Species Differentiation of Human Vaginal Lactobacilli

By M. ROGOSA

*National Institute of Dental Research, National Institutes of Health,
Public Health Service, U.S. Department of Health, Education
and Welfare, Bethesda, Maryland, U.S.A.*

AND M. ELISABETH SHARPE

National Institute for Research in Dairying, University of Reading

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SUMMARY

The designation 'Döderlein's bacillus' for human vaginal strains of the genus *Lactobacillus* is vague and unsatisfactory. Thirty-five strains of lactobacilli from twenty-one normal non-pregnant women aged 21-40 were isolated; c. 25% were heterofermentative. Of 21 isolates studied in detail, 14 were *L. acidophilus* and two were *L. casei* var. *rhannosus* (Rogosa *et al.* 1958). The heterofermentative species comprised four strains of *L. fermenti* and one of *L. cellobiosus* (Rogosa *et al.* 1958). Glycogen fermentation, which was found only in certain strains of *L. acidophilus*, was a variable characteristic. In addition, *Leuconostoc mesenteroides*, which on examination of smears can be confused with short rod forms, was also isolated.

INTRODUCTION

At birth the human vagina is sterile and the vaginal secretion is at pH 5.0 or less (Miura, 1928; Hardy, 1941; Cruickshank & Sharman, 1934*a, b*). It has been assumed that bacterial implantation takes place within a few hours and for 2 or 3 weeks postnatally 'a simple homogeneous flora of Gram-positive bacilli, known as Döderlein's vaginal bacillus, is established in the vagina in association with a slightly acid non-purulent secretion' (Cruickshank & Sharman, 1934*b*). Shortly after this the flora changes and until puberty normally comprises micrococci, streptococci, diptheroids, but seldom 'Döderlein's bacillus' (Döderlein, 1892). At puberty and before menstruation, the flora again changes to one predominantly of lactobacilli, and remains so in normal individuals throughout the reproductive period. At menopause there is a final change to the mixed flora found before puberty (Pettit & Hitchcock, 1933; Soeken, 1926; Dychno & Dertschinsky, 1930). These changes are thought to be correlated with the appearance of glycogen in the mucosal epithelium induced by oestrin formation (Miura, 1928; Cruickshank & Cruickshank, 1931; Cruickshank & Sharman, 1934*a, b*). Döderlein's (1892) original thesis, also maintained by others, was that the production of lactic acid by the Gram-positive bacilli served as one of the protective measures against infection.

Organisms assumed to be 'Döderlein's bacillus' have been variously designated as *Bacillus vaginae*, *B. vaginalis longus*, *B. vaginalis*, *Acidobacterium doederleinii*, *Plocamobacterium vaginae*, *Lactobacillus doederlein*, *Bacillus lacticus* and *Lactobacillus*

acidophilus (Bergey's Manual, 1948). The Gram-positive rods isolated in previous work are so incompletely described that it is impossible to know what they really were, except that they may often have been lactobacilli, or even diphtheroids. In a number of investigations diagnoses for 'Döderlein's bacillus' have been made from studies of smears only.

During an investigation at the U.S. National Institutes of Health by one of us (M.R.), the purpose of which was to explore the relation between bacterial and trichomonad metabolism, modern methods of study (Rogosa *et al.* 1953; Sharpe, 1955) were used to isolate and characterize vaginal lactobacilli from normal non-pregnant women. It was almost immediately apparent that the isolated strains did not form a homogeneous group, and certainly not within a species, since homofermentative and heterofermentative organisms were obtained.

METHODS

The vaginal secretions were obtained by the swab technique designed to minimize external contamination (Miura, 1928; Soeken, 1926; Cruickshank & Sharman, 1934*a, b*). The samples were plated on the selective lactobacillus medium of Rogosa, Mitchell & Wiseman (1951*a, b*) and incubated at 37° anaerobically for 4 days in an atmosphere of 90 % (v/v) N₂ and 10 % (v/v) CO₂. Isolations from representative colonies were made and replated as required to ensure purity.

The methods used for the identification of the isolates combined cultural, biochemical and serological techniques to establish correlations of characteristics. These methods have previously proved to be highly successful in characterizing lactobacilli from a variety of sources (Rogosa *et al.* 1953; Sharpe, 1955) and have been developed from the basic studies of Orla-Jensen (1919, 1943).

RESULTS

Thirty-five strains of lactobacilli from twenty-one individuals, aged 21–40 years, were isolated; 21 of these isolates were studied in detail to determine their species designation. The remaining 14 strains were lost, but not before tests for gas production had been completed. Heterofermentative lactobacilli for the total sampling were found in approximately the ratio of 1:4 as shown in Table 1.

Fourteen of the 21 isolates were typically *Lactobacillus acidophilus*, and were so designated by means of the criteria of Orla-Jensen (1919, 1943), Rogosa *et al.* (1953), Sharpe & Mattick (1957), Harrison & Hansen (1950), de Man (1956), Wheeler (1955), and others. Serological techniques for the confirmatory identification of *L. acidophilus* are not available since successful group antisera for either the agglutination or precipitin techniques have not yet been produced. The two strains of the second homofermentative species, *Lactobacillus casei* var. *rharnosus* (Rogosa *et al.* 1953; Sharpe & Wheeler, 1957) were typical in all respects, including possession of the group C antigen.

The remaining isolates were heterofermentative. Two of the strains of *Lactobacillus fermenti* had typical characteristics, including the group F antigen. However, two of the strains, although typical in other respects, did not react serologically and thus resembled many of the strains of intestinal origin.

In a previous examination by one of us (M. E. S.) of the faeces of twelve breast-fed infants 6–11 days old, in addition to the so-called *Lactobacillus bifidus* (Sundman, Björkstén & Gyllenberg, 1959), authentic lactobacilli were also isolated from three

Table 1. *Species characteristics and frequency distribution of human vaginal lactobacilli*

	No. of strains	Gas from glucose	NH ₃ from arginine	Configuration of lactic acid	Aesculin cleavage	Amygdalin	Arabinose	Cellobiose	Glycogen	Mannitol	Mannose	Melezitose	Melibiose	Raffinose	Rhamnose	Salicin	Sorbitol	Sorbose	Trehalose	Xylose
<i>L. acidophilus</i>	14	—	—	DL	+	+	—	+	±	—	+	—	—	±	—	+	—	—	+	—
<i>L. casei</i> var. <i>rhamnosus</i>	2	—	—	L(+)	+	+	—	+	±	—	+	—	—	—	—	+	—	—	+	—
<i>L. fermenti</i>	4	+	+	DL	—	—	+	—	—	—	—	—	+	+	—	—	—	—	—	—
<i>L. cellobiosus</i>	1	+	+	DL	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—

± = variable reaction. All strains fermented glucose, galactose, lactose, fructose and maltose. Half the strains of *L. acidophilus* and both strains of *L. casei* var. *rhamnosus* fermented dextrin slowly or slightly. All except one strain of *L. casei* var. *rhamnosus* fermented sucrose. Since the sucrose fermentation is adaptive for this organism, this is not uncommon. No strains fermented adonitol, erythritol, inulin, α-methyl D-mannoside, α-methyl D-glucoside was fermented by one strain of *L. casei* var. *rhamnosus* only.

The nutritional characteristics (not shown) were identical with typical species behaviour as described by Rogosa *et al.* (1953).

of the infants; these strains were all *L. fermenti*, giving the typical biochemical and serological reactions of this species. Witkowski (1985) showed that the mouth of the infant shortly after birth contains organisms in proportions corresponding closely to the flora of the mother's vagina. 'The intestine at birth is either sterile or contains a few bacteria; infection rapidly occurs, partly from above and partly from below' (Topley and Wilson's *Principles*, 1955). The strains of *L. fermenti* described here may also have had a similar origin. The heterofermentative strain of *L. cellobiosus*, a species previously isolated from the mouth, had the modal characteristics described by Rogosa *et al.* (1953).

Occasionally certain *Leuconostoc* species may be isolated from the medium designed for the selection of lactobacilli (Perry & Sharpe, 1960). It is of incidental interest that *L. mesenteroides* was isolated in the present work and found to be identical with the organism as described by Garvie (1960). This appears to be the first reported isolation of this heterofermentative lactic acid coccus from the human vagina. It is also of interest since in morphology this organism often resembles the short coccobacillary forms of lactobacilli, particularly in acidic environments. Thus examinations of smears for 'Döderlein's bacillus' might be misleading in underestimating the real coccal population.

DISCUSSION

Except that Döderlein (1892) had most probably isolated a lactobacillus, the species identities of the vaginal lactobacilli have not previously been satisfactorily established. In many instances any rod-like bacterium was identified as 'Döderlein's bacillus' on the basis of morphology alone. Although Miura (1928) had identified the lactic acid produced by his isolate as the DL enantiomorph, it is impossible to identify the organism from the fragmentary data. Many species of lactobacilli produce DL-lactic acid. Thomas (1928), who used inadequate and somewhat questionable tests, concluded that 'Döderlein's bacillus' was *Lactobacillus acidophilus*, but recognized that this terminology was loose and generic, as was the case for the wide spectrum of oral lactobacillus species also previously designated as *L. acidophilus* (Rogosa *et al.* 1953; Tilden & Svec, 1950).

Although Miura (1928) found that his isolate did not ferment glycogen, Wheeler (1955) observed that 43 % of her strains of *Lactobacillus acidophilus* did so. We have recently re-studied representative strains of Wheeler's collection and have confirmed her results. On originally testing our isolates, none of our strains fermented glycogen. However, it was noted that the initial pH value of the medium was relatively high for lactobacilli (pH 7.0). When the tests were repeated with 9 representative strains at more favourable pH values (5.0, 5.8, 6.5) five of them fermented glycogen, but usually weakly. This behaviour was not associated with consistent species activity but rather with individual strains and in repeated experiments seemed to be an adaptive and variable phenomenon, independent of anaerobiosis or aerobiosis. Thus it seems highly probable that these lactic acid bacteria obtain available carbohydrate from the enzymic conversion of glycogen if not other carbohydrates, by the tissues or by micro-organisms other than lactobacilli. Also a considerable heterofermentation in the vagina is strongly suggested by the present results.

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