International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes*

Minutes of Interim Meetings, 2 and 5 September 1980, Custer, South Dakota

Session 1. Closed meeting

Minute 1. Call to order. The meeting was called to order at 9:30 on 2 September 1980 by the Chairman, E. A. Freundt.

Minute 2. Record of attendance. Members present were: E. A. Freundt (Chairman), R. F. Whitcomb (Secretary), M. F. Barile, J. M. Bové, G. S. Cottew, H. Ernø, R. M. Lemcke, H. Neimark, S. Razin, D. Taylor-Robinson, J. G. Tully, and P. Whittlestone. G. Masover attended as a representative of L. Hayflick.

Minute 3. Report of the Chairman. The activities of the Subcommittee since the previous meeting were summarized by the Chairman. It was noted that although minutes of the 1978 sessions at Freiburg and Munich were prepared and forwarded through ICSB channels, they had not yet been processed for publication. The updated minimal standards document (Int. J. Syst. Bacteriol. **29**:172–180, 1979) appeared in print the preceding year. An advisory committee for the ninth edition of *Bergey's Manual of Systematic Bacteriology* was selected, and authors for the various taxa of *Mollicutes* were selected. The recommended name *Mycoplasma hyopneumoniae* was published, and the J strain was designated as a neotype (D. L. Rose, J. G. Tully, and R. G. Witter, Int. J. Syst. Bacteriol. **29**:83–91, 1979). The status of *Mycoplasma flocculare* also was confirmed (D. L. Rose, J. G. Tully, and R. G. Witter, Int. J. Syst. Bacteriol. **29**:83–91, 1979). Lists of approved names were forwarded to V. B. D. Skerman and published (ICSB, Int. J. Syst. Bacteriol. **30**:225–420, 1980) with other bacterial names in 1980. Unfortunately, the family name *Spiroplasmataceae* [I. G. Skripal, Mikrobiol. Zh. (Kiev) **36**:462–467, 1974] was inadvertently omitted from the final list. Although the name was validly published in 1974, it must now be revived. Unfortunately, original designations of type strains were not included in the list. Consequences of the appearance of the list were discussed. The Chairman reminded Subcommittee members to furnish names of effectively published taxa to the *International Journal of Systematic Bacteriology* for listing to ensure their acceptance under Rule 27 of the Code.

Minute 4. Higher taxonomy of *Mollicutes*. The Subcommittee discussed the taxonomic status of the class *Mollicutes*. The concept and name (D. G. Edward and E. A. Freundt, Int. J. Syst. Bacteriol. **17**:267–268, 1967) is now well established and appears in the Approved Lists of Bacterial Names (Int. J. Syst. Bacteriol. **30**:225–420, 1980) and in *Bergey's Manual*. The name *Mollicutes* has, however, also been proposed (N. E. Gibbons and R. G. E. Murray, Int. J. Syst. Bacteriol. **28**:1–6, 1978) for a division of procaryotes. Although the Subcommittee believes that a broad consensus exists on the desirability of divisional status for *Mollicutes*, proper nomenclature will be required to legitimize the concept. Accordingly, a working committee was appointed to draft a revised proposal for higher classification of *Mollicutes*. This committee is composed of E. A. Freundt, S. Razin, J. M. Bové, H. Neimark, and R. F. Whitcomb. The final draft will be submitted for publication by the Subcommittee.

In their deliberations on the taxonomic status of *Molli-*

cutes, the Subcommittee took note of the recent work of C. R. Woese and his colleagues regarding ribosomal ribonucleic acid cataloging, which indicates that phylogenetic origins of members of *Mollicutes* may be somewhat diverse (Fox et al., Science **209**:457–463, 1980; Woese, Maniloff, and Zablen, Proc. Natl. Acad. Sci. U.S.A. **77**:494–498, 1980). Although the Subcommittee felt that this work is substantial and important, it noted that it represented a single approach to taxonomy. In any event, organisms now classified in the *Mollicutes* appear to represent a cluster of descendants of gram-positive bacteria with pro-clostridial ancestors. Therefore, the Subcommittee unanimously agreed that the concept of *Mollicutes* should be retained in microbial classification.

Minute 5. *Ureaplasma* taxonomy. The Subcommittee reviewed recent advances in the taxonomy of *Ureaplasma*. Deoxyribonucleic acid (DNA)-DNA homology data has been obtained that indicates that *Ureaplasma urealyticum* can be divided into subgroups (C. Christiansen, F. T. Black, and E. A. Freundt, Int. J. Syst. Bacteriol. **31**:259–262, 1981). On the other hand, the status of the bovine ureaplasma strain A417 was reviewed in detail. This strain is distinct from *U. urealyticum* by serology and host range and is also distinct as measured by one-dimensional polyacrylamide gel electrophoresis (C. J. Howard, D. H. Pocock, and R. N. Gourlay, Int. J. Syst. Bacteriol. **31**:128–130, 1981). Experiments on two-dimensional electrophoresis, performed by Texier and colleagues at Bordeaux in response to a Subcommittee suggestion, were reported (Texier et al., Rev. Infect. Dis. **4**(Suppl.):S250, 1982) at this meeting and confirmed the distinctness of the A417 strain. The naming of strain A417 is therefore appropriate as soon as a description fulfilling the minimal standard requirements is available.

Minute 6. *Spiroplasma* working team. The ad hoc spiroplasma working team had progressed considerably, especially in determination of both one- and two-dimensional polyacrylamide gel electrophoretic profiles (Daniels et al., Curr. Microbiol. **4**:377–380, 1980; Mouches et al., Curr. Microbiol. **2**:69–74, 1979) and analysis of content of spiroplasma genomes (J. M. Bové and C. Saillard, In R. F. Whitcomb and J. G. Tully (ed.), The mycoplasmas, vol. 3, p. 83–153, 1979). The results of serological studies by deformation and metabolic inhibition were published (D. L. Williamson, J. G. Tully, and R. F. Whitcomb, Int. J. Syst. Bacteriol. **29**:345–351, 1979), and growth inhibition studies were in progress.

Minute 7. *Spiroplasma* taxonomy. Taxonomy of the genus *Spiroplasma* was discussed at great length, especially with regard to subgeneric groupings. A recent proposal for spiroplasma groupings by Junca et al. (C. R. Acad. Sci., Ser. D **290**:1209–1212, 1980) was reviewed. The recommendation of the Subcommittee (Int. J. Syst. Bacteriol. **32**:261–264, 1982) that the SMCA spiroplasma could (with appropriate characterization) be named was reaffirmed. Also, the flower spiroplasmas represented by groups III and IV of Junca et al., because they are distinct serologically and share no detectable DNA-DNA homology with *Spiroplasma citri*, are certainly new species and could be named. Note that group III has recently received the name *Spiroplasma floricola* (R. E.

Davis, I.-M. Lee, and J. F. Worley, *Int. J. Syst. Bacteriol.* **31**:456-464, 1981), and group V has been named *Spiroplasma mirum* (J. G. Tully, R. F. Whitcomb, D. L. Rose, J. M. Bové, *Int. J. Syst. Bacteriol.* **32**:92-100, 1982). However, in recent studies (J. G. Tully et al., *Curr. Microbiol.* **3**:369-372, 1980) it has been suggested that strains of group IV are serologically heterogeneous. The most difficult taxonomic problems were with the honey bee (subgroup I-2) and corn stunt (subgroup I-3) spiroplasmas. The honey bee spiroplasma hybridizes with a *S. citri* probe at 65%, whereas the corn stunt organism hybridizes at 53% (Junca et al., *C. R. Acad. Sci., Ser. D* **290**:1209-1212, 1980). However, growth inhibition tests show significant crossing between these subgroups. Further study is required to resolve the taxonomic status of these agents.

Minute 8. Taxonomic status of Acholeplasmataceae. The Subcommittee discussed the elevation of the family *Acholeplasmataceae* to ordinal rank. Such an elevation is suggested, for example, by the ribosomal ribonucleic acid RNA cataloging of C. R. Woese and colleagues (Woese, Maniloff, and Zablen, *Proc. Natl. Acad. Sci. U.S.A.* **77**:494-498, 1980), who have suggested that *Acholeplasmataceae* and the branch comprising members of the families *Mycoplasmataceae* and *Spiroplasmataceae* are representative of deep branches of mycoplasma phylogeny. Such indications support previous observations of fundamental differences. These include genome size, sterol utilization, localization of certain key enzymatic functions, content of superoxide dismutases, and incorporation of exogenous phospholipids.

Two views were expressed by members of the Subcommittee. One view was that elevation of *Acholeplasmataceae* to an order might not be useful and could cause future problems. In particular, the possibility that acholeplasmas are related to members of the family *Lactobacillaceae* was stressed, creating nonparallel taxa if acholeplasmas were accorded ordinal rank. A second view, held by the majority of the members, was that the profound differences between the two groups merited recognition at the ordinal level. The proposed elevation would provide formal recognition of the natural diversity to be found within the *Mollicutes*. It was agreed that a working group would draft a proposal to this effect for the possible approval of the Subcommittee.

Minute 9. Species concept in Acholeplasma. The present status of species of *Acholeplasma* was discussed. Some species in this genus exist as clusters of strains that have rather wide ranges of DNA-DNA homology. If strains of such clusters exhibiting the widest homology differences were named as species, it would be impossible to identify intermediate strains. Therefore, a conservative approach to the taxonomy of *Acholeplasma* species was advocated by the Subcommittee.

Minute 10. Subspecies concept in Mycoplasma. The Subcommittee briefly discussed the status of subspecies in *Mycoplasma*, with special reference to the status of *Mycoplasma mycoides*. It was agreed that the detailed statement in the 1978 minutes (*Int. J. Syst. Bacteriol.* **32**:261-264, 1982) accurately reflected the present status.

Minute 11. Generic concept in Mycoplasma. The generic concept in *Mycoplasma* was discussed with reference, in particular, to possible transfer of *Mycoplasma gallisepticum* to a new genus. However, it was felt that many of the unusual properties of this mollicute are, in fact, shared by other *Mycoplasma* species. Therefore, it seemed premature to split the genus *Mycoplasma* in any way at this time.

Minute 12. Status of Thermoplasma. The taxonomic status of the genus *Thermoplasma* was discussed by the Sub-

committee. The extremely unusual properties of members of this genus were acknowledged by the Subcommittee. These include optimal growth at extremely high temperatures and low pH, failure to utilize fatty acids or cholesterol, high guanine-plus-cytosine content, and possession of branched lipids unique among mollicutes but present in members of the *Archaeobacteria*. Although thermoplasmas meet the requirements in the class description, ribosomal ribonucleic acid studies clearly show that they are related to the *Archaeobacteria*. Since *Thermoplasma* is unrelated to all mollicutes, is being studied actively by others as an archaeobacterium, and has never been definitely classified as a mollicute, it was agreed that this genus should be excluded from the updated class description of *Mollicutes*.

Minute 13. Liaison with ASM Subcommittee on the Taxonomy of the Mollicutes. Although the ASM committee had been relatively inactive, some correspondence had been exchanged concerning classification of *Mollicutes*. The Subcommittee expressed the hope that it would continue to contribute to the activities of the ICSB Subcommittee in the future.

Minute 14. Report of the Board for the Program of Comparative Mycoplasmaology. The Chairman of the Board, P. Whittlestone, briefly reported the activities of the Board. During the past 2 years, links to the International Organization for Mycoplasmaology had been strengthened, but links with the World Health Organization were not emphasized. Working teams are actively cooperating on ureaplasma and spiroplasma projects and on *M. mycoides* subspecies studies.

Minute 15. Amendment of minimal standards. The Subcommittee received from R. Leach a suggestion for amendment of the minimal standards document. Leach commented upon the wisdom of requiring that "two clones should be examined and shown to have the same properties" before a species could be named. R. Leach felt that examination of a second clone was not necessarily relevant for taxonomic purposes and should not be required. It should be sufficient to examine a single clone of a candidate species and show that it fulfills the minimal standards. If so, it can be named. Especially, in R. Leach's words, "... the implication that each clone must separately be tested ... is unwelcome from a practical point of view and would seem to be a taxonomically empty gesture."

The Subcommittee felt that this amendment to the document was an improvement and agreed to incorporate the amendment in the minutes for the convenience of bench workers as a practical guideline in their work. At the same time, the Subcommittee felt that examination of different clones is a very useful means of observing purity and homogeneity among clones and recommended that two or more clones be used for selected tests.

Minute 16. Trivial class name. The appropriateness of the trivial term 'mycoplasma' for members of the class *Mollicutes* was discussed. The Subcommittee felt that, notwithstanding long-established conventional use of "mycoplasma," it was technically incorrect and should be replaced by "mollicute."

Minute 17. Mollicute "superspecies." The explicit status of clusters of mollicute strains within superspecies was discussed. These superspecies included, for example, *M. mycoides*, *Acholeplasma axanthum*, *Spiroplasma* subgroups I and IV and *U. urealyticum*. It was agreed that extensive reciprocal tests by serology, two-dimensional polyacrylamide gel electrophoresis, and DNA-DNA hybridization would be required before taxonomic conclusions

could be drawn. The Subcommittee noted that such problems occurred in all mollicute genera, with the possible exception of the genus *Anaeroplasma*; therefore, resolution of a problem in one genus strongly influences taxonomic decisions concerning other genera.

Minute 18. Election of officers and members. E. A. Freundt will remain Chairman and R. F. Whitcomb will remain Secretary. The membership of the Subcommittee will remain as listed in previous minutes.

Minute 19. Adjournment. The meeting was adjourned at 19:45.

Session 2. Open meeting

Minute 20. Call to order. An open meeting was called to order at 20:00 on 5 September 1980 by the Chairman at a session of the International Organization for Mycoplasmaology held in Custer, S.D.

Minute 21. Record of attendance. Members present were: E. A. Freundt (Chairman), R. F. Whitcomb (Secretary), M. F. Barile, J. M. Bové, G. S. Cottew, H. Ernø, R. M. Lemcke, H. Neimark, S. Razin, D. Taylor-Robinson, J. G. Tully, and P. Whittlestone. G. Masover attended as a representative of L. Hayflick.

Minute 22. Report of the chairman. The chairman welcomed about 80 observers to the meeting and expressed appreciation for continued interest in the Subcommittee functions. The proceedings of the closed meeting of 2 September 1980 were summarized. In particular, thanks were expressed to R. Leach for submission of a well-reasoned proposal that was discussed and accepted by the Subcommittee (see Minute 15). It was announced that the new (ninth) edition of *Bergey's Manual* will contain updated descriptions of all mycoplasma taxa. Mycoplasmaologists who wish to convey their suggestions on particular taxa are invited to do so. The authors of various chapters are: *Mollicutes*, E. A. Freundt and S. Razin; *Mycoplasma*, E. A. Freundt and S. Razin; *Ureaplasma*, D. Taylor-Robinson and N. Gourlay; *Acholeplasma*, J. G. Tully; *Spiroplasma*, R. F. Whitcomb and J. G. Tully; *Anaeroplasma*, I. Robinson; and *Thermoplasma*, T. Langworthy and P. Smith.

Minute 23. Current status of *Mollicutes*. The Subcommittee described the current status of *Mollicutes* as a preoccupied name for a division of the kingdom *Prokaryotae*. A brief discussion ensued, with participation by D. Pollack, R. Cole, and H. P. Chu. The absence of iron-sulfur proteins and cytochromes in the *Mollicutes* sets them apart from all other cells. Even though this may be only one of many functional losses, the discreteness of the group remains. The classification of organisms that may be somewhat polyphyletic in a single class may still be the most useful working classification. Despite evidence linking acholeplasmas to streptococci, the phylogenetic linkages of *Mollicutes* are not yet clear.

Minute 24. Status of *Thermoplasma*. The status of the

genus *Thermoplasma* was discussed. In view of its possession of flagella, cytochromes, histones, and unique dialkyl diglycerol tetraethers, ability to subsist on nutritionally simple media, and content of unique 16S ribosomal ribonucleic acid, the group is dissimilar to all other mollicutes, is recognized by others as an archaeobacterium, and, despite its lack of cell wall, should not be classified in *Mollicutes*.

Minute 25. Status of *Acholeplasmataceae*. The current status of acholeplasmas as a natural family and as a cluster of heterogeneous strains was summarized, and possible elevation of the group to ordinal rank was discussed.

Minute 26. Status of *Mycoplasma*. The current status of the genus *Mycoplasma* and its species was discussed. No consensus for change in taxonomic status exists at this time.

Minute 27. Species concept in *Spiroplasma*. A discussion was held on the current species concept in *Spiroplasma*. In particular, R. E. Davis restated the proposal that he had set forth with I. M. Lee (R. E. Davis and I. M. Lee, Rev. Infect. Dis. 4(Suppl.):S122-S128, 1982) for separation of group I spiroplasmas into separate species. Special emphasis was placed on their biological mutual independence, levels of DNA-DNA homology that fall within the range accepted for bacterial species, feasibility of fulfillment of minimal standard requirements, and the desirability for binary names as useful instruments in formulating and enforcing quarantine policies. In particular, it was noted that levels of 40% homology existed between existing *Mycoplasma* species, such as *Mycoplasma agalactiae* and *Mycoplasma bovis*, and that homology differences less than those observed in group I spiroplasmas were recognized taxonomically in descriptions of subspecies of *M. mycoides*. A discussion by R. M. Cole, R. Del Giudice, and D. Pollack and Subcommittee members explored the difficulties in species concepts within the group I complex. Apparently there is a wide divergence of opinion concerning the best taxonomic course to follow at this time.

Minute 28. Adjournment. With special thanks to observers, the meeting was adjourned at 22:40.

Minute 29. Current membership. 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. M. Bové, Bordeaux, France; G. S. Cottew, Parkville, Victoria, Australia; H. Ernø, Aarhus, Denmark; Ruth Lemcke, Compton, United Kingdom; J. Maniloff, Rochester, N.Y.; H. Neimark, Brooklyn, N.Y.; S. Razin, Jerusalem, Israel; D. Taylor-Robinson, Harrow, United Kingdom; J. G. Tully, Bethesda, Md.; R. M. Chanock, Bethesda, Md.; J. Fabricant, Ithaca, N.Y.; L. Hayflick, Stanford, Calif.; and N. L. Somerson, Columbus, Ohio.

R. F. WHITCOMB, *Secretary*