International Committee on Systemic Bacteriology Subcommittee on Gram-Negative Anaerobic Rods

Minutes of the Meeting, 26 and 27 July 1992, Los Angeles, California

Minute 1. Call to order. Chairman Walter Loesche called the meeting to order at 2:30 p.m. on 26 July 1992. The meeting ended at 5 p.m. that day and resumed on 27 July 1992 at 9:30 a.m.

Minute 2. Record of attendance. The members present were W. J. Loesche (Chairman), A. Tanner (Secretary), H. Beerens, J. Bittner, S. Finegold, H. S. Goldberg, T. Hofstad, J. L. Johnson, D. N. Love, H. N. Shah, and K. Ueno. Invited guests and observers present at the meeting were E. J. Baron (California), J. Brazier (United Kingdom), S. H. Gerardo (California), S. E. Gharbia (Canada), E. J. C. Goldstein (California), M. Marina (Bulgaria), T. V. Riley (Western Australia), H. Jousimies-Somer (Finland), C. Strong (California), P. Summanen (California), and H. Wexler (California).

Minute 3. Changes in members. S. E. Gharbia (Canada) was proposed and elected as a new member to the committee.

Minute 4. Status of committee mandate. A suggestion was made to broaden the range of species considered by this committee from mainly human clinical isolates. Proposed species for consideration included species isolated from breweries and species colonizing subjects infected with the human immunodeficiency virus, not previously recognized as human isolates, and animal isolates.

Minute 5. Genus marker, use of 16S rRNA sequences to define species. The committee discussed the need for useful markers for genus definition in addition to data from DNA and rRNA. Suggested criteria for genus definition included 16S RNA sequencing, (cell wall) peptidoglycan, and cellular fatty acids (CFA), with use of type cultures of existing species. Limitations of Exclusive use of 16S rRNA sequencing were mentioned, with reference to the study by Fox et al. (G. E. Fox, J. D. Wisotzkey, and P. Jurtshuk, Jr., *Int. J. Syst. Bacteriol.* **42**:166–170, 1992). Dr. Bereens reviewed the utility of SDS outer membrane proteins for strain characterization.

Minute 6. Status of nonmotile species with a formate and fumarate metabolism (*Bacteroides gracilis* and *Bacteroides ureolyticus*). The proposal to transfer the clinical motile species *Wolinella recta* and *Wolinella curva* that have a formate and fumarate metabolism to the genus *Campylobacter* (Vandamme et al., *Int. J. Syst. Bacteriol.* **41**:88–103, 1991) was noted and approved by the subcommittee. The taxonomic status of *Bacteroides gracilis* and *Bacteroides ureolyticus* remains unresolved. These species share characteristics with *Campylobacter* species, including microaerophilic growth (Y.-H. Han, R. M. Smibert, and N. R. Krieg, *Int. J. Syst. Bacteriol.* **41**:218–222, 1991) and overlap in 16S rRNA sequences (B. R. Paster and F. E. Dewhirst, *Int. J. Syst. Bacteriol.* **38**:56–62, 1988; Vandamme et al., *Int. J. Syst. Bacteriol.* **41**:88–103, 1991). A suggestion was made to contact the *Campylobacter* subcommittee, so that the *Campylobacter* subcommittee might review the taxonomic status of *Bacteroides gracilis* and *Bacteroides ureolyticus*.

Minute 7. Status of *Centipeda periodontii*. 16S rRNA sequencing data indicate a close relationship between *Centipeda periodontii*, a one-species genus, and the genus *Selenomonas* (Hespell, Paster, Dewhirst, The genus *Selenomonas*, chapter 92, in *The prokaryotes*, 1992). The committee was unaware of other data (e.g., cellular fatty acids and cell wall lipopolysac-

charides) comparing *C. periodontii* with *Selenomonas* species, which would be desirable before taxonomic revision of *Centipeda*. This opinion was shared by C. H. Lai, who proposed *Centipeda periodontii* (C.-H. Lai, B. M. Males, P. A. Dougherty, P. Berthold, and M. A. Listgarten, *Int. J. Syst. Bacteriol.* **33**:628–635, 1983).

Minute 8. New species of gram-negative anaerobic rods from breweries. One new genus and four new species isolated from breweries have been described (R. H. Schleifer, M. Leuteritz, N. Weiss, W. Ludwig, G. Kirchhof, H. Seidel-Rüfer, *Int. J. Syst. Bacteriol.* **40**:19–27, 1990). The new genus, *Zymophilus*, includes two new species, *Zymophilus raffinosivorans* and *Zymophilus paucivorans*. Additional new species from breweries are *Pectinatus frisingensis* and *Selenomonas lacticifex*, with emendation of *Pectinatus cerevisiiphilus*.

Minute 9. Valid publication of *Bilophila*. The new genus *Bilophila* (Baron et al., *J. Gen. Microbiol.* **135**:3405–3411, 1989) has been validly published (*Int. J. Syst. Bacteriol.* **40**:320, 1990).

Minute 10. Status of *Porphyromonas* species. A new dark-pigmented species, *Porphyromonas circumdentaria*, isolated from gingival margins or mouth-associated diseases of cats was named (D. N. Love, G. D. Bailey, S. Collings, and D. A. Briscoe, *Int. J. Syst. Bacteriol.* **42**:434–438, 1992). The same publication reassigned *Bacteroides salivus* to *Porphyromonas salivosa*. The possibility of two subspecies of *Porphyromonas gingivalis* differing in catalase activity was raised. Catalase-positive *P. gingivalis* also has superoxide dismutase.

Minute 11. Status of *Prevotella* species. Two genotypes of *Prevotella intermedia* were recognized by DNA-DNA homology (J. L. Johnson and L. V. Holdeman, *Int. J. Syst. Bacteriol.* **33**:15–25, 1983). A new proposal creates a new species, *Prevotella nigrescens*, for genotype II, which does not include the *P. intermedia* type strain (H. N. Shah and S. E. Gharbia, *Int. J. Syst. Bacteriol.* **42**:542–546, 1992). Differences in the lengths of carbon chains of fatty acid esters and multifocus enzyme electrophoresis of glutamate dehydrogenase and malate dehydrogenase, in addition to differences in protein profiles on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) differentiate *P. nigrescens* from *P. intermedia*. A question was raised about the prevalence of *Prevotella oralis*. Committee members were not aware of the recent isolation of this species.

Minute 12. Taxonomy of *Fusobacterium necrophorum*. The reclassification of *Fusobacterium necrophorum* biovars to *Fusobacterium pseudonecrophorum* (biovar C), *F. necrophorum* subspecies *necrophorum* (biovar A), and *F. necrophorum* subspecies *funduliforme* (biovar B) based on GC (mole percent) content and DNA homology data (Shinjo et al., *Int. J. Syst. Bacteriol.* **40**:71–73, 1990, and **41**:395–397, 1991) was discussed. It was considered that the classification of the biovars of *F. necrophorum* to separate species or subspecies may be premature. It was considered that the species designation of JCM 3722^T and JCM 3723 as *F. pseudonecrophorum* may be premature and that the subspecies designation for *F. necrophorum* requires further investigation.

Dr. Bereens reviewed the data of Leon Fievez (1963), who named the species *Spherophorus necrophorum*, which was

divided into three subgroups, biovars A, B, and C. The source of biovar B strains was humans, whereas animal hosts may harbor biovars A, B, and C. Biovar A is pathogenic in mice and retains that pathogenicity. Biovar B strains either were non-pathogenic or lost pathogenic potential.

Dr. Love reviewed the data of Shinjo et al. which indicated inconsistencies in the reported differentiation of the biovars of *F. necrophorum*. Dr. Love's laboratory found that JCM 3722^T and JCM 3723 gave results at variance with those reported previously for lactate conversion to propionate, susceptibility to penicillin, growth on bile, and glucose and fructose fermentation. In addition, they found that *F. necrophorum* subspecies *funduliforme* JCM 3722^T was lipase positive, as reported by Shinjo et al. in *Int. J. Syst. Bacteriol.* **40**:71–73, 1990, but at variance with the data that they reported in *Int. J. Syst. Bacteriol.* **41**:395–397, 1991. The sources of strains were considered to include the alimentary tract and other sites.

Minute 13. *Dissimilarity of type strain of "Fusobacterium naviforme" with clinical isolates.* The *Fusobacterium naviforme* type strain had a GC content of over 49 mol%, which differentiates it from human clinical isolates of *F. naviforme*.

Minute 14. *Metabolism of Fusobacterium nucleatum.* *Fusobacterium nucleatum* subspecies *nucleatum* strains generally do not ferment glucose but can collect glucose and save this substrate for energy later. Energy sources appear to be nitrogen and amino acids. Different pathways for glutamate catabolism were reviewed (Gharbia and Shah, *J. Gen. Microbiol.* **137**:1201–1206, 1991).

Minute 15. *Subspeciation of Fusobacterium nucleatum.* *Fusobacterium nucleatum* was divided into subspecies by two sets of investigators (Dzink et al., *Int. J. Syst. Bacteriol.* **40**:74–78, 1990; S. E. Gharbia and H. N. Shah, *Lett. Appl. Microbiol.* **10**:105–108, 1990; and S. E. Gharbia and H. N. Shah, *Int. J. Syst. Bacteriol.* **42**:296–298, 1992). The taxonomic relationship between *F. nucleatum* subspecies *vincentii* and *F. nucleatum*

subspecies *fusiforme* remains unresolved. Gharbia and Shah differentiated the *fusiformis* and *vincentii* subspecies, using glutamate dehydrogenase (GDH) migration patterns. This method did not, however, distinguish between the *vincentii* and *nucleatum* subspecies, which differ in DNA homologies and SDS-PAGE (Dzink et al., *Int. J. Syst. Bacteriol.* **40**:74–78, 1990). Comparison of the two subspecies by DNA-DNA homology experiments was proposed.

Minute 16. *Status of Leptotrichia buccalis.* *Leptotrichia buccalis* strains do not appear to be strict anaerobes but grow under microaerobic conditions and slowly in air in the presence of carbon dioxide. Differences were noted among strains in API ZYM test reactions for glycosidases (Hofstad and Naess, *APMIS* **100**:116–118, 1992). Dr. Gharbia reported that superoxide dismutase which may play a role in oxygen tolerance, was detected in *Leptotrichia* strains. Three groups within *L. buccalis* were detected on the basis of multifocus enzyme electrophoretic patterns. Furthermore, she reported that this species had pathogenic potential on the basis of the production of D-lactic acid, which is extremely toxic. *L. buccalis* has been associated with bacteremia in neutropenic patients (Hofstad and Naess, *APMIS* **100**:116–118, 1992).

Minute 17. *Present subcommittee membership.* W. J. Loesche (Chairman), United States; A. Tanner (Secretary), United States; H. Beerens, France; J. Bittner, Romania; S. Finegold, United States; H. S. Goldberg, United States; T. Hofstad, Norway; J. L. Johnson, United States; D. N. Love, Australia; T. Mitsuoka, Japan; M. Sebald, France; H. N. Shah, Canada; K. Ueno, Japan; and H. Werner, Germany. S. E. Gharbia (Canada) is a new member of this committee.

Minute 18. *Adjournment.* The meeting was closed at 2:30 p.m. on 27 July 1992.

Anne Tanner, Secretary