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

Minutes of the Meetings, 18 and 20 September 1990, Osaka, Japan

Session 1. Closed meeting.

Minute 1. Call to order. The closed meeting was held at 6:00 p.m. on 18 September 1990 in Room D of International House, Osaka. The meeting was called to order by the Chairman, R. Sakazaki.

Minute 2. Record of attendance. The members present were R. R. Colwell, B. Holmes, K. Kogure (as an alternate for A. Huq), Y. Kosako, B. Nair, and R. Sakazaki (Chairman).

Minute 3. Approval of agenda. The written agenda previously mailed to members was presented and approved. The minutes of the previous meetings which were held on 8 and 11 September 1986 in Manchester, England, had been approved by mail and have been published (Int. J. Syst. Bacteriol. **39**:210–212, 1989). A copy was given to each member.

Minute 4. Apologies for absence. Apologies were received from D. J. Brenner, P. Desmarchelier, J. J. Farmer III, R. Hugh, A. Huq, J. V. Lee, and R. Schubert for being unable to attend.

Minute 5. Changes in membership and officers. A resignation had been received in 1990 from M. S. Hendrie as she had retired. J. J. Farmer III had resigned as Secretary after serving two terms, and R. Sakazaki announced his wish to resign after serving three terms as Chairman. The willingness of both to remain as members was greatly appreciated, as were their services to the Subcommittee. R. Sakazaki chaired the meeting for the last time, and nominations were called for both positions. R. Sakazaki proposed J. J. Farmer III as Chairman and B. Holmes as Secretary; both were duly elected. B. Holmes recorded the minutes. The Subcommittee voted to seek approval for election to full membership of S. Shinoda (Okayama, Japan).

Minute 6. Current membership. The current voting membership and officers (see Minute 22) of the Subcommittee is as follows: D. J. Brenner, Atlanta, Ga.; R. R. Colwell, College Park, Md.; P. Desmarchelier, St. Lucia, Australia; J. J. Farmer III, Atlanta, Ga. (Chairman); B. Holmes, London, England (Secretary); A. Huq, College Park, Md.; M. T. Kelly, Vancouver, Canada; Y. Kosako, Saitama, Japan; J. V. Lee, Salisbury, England; B. Nair, Calcutta, India; R. Sakazaki, Tokyo, Japan; R. Schubert, Frankfurt/Main, Germany; and S. Shinoda, Okayama, Japan. In addition, R. Hugh, Washington, D.C., is an honorary member.

Minute 7. J. Shewan. The death on 23 June 1988 of J. Shewan, who had a keen interest in vibrios and who had performed much work on the vibriostatic agent 0/129, was deeply regretted.

Minute 8. Concept and definition of the family *Vibrionaceae* and its relatives. The proposal to remove the genus *Aeromonas* from the family *Vibrionaceae* and to establish a new family, *Aeromonadaceae*, with the single genus *Aeromonas* (R. R. Colwell, M. T. MacDonell, and J. De Ley, Int. J. Syst. Bacteriol. **36**:473–477, 1986) had been discussed in detail at the previous meeting. The members voted to accept the proposal, and it was suggested that the name of the Subcommittee might also be changed. The matter was further discussed by correspondence in which it was noted that

the traditional family *Vibrionaceae* is heterogeneous, that additional families may be warranted, and that some species and/or genera may be excluded when the results of full 5S and 16S rRNA sequencing are available. The creation of *Aeromonadaceae* and the transfer of *Plesiomonas* to the family *Enterobacteriaceae* are only the first of such proposals to be discussed and considered. Accordingly, it was considered premature to change the name of the Subcommittee or to reassign responsibility for any of the organisms traditionally dealt with by the *Vibrionaceae* Subcommittee.

Minute 9. Classification of the genus *Allomonas*. The genus *Allomonas* had been placed in the family *Vibrionaceae* (G. P. Kalina, A. S. Antonov, T. P. Turova, and T. I. Grafova, Int. J. Syst. Bacteriol. **34**:150–154, 1984). As reported at the previous meeting, several members had studied the type strain of *Allomonas enterica* (ATCC 35843) and found it essentially phenotypically identical, and highly related by DNA-DNA hybridization, to *Vibrio fluvialis*. Those who have compared these two organisms were encouraged to publish their detailed findings.

Minute 10. Nomenclatural status of *Vibrio alginolyticus* subsp. *iophagus*. This problem had been discussed at length at the previous meeting. An erratum was published (Int. J. Syst. Bacteriol. **39**:95, 1989) stating that *Vibrio alginolyticus* subsp. *iophagus* is an illegitimate name and has no standing in nomenclature. Similarly, this name will not be included in future editions of lists of names with standing in nomenclature.

Minute 11. Identification of *Aeromonas media*. It was pointed out that it is difficult to identify this organism because for most of the characteristics given by Allen et al. (Int. J. Syst. Bacteriol. **33**:599–604, 1983) different strains of the species gave different results. R. Sakazaki suggested that the Secretary write to B. Austin to see whether any further work has been done in this area.

Minute 12. Genospecies of *Aeromonas*. Although there are now eight named genospecies in the genus (*A. caviae*-*A. punctata*, *A. eucrenophila*, *A. hydrophila*, *A. media*, *A. salmonicida*, *A. sobria*, *A. schubertii*, and *A. veronii*), R. Sakazaki stated that there were at least another three or four to be named (a name has since been proposed for one of these: *A. jandaei* [J. Clin. Microbiol. **29**:560–564, 1991]). The taxonomic position of each genospecies needed to be clarified and additional phenotypic characteristics needed to be found for differentiating among them.

Minute 13. Priority of *Aeromonas punctata* over *Aeromonas caviae*. This problem was well documented in Minute 17 of the previous meeting. It was considered that a request should be made to the Judicial Commission to correct the type strain of *Aeromonas punctata* so that a recommendation can be made by the Subcommittee on the classification of these two organisms. Since *Aeromonas caviae* and *A. punctata* currently share the same type strain, they are objective synonyms and the latter has priority since it was published first. Correction of the type strain of *A. punctata* would be the first step in the resolution of this problem.

Minute 14. Taxonomic status of the three subspecies of *Aeromonas salmonicida*. Y. Kosako agreed to perform

DNA-DNA hybridizations between strains representing the three subspecies of *Aeromonas salmonicida* to determine whether they could be differentiated on other than purely phenotypic criteria.

Minute 15. Status of the genus *Beneckea*. The genus *Beneckea*, originally proposed by Baumann, had been rejected by him (Curr. Microbiol. 4:127-132, 1980) as well as by most others in the field, including the members of the Subcommittee. Since the use of *Beneckea* or *Vibrio* is a matter of classification and usage, rather than nomenclature, no further action is needed. However, if a group of *Vibrio* species is subsequently found to be highly related to *Vibrio campbellii* (*Beneckea campbellii*), then this group could be distinguished at the genus level by including them in a redefined genus *Beneckea*, whose definition would be centered around its type species, *Beneckea campbellii*.

Minute 16. Status of the genus *Colwellia*. The name *Colwellia hadaliensis* was proposed for a single strain isolated from the deep sea (J. W. Deming, L. K. Somers, W. L. Straube, D. G. Swartz, and M. T. MacDonell, Syst. Appl. Microbiol. 10:152-160, 1988). Given the extreme environmental conditions from which the isolate came, little phenotypic characterization was possible, but analysis of 5S rRNA sequences demonstrated a close phylogenetic relationship to the vibrio-enteric group. The organism formerly known as *Vibrio psychroerythrus* was also transferred to the new genus, where it became the type species. The Subcommittee members would consider whether the genus *Colwellia* (and/or *Shewanella*) should be included in the family *Vibrionaceae*, especially as its inclusion would require a change in the family definition. If excluded, then one might have to consider a new family with a single genus (in each case); also, such organisms may or may not be the responsibility of this Subcommittee, which has traditionally dealt only with fermentative organisms.

Minute 17. Working Group on Minimal Standards. R. Sakazaki distributed copies of a report prepared by J. J. Farmer III in his capacity as Chairman of the Working Group on Minimal Standards (the other members of the working group being R. R. Colwell, J. V. Lee, R. Sakazaki, and R. Schubert). The answers to the questionnaire described in the previous minutes had been considered and could be summarized as follows. All members considered phenotypic characterization minimal, and only one felt that DNA-DNA hybridization, rRNA-DNA hybridization, or RNA sequencing was minimal. Although many items were listed that would require expensive equipment (electron microscope, gas chromatograph, etc.) or specialized reagents (typing antisera), there were only a few votes for any of these as minimal. It could be concluded that, in agreement with the *Enterobacteriaceae* Subcommittee, a new taxon could be described by using simple methods available in most microbiology laboratories. More-complex methods would be regarded as desirable but not as minimal. Whereas there were several phenotypic tests that all members considered minimal, there were differences in opinion, with some considering 40 to 60 tests as minimal and others considering that fewer tests were necessary. To progress the matter further it was decided that a more formal working group should be established with additional members who would then seek the opinions of members who had joined the Subcommittee since 1986. This new working group would then produce a draft minimal standards document.

Minute 18. Working group on serotyping. The members of this working group are E. Aldova, Czechoslovakia; R. J. Gross, United Kingdom; and T. Shimada, Japan (Chairman).

P. A. M. Guinee (The Netherlands) had been a member but died on 29 November 1989. T. Shimada presented a report of the working group. The two O-serogrouping systems for mesophilic *Aeromonas* strains (National Institute of Health, Japan, and National Institute of Public Health and Environmental Hygiene, The Netherlands; 44 and 30 reference strains, respectively) had been compared. Nine strains in each system were considered identical; 34 unique strains were identified, and an additional 22 strains of the two systems were in an a,b-a,c type of relationship to each corresponding O-group. Progress in adoption of a universal scheme would be delayed following the death of P. A. M. Guinee. The two O-serogrouping systems for *Plesiomonas shigelloides* strains (National Institute of Health, Japan, and Institute of Hygiene and Epidemiology, Czechoslovakia; 50 and 34 reference strains, respectively) had been compared. Twenty-eight strains in each system were considered identical; four unique strains were identified, and an additional two strains of the two systems were in an a,b-a,c type of relationship to each corresponding O-group. T. Shimada would continue contact with E. Aldova to see whether progress could be made towards the adoption of a universal scheme. It had been concluded that a serogroup of *Vibrio cholerae*, tentatively named Hakata, which possesses the C (Inaba) factor but not the A and B (Ogawa) factors of *Vibrio cholerae* O1, should be included in *V. cholerae* non-O1; it had not been given a new O-serogroup yet.

Minute 19. Taxonomic significance of pteridine (0/129) susceptibility. This is a test long established for the differentiation of *Vibrio* strains from those of related genera; clinically, the test is particularly useful for separating *Aeromonas* from *Vibrio fluvialis*. Unfortunately, reports of resistance are increasing, with many *V. cholerae* strains showing resistance to both concentrations used. The resistance to 0/129 appeared to be linked to transferable resistance (possibly plasmid mediated, possibly transposon mediated, or both) to trimethoprim (both agents share a common structure). B. Nair reported that resistance to 0/129 had first been seen in 1983 in Bangladesh but had now also been seen in India, South-East Asia, and Japan; his own experience was that 97.3% of strains were resistant to the 10- μ g disk and 90.5% were resistant to the 150- μ g disk. The Subcommittee were asked to consider dropping the test from those recommended. B. Nair provided the following references to the problem: G. Gerbaud, A. Dodin, F. Goldstein, and P. Courvalin, Ann. Inst. Pasteur/Microbiol. 136B:265-273, 1985; M. V. Jesudason and T. J. John, Trans. Soc. Trop. Med. Hyg. 84:136-137, 1990; S. Matsushita, Y. Kudoh, and M. Ohashi, Microbiol. Immunol. 28:1159-1162, 1984; and S. Sundaram and K. V. Murthy, FEMS Microbiol. Lett. 19:115-117, 1983.

Minute 20. Adjournment. The meeting was adjourned at 8:30 p.m. on 18 September 1990.

Session 2. Open meeting.

Minute 21. Call to order. The open meeting was held at 6:00 p.m. on 20 September 1990 in Room B of International House, Osaka. The meeting was called to order by the Secretary, B. Holmes.

Minute 22. Record of attendance. The members present were B. Holmes, Y. Kosako, B. Nair, R. Sakazaki, T. Shimada, and S. Shinoda. K. Yamamoto was welcomed to the meeting.

Minute 23. Summary of the closed meeting. B. Holmes read a summary of the closed meeting. The open meeting

was chaired by B. Holmes, who also recorded the proceedings.

Minute 24. Taxonomic significance of pteridine (0/129) susceptibility. It was pointed out that resistance to 0/129 in *Vibrio* strains had been seen in Japan only from travellers from South-East Asia. The indiscriminate use of trimethoprim, an inexpensive drug, seemed to be the likely root of the problem.

Minute 25. Working group on serotyping. Except for P. A. M. Guinee, all other members of this working group were still active, and it was agreed to correspond with H. Lior, who used the system used in Japan. The question of the Hakata serogroup of *Vibrio cholerae* would be further considered by the working group.

In conventional serotyping of *V. parahaemolyticus*, the antibody is prepared against flagella. S. Shinoda stated that if antibody is prepared only against purified flagellin, then 13 flagellum types can be recognized (although serotypes 11 to 13 had not yet been published). These 13 flagellar antigens are species specific and so can be used to detect the organism.

Minute 26. Classification of the genera *Colwellia*, *Listonella*, and *Shewanella*. Whereas *Listonella* was acceptable to several members of the Subcommittee as a member of the *Vibrionaceae*, it was felt that more data were needed before its acceptability could be determined; all members thought

that this was especially so in the case of *Colwellia* and *Shewanella*.

Minute 27. Classification of *V. cholerae* and *V. mimicus*. These two species were distinguishable by DNA-DNA hybridization (only 50 to 65% related), but some sequencing data have indicated that they are close enough to be a single species. The Subcommittee was asked to reexamine the classification of these species together with that of *V. alginolyticus*, *V. harveyi*, and *V. parahaemolyticus*. However, the matter was debated in subsequent correspondence, in which it was pointed out that in the case of, for example, *V. carchariae* and *V. harveyi* they are indistinguishable by DNA-DNA hybridization although they have some sequence differences. The members of the Subcommittee declined to reexamine the question and supported the recommendations that species can and should be defined on the basis of DNA-DNA hybridizations (L. G. Wayne et al., *Int. J. Syst. Bacteriol.* **37**:463–464, 1987; R. G. E. Murray et al., *Int. J. Syst. Bacteriol.* **40**:213–215, 1990).

Minute 28. Vote of thanks. B. Holmes proposed a vote of thanks to R. Sakazaki and J. J. Farmer III for all of their efforts on behalf of the Subcommittee.

Minute 29. Adjournment. The meeting was closed at 7:15 p.m. on 20 September 1990.

Barry Holmes, Secretary