The Morphology and Relationships of
'Lactobacillus' bifidus

By A. C. Hayward, C. M. F. Hale and K. A. Bisset

Department of Bacteriology, University of Birmingham

SUMMARY: Strains of 'Lactobacillus' bifidus were found to vary in morphology from large globules to more slender septate rods and filaments, often very profusely branched. Adaptation of anaerobic strains to growth under aerobic conditions is usually accompanied by a diminution in branching. The organism has little or no morphological resemblance to a true Lactobacillus species, but strongly resembles certain strains of Corynebacterium.

The systematic position of the branched Gram-positive bacterium found in the faeces of infants and described in Bergey's Manual (1948) as Lactobacillus bifidus, according to the classification of Weiss & Rettger (1934), has been the subject of considerable controversy. It is defined as a member of the genus Lactobacillus on the grounds that it is non-motile, non-sporing, catalase-negative, does not reduce nitrates, and ferments carbohydrates with the production of lactic and acetic acids. It will be observed that these characters are preponderantly negative, which is rather unsatisfactory. On the other hand, it differs from typical members of the genus in being an obligate anaerobe, at least on primary isolation, in exhibiting true branching, and in having a very restricted range of habitat and temperature requirement (Norris, Flanders, Tomarelli & György, 1950). Olsen (1949) considered it to be a species of Corynebacterium, and the generic names Bacteroides, Nocardia and Actinomyces have at various times been conferred upon it. In the course of a recent investigation of the morphology of Lactobacillus species in this laboratory (Davis, Bisset & Hale, 1955), strains of this organism were examined, and were considered to merit a separate description.

METHODS

Fourteen strains of organisms corresponding to the foregoing description were used. Of these one strain was supplied by courtesy of Dr A. T. R. Mattick, one was isolated from turkey faeces, and the remainder from faeces of newborn infants.

Primary isolations were made upon the tomato-juice agar described by Davis et al. (1955) in the form of plates, incubated at 37° in a McIntosh and Fildes jar. Subsequent cultures were grown in stab tubes of the same medium or in tubes of broth covered with vaseline. Lactobacillus bifidus is difficult to maintain in culture, and it was found advisable to subculture at intervals of 24 hr.

The organisms were examined cytologically by Hale's (1953) method for cell walls.
RESULTS
When successfully subcultured for a period of c. 10 days isolates of this bacterium became progressively adapted to existence in artificial medium, grew more freely and gradually lost their sensitivity to oxygen. The degree of sensitivity varied considerably in different strains, but the majority, when newly isolated, showed after 24 hr. only a faint line of growth in the lower part of the stab. Later subcultures showed a much heavier growth, extending almost to the surface of the medium. This diminution in sensitivity to oxygen was reported by Weiss & Rettger (1934) as characteristic of their Lactobacillus bifidus, type I.

The various types of morphology observed are illustrated in Pl. 1, figs. 1–5. Characteristically the organisms resembled corynebacteria, as described by Bisset (1949), and included branched and unbranched septate rods with expanded terminal cells (Pl. 1, figs. 1, 3). Some were filamentous, or alternatively, so profusely branched as to cease to bear any resemblance to rods; examples of both are seen in Pl. 1, fig. 2. A different morphological form appeared also in some cultures; these cells were unbranched but swollen and irregular (Pl. 1, figs. 4, 5). As the strains became adapted to artificial culture, the branched and swollen forms became less and less frequent, and the simple corynebacterium-like rods predominated. Although the observation is of necessity subjective, there appeared at all stages of culture to be a correlation between the preponderance of branched or swollen organisms and the degree of sensitivity to oxygen of the culture (cf. Friedman & Black, 1941; Prévot & Raynaud, 1955). Thus a well-adapted culture might superficially resemble species of Lactobacillus, but a comparison of the cytological structure of these rods (e.g. the unbranched forms in Pl. 1, figs. 1–3) and of true lactobacilli (Davis et al. 1955) reveals the difference very clearly.

DISCUSSION
It appears that the resemblances between 'Lactobacillus' bifidus and the true lactobacilli are almost entirely negative, and do not extend to the details of morphology as these are demonstrable by cytological examination. Morphologically 'L'. bifidus appears to belong to the Actinomycetales, suborder Mycobacteriineae, as defined by Bisset (1952). It is not, perhaps, quite justifiable to assign it to the genus Corynebacterium, but it may be permissible to speculate whether it might not have originated in an organism of this type, adapted, as so many are, to parasitism upon mucous membranes, in this case that of the vagina, thence passing to the lower gut of newborn infants, and subsequently to a wider, but still restricted, faecal habitat.

The generic title Bifidobacterium (Orla-Jensen, 1924) is considered to be valid and applicable to the organism studied.
REFERENCES


EXPLANATION OF PLATE

Figs. 1–5. Lactobacillus bifidus, stained by Hale's method for cell walls; x3000. 24 hr. cultures. Figs. 1–3. Filamentous and branched forms. Figs. 4, 5. Globular forms.

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*Lactobacillus bifidus*. Plate 1

(Facing p. 294)