SHORT COMMUNICATION

Characterization of Two Strains of Cariogenic Lactobacilli

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Two strains of lactobacilli that initiate dental caries in conventional animals were examined for their physiological and serological characteristics. The strain designated V CL-25 was identified as *Lactobacillus fermentum* and belonged to serological group F. The strain designated IV CL-37 was a *Lactobacillus salivarius*, but it could not be further identified as either of the known subspecies, nor did it belong to serological group G.

INTRODUCTION

Dental caries results from the production of acid by bacteria in the dental plaque. Accordingly, there has been prolonged interest in the role of streptococci and lactobacilli in the aetiology of the disease (Burnett & Scherp, 1968). The ability of certain strains of lactobacilli to cause dental caries has been shown in animal experiments. Mono-infection of gnotobiotic rats by *Lactobacillus casei* (Wagner, 1966; Rosen *et al.*, 1968) and *Lactobacillus acidophilus* (Fitzgerald *et al.*, 1966), in conjunction with diets high in fermentable carbohydrates, resulted in extensive cavitation of the molar teeth. Of greater significance is the report that two lactobacilli isolated from caries-active sites in humans induce significant caries in conventional hamsters harbouring a normal microflora (Fitzgerald *et al.*, 1980). The aim of the present study was to identify these two strains on the basis of their physiological characteristics and also to examine their serological properties. Lactobacilli can be divided into seven serological groups (Sharpe, 1955; Rogosa & Sharpe, 1959; Sharpe, 1970), and the respective antigens from groups A to F (Knox & Wicken, 1976) and group G (Knox *et al.*, 1980) have been identified.

METHODS

Organisms. *Lactobacillus* strains V CL-25 and IV CL-37 were isolated from caries-active sites in first permanent molars of 9-year-old schoolchildren from Puerto Rico (Fitzgerald *et al.*, 1980). Strains *L. salivarius* subsp. *salivarius* ATCC 11741, *L. salivarius* subsp. *salicinus* ATCC 11742 and *L. fermentum* NCTC 6991 were employed for comparative studies.

Growth conditions. Unless otherwise stated, lactobacilli were grown under anaerobic conditions (95% N₂/5% CO₂) without pH control at 37 °C in MRS medium (de Man *et al.*, 1960) containing 2% (w/v) glucose. Preliminary identification of the two unknown lactobacilli was made by using the API-

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Table 1. *Identification of lactobacilli by cultural tests*

Test	Strain V CL-25	Strain IV CL-37
Aerobic growth	+	+
Final pH in broth	4.7	3.8
Gas from glucose	+	—
Gas from gluconate	—	—
NH ₃ from arginine	+	—
Catalase production	—	—
Slime from sucrose	—	—
Hydrolysis of aesculin	—	—
Fermentation of:		
Ribose	—	—
Rhamnose	—	—
Fructose	+	+
Glucose	+	+
Mannose	+	+
Galactose	+	+
Sucrose	+	+
Trehalose	—	+
Maltose	+	+
Cellobiose	—	—
Melibiose	+	+
Lactose	+	+
Raffinose	+	+
Sorbitol	—	—
Mannitol	—	+
Salicin	—	—

Both strains gave a negative reaction with xylose, arabinose, sorbose, melezitose, glycerol, inositol, amygdalin, inulin, dextran, glycogen and liquid starch; strain IV CL-37 gave a positive reaction (2 mm zone) in the more sensitive starch plate method.

System-Lactobacillus (Analytab Products, Plainview, N.Y., U.S.A.). As the strains differed in one or more diagnostic criteria from strains of prototype standard species, a more comprehensive study of the fermentation characteristics was undertaken using previously described physiological tests (Sharpe *et al.*, 1966; Sharpe, 1979).

Serological methods. Antisera to *L. salivarius* subsp. *salivarius* ATCC 11741, *L. fermentum* NCTC 6991 and strain IV CL-37 were prepared from heat-killed cells as previously described (Knox *et al.*, 1979, 1980). Whole-cell extracts were obtained by autoclaving (Campbell *et al.*, 1978), and soluble wall fractions were obtained by the action of M-1-*N*-acetylmuramidase from *Streptomyces globisporus* 1829 on wall preparations (Jacques *et al.*, 1979). Antigens were detected by the ring precipitin test.

RESULTS

Identification of strain V CL-25

Strain V CL-25 produced round shiny grey opaque colonies of 1.5 mm diameter after 24 h growth on MRS agar. Cells were Gram-positive rods of medium length, either singly or in pairs. In liquid medium containing glucose, the organism grew at 45 °C but not at 15 °C and was heterofermentative, producing gas from glucose.

Studies employing the API-System-Lactobacillus indicated that the strain had the general physiological characteristics of *Lactobacillus fermentum* as it hydrolysed arginine and fermented a range of sugars compatible with the identification; the exception was ribose, which is usually fermented by strains of this species. The designation of strain V CL-25 as *L. fermentum* was confirmed by more detailed physiological studies, the results of which are summarized in Table 1.

Many strains of *L. fermentum* belong to serological group F where the group antigen is a lipoteichoic acid (Knox *et al.*, 1970) which can be extracted with hot water (Wicken *et al.*,

1973). An extract from strain V CL-25 was prepared by autoclaving cells for 1 h and tested for its reactivity with antiserum containing group-specific antibodies raised against *L. fermentum* NCTC 6991. A positive reaction was obtained in the ring precipitin test, supporting the conclusion that the strain is *L. fermentum* (Sharpe, 1970; Knox & Wicken, 1976).

Identification of strain IV CL-37

Strain IV CL-37 produced round shiny grey opaque colonies of 1.5 mm diameter after 24 h growth on MRS agar. Cells were Gram-positive coccobacilli. The strain could grow at 45 °C, but not at 15 °C, with a homofermentative utilization of glucose. The absence of ribose fermentation and the lack of production of CO₂ from gluconate placed this strain in the subgenus *Thermobacterium*. The fermentation characteristics of strain IV CL-37 were those of *L. salivarius* (Table 1), a designation that was specifically supported by the fermentation of mannitol and melibiose but not cellobiose. Two subspecies of *L. salivarius* are recognized, namely subsp. *salivarius* and subsp. *salicinus* (Rogosa *et al.*, 1953). Strain IV CL-37 differed from *L. salivarius* subsp. *salivarius* in that it did not ferment sorbitol and rhamnose, and from *L. salivarius* subsp. *salicinus* in that it did not utilize salicin, aesculin and sorbitol.

The type strains of *L. salivarius* belong to group G (Rogosa & Sharpe, 1959; Sharpe, 1970) and the antigen isolated from strain ATCC 11741 has been identified as a phosphate-containing polysaccharide (Knox *et al.*, 1980). Antisera prepared against strain ATCC 11741 gave a positive reaction with enzymic lysates of homologous wall but failed to give a detectable reaction with a comparable preparation from strain IV CL-37.

It is concluded that strain IV CL-37 is *L. salivarius* although it does not conform to either of the accepted subspecies and it does not contain the group G antigen.

DISCUSSION

The identification of the two cariogenic strains as *L. fermentum* and *L. salivarius* is relevant to earlier studies on the incidence of oral lactobacilli. Bowen (1967) reported that the homofermentative lactobacilli most prevalent in dental plaque and carious dentine of monkeys were *L. casei*, *L. plantarum* and *L. salivarius* and that *L. fermentum* was the predominant heterofermenter. *Lactobacillus salivarius* has also been identified as one of the more common species in infected human dental root canals (Sundqvist & Carlsson, 1974).

The potential significance of lactobacilli in the advanced stages of caries progression is related to the prior establishment of environmental conditions within the lesion more favourable to the growth of lactobacilli and less favourable to the survival of other organisms. One of the important factors in this regard may be the local change in pH towards the more acidic condition (Fitzgerald *et al.*, 1980). Such acidic condition may actually aid the growth of certain lactobacilli. Tween 80 is a component of MRS medium and provides a source of oleic acid, which is essential for the growth of certain lactobacilli under standard conditions (de Man *et al.*, 1960). However, studies with strains of *L. salivarius*, particularly IV CL-37, indicate that while Tween 80 is necessary for growth at pH 7.0 it is not essential for growth at pH 6.0 or below (Jacques *et al.*, 1980).

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