board were forwarded to the ICSB chairman and other officials of that organization. As yet, the ICSB had not reacted to the request of the Subcommittee to change its name to "Subcommittee on the Mollicutes."

Following recommendations by the Subcommittee (4), a recent publication has reconsidered the taxonomic status of the "Donetta-like" strains (1). On the basis of data derived from serology and nucleic acid hybridization studies, the authors proposed that *Mycoplasma agalactiae* subsp. *bovis* was sufficiently distinct to be elevated to species rank under the name of *M. bovis* and with the type strain Donetta (PG45). In the discussion that followed, the Subcommittee expressed general support for the elevation of *M. agalactiae* subspecies *bovis* to species rank. Some members, however, although acknowledging that Rule 50a of the *Bacteriological Code* dictated application of the species designation *M. bovis* in this case, expressed concern that automatic application of Rule 50a could have very unfortunate results. For example, it would be possible that authors who supplied a completely adequate characterization of a new species might fail to achieve priority because their taxonomic description had been predated by an inadequate description that had been accompanied by the incorrect

application of a new subspecies epithet to an existing species name. Although the Subcommittee agreed that this particular case did not pose a clear-cut example of such an injustice, it was felt that the specifics of this particular case should be conveyed to the ICSB for their consideration in future revisions of the *Code*.

Minute 4. Taxonomic status of *Spiroplasma citri* and related organisms. The Subcommittee noted the proposal of Skripal (8) to establish a new family, *Spiroplasmataceae*, in the order *Mycoplasmatales*. This proposal took "into account such properties as ability to active movement, staining by Gram, a peculiar spiral-like shape of the cells with a distinctly developed main body parallel with such characters as the absence of the cell wall, polymorphism and pleomorphism of cells," plus the ability to form fried-egg colonies, filtration through 220- to 450-nm pores, inhibition by homologous sera and tetracycline antibiotics, high resistance to penicillin, and lack of reversion to cells with true cell walls. Although expressing reservations about the reliability of currently used Gram-staining methods in spiroplasma identification, and expressing serious reservations about the significance of the "main body," the Subcommittee expressed agreement with the principle and concept of a new family, agreed that all data published since the previous Subcommittee meeting (4) supported the placement of the family in the order *Mycoplasmatales*, and further agreed that the proposal was validly published under the existing provisions of the ICSB code. However, the Subcommittee also expressed the view that future proposals of this nature should be seriously scrutinized to determine whether they are in conformance with the provisions of the revised code.

The Subcommittee noted recent rapid advances in isolation of spiroplasmas and congratulated discoverers of new strains. However, the Subcommittee also noted confusion relating to the spiroplasma species concept. For example, sodium dodecyl sulfate (SDS) gel electrophoresis, growth inhibition, and deformation tests have yielded conflicting data on the inter-relatedness of spiroplasma isolates tested. It was felt that such issues should be resolved before any currently cultivated spiroplasmas are assigned taxonomic epithets. The Subcommittee, therefore, suggested the creation of an ad hoc working team on spiroplasmas under the general guidance of the Subcommittee to assist in rapid resolution of some of the problems. This Working Team, with J. M. Bové as Chairperson and R. F. Whitcomb as Vice-Chairperson, will be asked to carry out a comparative study of as many triply-cloned spiroplasma strains as possible by such techniques as restriction-enzyme patterns, SDS gel

patterns, deoxyribonucleic acid hybridization tests, and deformation, metabolism-inhibition, and growth-inhibition serological tests.

Minute 5. *Taxonomic implications of the inclusion of sterol-requiring as well as non-sterol-requiring organisms in genus Anaeroplasm.* The Subcommittee took note of a recent proposal (7) for "the establishment of a new genus for anaerobic mycoplasmas, the proposed genus to include . . . strains that either require or do not require sterols." The Subcommittee also noted that two of the non-sterol-requiring strains, designated as strain numbers 161 and 162, were reported (7) to have guanine-plus-cytosine contents of 40.2 and 40.3 mol%, respectively, in contrast to the 29.3 mol% reported for the type strain of *Anaeroplasm abactoclasticum* Robinson et al. The proposed inclusion of both sterol-dependent and sterol-nonrequiring organisms in a single genus would necessitate a major revision of taxonomic concepts within *Mollicutes* because sterol dependence or independence is now used to separate families of the order *Mycoplasmatales*. The Subcommittee therefore agreed that new strains exhibiting properties as widely divergent as *A. abactoclasticum* and strains 161 and 162 should not at present be placed within a single genus.

Minute 6. *Taxonomic status of non-cultivable mycoplasmas.* The taxonomic status of fastidious, non-cultivable mycoplasma strains was reviewed. For example, certain strains of *Mycoplasma hyorhinis* will not grow on artificial broth agar media, but can be isolated, identified, and studied by use of cell culture procedures commonly used for the classification of rickettsial, chlamydial, and viral agents (3). Although the existing proposal for minimal standards (5) requires that an organism belonging to the class *Mollicutes* must be shown to produce typical colonies on artificial agar medium, these results suggest that mycoplasma species, or strains of existing species, may exist that could not be studied in agar culture. However, after some deliberation, it was agreed that the requirement for growth on agar was necessary at present to assure and encourage proper and careful studies for the characterization of candidate mycoplasmas. Retention of this requirement does not preclude eventual classification in *Mollicutes* of non-cultivable wall-free procaryotes, especially if biochemical deletions could be demonstrated to explain their non-cultivability. However, extensive and careful documentation, as is available for *Chlamydia psittaci* (6), would be necessary.

Minute 7. *Taxonomic status of the Mollicutes.* The Subcommittee reviewed and discussed the arguments that were originally advanced to support the segregation of the mycoplasmas from the Class *Schizomycetes* (2, 9). Also, the Subcommittee noted with interest studies on the phylogenetic relationships between mycoplasmas and other procaryotes based upon the electrophoretic behavior of their ribosomal ribonucleic acids (M. E. Reff, E. J. Stanbridge, and E. L. Schneider, unpublished data). The data obtained from these studies provide additional evidence to support the assignment of the mycoplasmas and bacteria to separate taxonomic classes.

In a letter that had been communicated to the Subcommittee, Professor J. C. Senez, Secretary Gen-

eral of IAMS, had made the proposal that "the denomination and the attributions of the ICSB should be modified as to cover both bacteria and mollicutes," provided the *Mollicutes* be accepted as a Class. The Subcommittee would strongly endorse such a proposal, should it be formally advanced.

Minute 8. *List of approved names of species of Mycoplasmatales and affiliated organisms.* A revised proposal of approved names of species of the Class *Mollicutes* was circulated and approved for transmittal to Professor V. B. D. Skerman, Chairman of the ICSB, in conformance with an earlier request.

Minute 9. *Proposal for revision of "Minimal Standards for Descriptions of New Species of the Order Mycoplasmatales."* A working draft of a proposed revision of the published document, which had been distributed in advance of the meeting to the Subcommittee members, was discussed. Further drafts will be circulated by the new Secretary to the members until concurrence is reached.

Minute 10. *Report of the Board for the WHO/FAO Programme on Comparative Mycoplasmaology.* J. G. Tully, Chairman of the Board, presented a brief report describing some current activities of the Board and of working teams established to study mycoplasmas of various host groups. The Working Team on Avian Mycoplasmas should complete studies shortly on the five unnamed "serogroups" of avian mycoplasmas and will propose specific taxonomic designations for these organisms in accordance with currently accepted minimal standards. The Board has recommended close liaison between the Working Team on Ureaplasmas and the Subcommittee to develop international agreement on retention of "serogroup" designations within ureaplasmas, and until more adequate information is available on their classification and taxonomy.

The Board has also approved the formation of a new Working Team on Plant and Insect Mycoplasmas to be led by M. J. Daniels of Norwich, United Kingdom. The Chairman informed the Subcommittee of the Board's interest in the taxonomic status of newly isolated spiroplasmas and of the Board's desire to participate in various international cooperative endeavors to develop adequate procedures and minimum standards for proper classification of these organisms.

Minute 11. *Liaison with ASM Subcommittee on the Taxonomy of the Mycoplasmas.* The Chairman of the American Society for Microbiology (ASM) Subcommittee, J. G. Tully, discussed very briefly the aims and programs of this group. The members of the ASM Subcommittee have agreed to submit appropriate recommendations and comments on various taxonomic matters relating to the mycoplasmas to both the ASM and to the ICSB Subcommittee concerned with these organisms. In this way it was felt that the efforts of the ASM Subcommittee would enlarge the expertise available to the ICSB Subcommittee and provide additional means of enhancing international discussion and agreement on the taxonomy of mycoplasmas.

Minute 12. *Election of officers.* E. A. Freundt was elected Chairman and R. F. Whitcomb was elected Secretary. The Subcommittee formally elected the Chairman of the Board for the WHO/FAO Programme on Comparative Mycoplasmaology, P. Whittle-

stone, an ex officio member of the Subcommittee. The following new members were elected to the Subcommittee: H. Neimark, Brooklyn, N.Y.; J. Bové, Bordeaux, France; and H. Ernø, Aarhus, Denmark.

Minute 13. Membership of the Subcommittee. The present membership of the Subcommittee is as follows: E. A. Freundt (Chairman), Aarhus, Denmark; R. F. Whitcomb (Secretary), Beltsville, Md.; M. F. Barile, Bethesda, Md.; J. Bové, Bordeaux, France; G. S. Cottew, Parkville, Victoria, Australia; H. Ernø, Aarhus, Denmark; Ruth Lemcke, Compton, England; H. Neimark, Brooklyn, New York, N. Y.; S. Razin, Jerusalem, Israel; D. Taylor-Robinson, Harrow, England; and J. G. Tully, Bethesda, Md. Corresponding members are R. M. Chanock, Bethesda, Md.; D. G. ff. Edward, Kent, England; J. Fabricant, Ithaca, N.Y.; L. Hayflick, Stanford, Calif.; and N. L. Somerson, Columbus, Ohio.

Minute 14. Adjournment. The meeting was adjourned at 5:00 p.m., 22 September 1976.

Robert R. Whitcomb, Secretary

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