International Committee on Nomenclature of Bacteria

Subcommittee on Lactobacilli and Closely Related Organisms

Minutes of the Meeting, 30 June and 1 July 1968

Canterbury, England

Minute 1. Call to order. The meeting was convened 30 June by the Chairman, G. Mocquot, in Slatter's Hotel.

Minute 2. Record of attendance. The members present were P. A. Hansen, O. Kandler, K. Kitahara, C. W. Langston, T. Mitsuoka, G. Mocquot, G. Reuter, M. Rogosa, Elisabeth Sharpe, and P. H. A. Sneath. P. Raibaud of Jouy-en-Josas was present as an observer.

Minute 3. Minutes of previous meeting. The minutes of the 1966 (Moscow) meeting were approved as read.

Minute 4. Membership. J. Dehnert resigned from the Subcommittee, and R. F. Norris and N. B. Williams resigned from the Bifidus Subgroup. R. Tittsler is deceased. K. Kitahara and T. Mitsuoka were added as members of the Subcommittee, and G. H. G. Davis and W. E. C. Moore were added to the Subgroup.

Minute 5. Report on study set of strains. The Secretary reported on the findings of the members on the set of strains distributed for study. The data are to be published shortly in book form.

Minute 6. Type species of *Lactobacillus*. *Lactobacillus helveticus* was recommended to be the type species of the genus, subject to the approval of the Judicial Commission.

Minute 7. *Lactobacillus buchneri*. The nomenclatural type of *L. buchneri* was discussed. The original strain has been lost and no strain corresponding to the original has ever been isolated. The description of *L. buchneri* is probably based on an impure culture. ATCC 4005, a strain labeled *L. buchneri* and examined by the Subcommittee, does not grow at 45 C and corresponds to *L. buchneri* as defined by Rogosa and Sharpe (1959); it may be considered a variety of *Lactobacillus brevis*. Sneath suggested that *L. buchneri* be considered a nomen dubium.

Minute 8. *Lactobacillus bulgaricus*. Hansen reviewed briefly the case of *L. bulgaricus*. The members voted in favor of applying this name to Orla-Jensen's organism, that is the D(-)-lactic acid-producing lactobacillus, rather than to the one which forms DL-lactic acid, the *L.*

bulgaricus of Rogosa et al., 1953. The type strain is ATCC 11842 (Thermobacterium No. 14 in the collection of Orla-Jensen).

Minute 9. *Lactobacillus leichmannii*. The status of *L. leichmannii* was discussed, and the characters of ATCC 4797 were reexamined. Kitahara considers ATCC 4797 to be a variety of *L. delbrueckii*, and Kandler calls it *L. lactis*. It was agreed that *L. leichmannii* is poorly described and not recognizable. Kitahara pointed out that *L. leichmannii*, according to the original description, should be mesophilic with a maximum of 40 to 46 C, whereas the present strain grows at 48 C.

It is possible that ATCC 4797 may represent a new species. It seems that the species *L. leichmannii* cannot be recognized, and consequently a neotype cannot be defined.

Reuter's strain R 13, proposed as a neotype of *L. leichmannii*, produced DL-lactic acid according to observations by Hansen and Kandler. It grew at 15 C but not at 45 C. No further studies of the strain by the Subcommittee are contemplated.

Minute 10. *Lactobacillus jugurt*. It was agreed to propose ATCC 521 as a neotype strain for *L. jugurt*. If *L. jugurt* is united with *L. helveticus* into one species, *L. helveticus* 15009 will typify the taxon.

Minute 11. Status of names attributed to Holland. Hansen pointed out that many of the names attributed to Holland cannot be considered as validly published because she did not attach a description or reference to a previously published description of the species. The assistance of the Judicial Commission has been requested. Sneath stated that it would not be possible to validate some of Holland's names on the recommendation by the Subcommittee, but he suggested that they should be republished. He further stated that all types and neotypes should be published and the corrected author citation given.

Minute 12. *Lactobacillus fermentum*. There is considerable margin for uncertainty in the description of *L. fermentum*. The original definition by Beijerinck is too vague to permit

placing the presently suggested neotype strain in his species *L. fermentum* with certainty. However, a later description by Smith (1915), also of the Delft laboratory, agrees in the main with the characters of the strain here proposed as the neotype (ATCC 14931). Because *L. fermentum* is now uniformly used for a species having the general characters of ATCC 14931 and as, in fact, no disagreement seems to exist, the name *L. fermentum* Beijerinck ought to be conserved with the neotype ATCC 14931. Hansen pointed out that further work is needed, particularly on the betabacteria and possibly utilizing taxometrics to obtain a better definition of the species. The present arrangement should be considered tentative.

Minute 13. *Lactobacillus acidophilus*. The name *L. acidophilus* should be conserved, and ATCC 4356 should be designated as the neotype.

Minute 14. Mocquot and Raibaud reported the following:

(i) *Taxonomic significance of the 2,3,5 triphenyltetrazolium chloride (TTC) test* (P. Raibaud, J. Fournaud, and G. Mocquot, 1967, C. R. Acad. Sci. 264:779-782). Under anaerobic conditions the TTC is reduced in the presence of lactate or pyruvate ions by different strains of the genus *Lactobacillus*.

(ii) *Fermentation of pentoses*. In a study of 606 strains of thermobacteria, none of which developed at 15 C, it was demonstrated that 253 fermented D-ribose and 127 D-ribose and L-arabinose. A small amount of glucose (0.01%) added to the medium containing ribose made the strains which fermented D-ribose only enhance the fermentation of this substrate.

(iii) *Fermentation mutants*. When the Whittenbury method was employed in the fermentation studies, a large number of thermobacteria showed fermentation mutants: anaerobic mutants for the carbohydrates cellobiose, melibiose, sucrose, salicin, amygdalin, trehalose, L-arabinose, and D-ribose; anaerobic and microaerophilic mutants for raffinose and mannitol.

Minute 15. *Additional organisms for study*. It was deemed desirable that new species and species which have so far not been considered should be studied by several members, e.g., *Sporolactobacillus inulinus*, which has no cell wall teichoic acid.

Minute 16. *National representation on Subcommittee*. In response to an inquiry about national representation on the Subcommittee, Sneath stated that it was important that the most knowledgeable workers in the field should

be included. There is, in fact, no prescribed proportion of representation of nations.

Minute 17. *Subgenera*. Rogosa defended the subdivision of lactobacilli into the three subunits (subgenera) of Orla-Jensen and elaborated further on the differences between these. Betabacteria ferment ribose and produce 1 mole of lactic acid and 1 mole of acetic acid; gas is formed from both glucose and from gluconate; aldolase is absent. Streptobacteria ferment ribose to lactate and acetate; gas from gluconate but not from glucose; aldolase is present. Thermobacteria do not produce gas from glucose or from gluconate; aldolase is present. Kandler's cell wall studies also separate the three taxa.

Minute 18. *Serology of Lactobacillus acidophilus*. Sharp reported on serological reactions of *L. acidophilus* strains. She stated: "Thirty-five strains of *L. acidophilus* have been examined serologically by ring precipitin and gel diffusion tests. It was possible to divide them into 2 groups by their serological reactions, and a third group in which the antigens were not characterized. The 10 Group I strains appeared to possess 2 antigens, a and b; and the 7 Group II strains to possess 2 antigens, b and c. These were found to be cell wall antigens. The serological groups were in good agreement with the electrophoretic patterns of lactic dehydrogenases as determined by Dr. Gasser in California."

Hansen called attention to the lack of antigenic studies by Subcommittee members and recommended that Sharpe distribute a small amount of standard sera to members. It was decided to limit the distribution of sera only to members who actually intend to do serological work. It was agreed that antigenic structure is of considerable interest and that further studies should be carried out. Kandler warned against placing too much importance on the so-called group antigens as it is entirely possible that dissimilar organisms may share specific cell wall polysaccharides. The classification of streptococci by group antigens only has led to certain unnatural arrangements and ought not serve as a model for the classification of lactobacilli.

Adjournment. The meeting was adjourned at 6:25 PM and was reconvened on 1 July at 10:15 AM at Slatter's Hotel. Present were H. Beerens, P. A. Hansen, O. Kandler, K. Kitahara, C. W. Langston, T. Mitsuoka, G. Mocquot, G. Reuter, M. Rogosa, Elisabeth Sharpe, and P. H. A. Sneath. P. Raibaud was present as an observer.

Minute 19. Unusual lactobacilli. Langston gave a brief account on some unusual lactobacilli, and it was generally agreed that representative strains should be deposited with the Subcommittee, e.g., strains which are motile, reduce nitrates, or produce catalase.

Kandler elaborated on *L. inulinus*, which forms spores but which is quite unlike *Bacillus*, having no molecular oxygen uptake and no catalase. It has, like streptobacteria, diamino-pimelic acid in the cell wall. *L. inulinus* may be an intermediate form between *Lactobacillus* and *Bacillus*. *L. inulinus* forms D(–)-lactic acid.

Minute 20. Bifidus Subgroup. The meeting was turned over to the Bifidus Subgroup. The type species of *Bifidobacterium* is *B. bifidum*. Beerens stated that he has Tissier's original culture. It often forms Y-shaped cells but does not produce a mycelium. It is a strict anaerobe, and it does not produce gas. It falls into Dehnert's type I or Reuter's III. Its relation to other organisms was discussed. Glucose-phosphate dehydrogenase is absent and possibly also aldolase. The guanine plus cytosine (GC) percentage is given as 57.2 to 60.8, which is far from the values found for lactobacilli.

Minute 21. Study of serotypes of Bifidobacterium. Reuter's and Werner's types of bifidobacteria ought eventually to be studied by the Subcommittee.

Minute 22. Study set of bifidobacteria. Beerens has distributed a collection of bifidobacteria, but very few of the members were in a position to report. The data by Beerens, Hansen, Kandler, and Reuter were discussed. A more detailed report has been furnished to the members of the Subgroup by Beerens.

Minute 23. Status of the name Eubacterium. Hansen suggested that an Opinion should be requested to reject the name *Eubacterium* on the grounds that the type, *Eubacterium foedans*, cannot be recognized from the description and no original strains are extant. The majority was in favor of declaring it a nomen dubium. Sneath remarked that some of the organisms which may have been placed in the genus could be united in a new genus; however,

this is outside the activity of the Subgroup and is the responsibility of individual research workers.

Minute 24. Designation of Bifidobacterium serotypes. The Subcommittee should at some future date eventually decide whether it will follow the system of designating *Bifidobacterium* serotypes proposed by Dehnert or the one proposed by Reuter.

Minute 25. New set of strains to be studied. A list of strains to be distributed to the Subcommittee members for consideration as type or neotype strains was prepared. It was agreed that the first set of strains ought to be limited to very important strains. All strains will be subject to an investigation in depth carried out as a cooperative effort and embracing not only the usual morphological and cultural studies but also fermentation balances, GC ratios, cell wall analyses, genetic compatibilities (if possible), antigenic structures, and other critical procedures. Rogosa pointed out that the determination of the products of fermentation without reference to the substrate utilized, although possibly useful for the screening of the organisms, would be insufficient for the purpose of serious classification. The complete fermentation balance is indispensable. Everybody concurred. A tentative list of 18 strains was agreed upon from the genera *Catenabacterium*, *Ramibacterium*, *Propionibacterium*, *Bifidobacterium*, *Butyribacterium*, and *Actinomyces*.

Minute 26. "Anaerobic corynebacteria." It was recommended that the term "anaerobic corynebacteria" be abandoned and that the organisms so designated be placed in appropriate genera. Most would probably fall into *Propionibacterium*.

Minute 27. T. Mitsuoka was added to the membership of the Bifidus Subgroup.

Adjournment. The meeting was adjourned at 6:45 PM on 1 July 1968.

P. A. Hansen
Secretary