Lactobacillus helveticus

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SUMMARY: Two of the *Lactobacillus* species previously defined, *Lactobacillus helveticus* and the 'intermediate group', have been renamed *L. casei* var. *rhannosus* and *L. helveticus* (Orla-Jensen) Holland, respectively. Antisera prepared against *L. bulgaricus* and *L. helveticus* (Orla-Jensen) Holland show that these species belong to the same serological group. The names of the serological groups previously defined have been replaced by letters.

Much confusion exists concerning the identity of '*Lactobacillus helveticus*'. Orla-Jensen (1919) renamed Freudenreich's *Bacillus casei* ϵ as *Thermobacterium helveticum*. Later (Holland, 1920) this name was amended to *Lactobacillus helveticus* (Orla-Jensen). Orla-Jensen described it as a high-temperature organism, fermenting glucose, fructose, galactose, lactose, mannose, maltose and dextrin, but not mannitol, sucrose, salicin, glycerol, rhamnose, arabinose, xylose, raffinose, inulin or sorbitol. The description of *L. helveticus* (Orla-Jensen) Holland in *Bergey's Manual of Determinative Bacteriology* (1948) agrees with this. Wheeler (1955*a*) described the species as a high-temperature *Lactobacillus* fermenting those same sugars and in addition mannitol, sucrose, salicin, glycerol and rhamnose. This classification was based on named strains of '*L. helveticus*' in our collection. Of 21 such strains 3 were identified (Wheeler, 1955*a, b*; Sharpe, 1955) as *L. casei*, 2 as *L. bulgaricus*, 4 as 'intermediate group' (intermediate between *L. acidophilus* and *L. bulgaricus*), 2 as *L. lactis*, and the remaining 10 strains formed the nucleus of our group '*L. helveticus*'. These organisms were all strains labelled *L. helveticus* when received from other workers and from national type collections; none was a fresh isolate named only by us. Since then, however, we have learnt in a private communication from Dr R. G. Jensen that the original strain of *L. helveticus* described by Orla-Jensen is still extant, and is indeed one of the strains of lactobacilli in our collection. This strain, *L. helveticus* ATCC 8018, NCIB 8025, was previously identified by Wheeler (1955*b*) as a member of her 'intermediate group'; she regarded this group as being possibly related to *L. bulgaricus*, and had not defined it as a species. The physiological characteristics of this 'intermediate group', including strain ATCC 8018 and 3 other named strains of *L. helveticus* in our collection, agree completely with those of Orla-Jensen's original description of *L. helveticus*. In view of these findings, which are based on the original strain of *L. helveticus*, we propose to rename Wheeler's 'intermediate group' as *L. helveticus* (Orla-Jensen) Holland.

The characteristics of *Lactobacillus helveticus* (Orla-Jensen) Holland are

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given below, and are those of the group previously known as the 'intermediate group', namely:

No growth at 15° or below. Maximum temperature between 45° and 50°. Homofermentative. Ferments glucose, fructose, galactose, mannose, maltose, lactose, dextrin. Does not ferment mannitol, sucrose, salicin, glycerol, inositol, rhamnose, arabinose, xylose, raffinose, inulin, sorbitol, amygdalin, cellobiose, melibiose, melezitose. Grows in the presence of 2% (w/v) but not 4% (w/v) NaCl. Does not grow in 2% (w/v) bile salt.

Wheater (1955*a*) and Davis (1955) concluded that *Lactobacillus casei* var. *rhamnosus*, described by Rogosa, Wiseman, Mitchell, Disraely & Beaman (1953), and our organism previously designated '*L. helveticus*' were the same organism. Now that we have redesignated our 'intermediate group' as *L. helveticus* (Orla-Jensen) Holland we consider that our strains previously named *L. helveticus* should be reclassified as *L. casei* var. *rhamnosus* as the descriptions of the two agree so nearly. It was suggested previously that this variety differs sufficiently from *L. casei*, i.e. in its ability to grow at 45° and to ferment rhamnose, sorbitol and inositol, to justify giving it species rank. However, we have since isolated several strains of *L. casei* which, although they were able to grow at 45°, were not var. *rhamnosus*, and the separation of *L. casei* var. *rhamnosus* as a new species solely on these few sugar fermentations is not considered justified. *L. casei* and *L. casei* var. *rhamnosus* belong to the same serological group (Sharpe, 1955), but strains having the physiological characteristics of *L. casei* var. *rhamnosus* usually possess a specific type antigen (Orland, 1950; Sharpe, 1955).

Further confusion is attached to the *Lactobacillus* strain ATCC 7469 which for many years has been used extensively for microbiological assay work and widely issued for this purpose. Tittsler, Rogosa & Whittier (1942) and Orland (1950) described how this organism became known variously as *L. casei* *e*, *L. casei* and *L. helveticus*; Tittsler *et al.* (1942) also showed that it was a strain of *L. casei* which could be distinguished from *L. helveticus* by physiological characters, and the name of the culture was then amended by the American Type Culture Collection to *L. casei*. Since then it has been further identified as *L. casei* var. *rhamnosus*. It appears, however, from a study of the many named cultures sent to us as *L. helveticus*, but which were in fact *L. casei* var. *rhamnosus*, that strains of this organism, or of organisms identified by the same characteristics, and labelled *L. helveticus* are still to be found in many collections. We possess both *L. casei* ATCC 7469 and *L. helveticus* ATCC 7469. Two of the strains received by us, namely ATCC 11981 (NCIB 6557) and ATCC 11982 (NCIB 8103) which are at present listed in both the American Type Culture Collection and the National Collection of Industrial Bacteria catalogues as *L. helveticus*, are in fact *L. casei* var. *rhamnosus*. It is therefore suggested that workers who include strains labelled '*L. helveticus*' in their culture collections might be wise to check the physiological properties of these organisms. *L. casei* var. *rhamnosus* ferments mannitol, sucrose, salicin, glycerol, inositol, rhamnose, cellobiose and melezitose, grows at 15° and hydrolyses aesculin; *L. helveticus* (Orla-Jensen) Holland has none of these properties.

Serological position of Lactobacillus helveticus

Further work has now shown that *Lactobacillus helveticus* (Orla-Jensen) Holland is a member of the serological group *L. bulgaricus*. Using methods previously described (Sharpe, 1955), a more potent antiserum against *L. bulgaricus* has now been prepared. This antiserum reacted by precipitin test with HCl extracts of all strains previously classified as *L. bulgaricus*, and in addition with extracts of all 15 of the strains now physiologically identified by us as *L. helveticus* (Orla-Jensen) Holland. A serum has also been prepared against a strain of *L. helveticus*, which has been found to react with extracts of *L. bulgaricus* strains as well as with those of *L. helveticus* strains, so that

Table 1. *Proposed nomenclature for serological groups of lactobacilli*

New designation	Former designation*	Species included in group
Group A	<i>L. bulgaricus</i>	<i>L. bulgaricus</i> and <i>L. helveticus</i>
Group B	<i>L. casei</i>	<i>L. casei</i>
Group C	<i>L. casei-helveticus</i>	<i>L. casei</i> and <i>L. casei</i> var. <i>rhamnosus</i>
Group D	<i>L. plantarum</i>	<i>L. plantarum</i>
Group E	<i>L. lactis-brevis</i>	<i>L. lactis</i> , subgroup <i>L. brevis</i>
Group F	<i>L. fermenti</i>	<i>L. fermenti</i>

* As given in Sharpe (1955).

these two species belong to the same serological group. It was observed, however, that each species reacted somewhat differently with the group antisera. Extracts of strains of *L. bulgaricus* (including different serological types) generally gave a stronger reaction with *L. bulgaricus* antiserum and a weaker reaction with *L. helveticus* antiserum, whereas extracts of *L. helveticus* gave a stronger reaction with *L. helveticus* antiserum and a weaker one with *L. bulgaricus* antiserum. There appeared to be a qualitative difference here. Differential absorption tests were attempted but neither of the antisera, particularly the rather weak *L. helveticus* one, were potent enough to give clear-cut results; any absorption, even with heterologous strains, greatly weakened the precipitin reactions. Although the two species belong to the same serological group, these differences in degree of reaction suggest some difference in antigenic components.

Other workers (Dr R. G. Jensen, private communication; Davis, 1955) have suggested a close relationship between these two species and the serological results bear this out. Further work on the characteristics of the two groups is still needed before it can be decided whether *Lactobacillus helveticus* (Orla-Jensen) Holland deserves species rank or whether it should be regarded as a variety of *L. bulgaricus*. Until then we prefer to retain its specific rank.

Nomenclature of the serological groups of lactobacilli

We would like to take this opportunity of renaming the serological groups of lactobacilli by letters instead of by the names of the physiologically defined species contained in them. The work described above confirms our previous

findings (Sharpe, 1955) that a serological group may contain more than one species, and the present method may become very cumbersome. We propose, therefore, to name the serological groups as shown in Table 1. This should eliminate any confusion which might arise between the serological and physiological methods of classification and allow for the definition of further species within a serological group. It has not yet been possible to prepare a serum against *Lactobacillus acidophilus*, which has been physiologically defined as a species.

REFERENCES

- Bergey's *Manual of Determinative Bacteriology* (1948). 6th ed. Edited by Breed, R. S., Murray, E. G. D. & Hitchens, A. P. London: Baillière, Tindall and Cox.
- DAVIS, G. H. G. (1955). The classification of lactobacilli from the human mouth. *J. gen. Microbiol.* **13**, 481.
- HOLLAND, D. F. (1920). Generic index of the common forms of bacteria. *J. Bact.* **5**, 223.
- ORLA-JENSEN, S. (1919). *The Lactic Acid Bacteria*. Copenhagen: Andr. Fred. Host and Son.
- ORLAND, F. J. (1950). A correlation of antigenic characteristics among certain bacteria of the Lactobacillus group. *J. infect. Dis.* **86**, 63.
- ROGOSA, M., WISEMAN, R. F., MITCHELL, J. A., DISRAELY, M. N. & BEAMAN, A. J. (1953). Species differentiation of oral lactobacilli. *J. Bact.* **65**, 681.
- SHARPE, M. E. (1955). Serological classification of lactobacilli. *J. gen. Microbiol.* **12**, 107.
- TITSLER, R. P., ROGOSA, M. & WHITTIER, E. O. (1942). The nomenclature and characteristics of the lactobacillus employed for the microbiological assay of riboflavin. *J. Bact.* **43**, 56.
- WHEATER, D. M. (1955*a*). The characteristics of *Lactobacillus plantarum*, *L. helveticus* and *L. casei*. *J. gen. Microbiol.* **12**, 133.
- WHEATER, D. M. (1955*b*). The characteristics of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. *J. gen. Microbiol.* **12**, 123.

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