A STUDY OF NON-MOTILE AND MOTILE STRAINS OF GROUP D STREPTOCOCCI: SEROLOGICAL GROUPING AND IDENTIFICATION AS ENTEROCOCCI

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SUMMARY

Sixty-seven strains of streptococci belonging to Lancefield Group D—57 from animals or birds and eight from humans—were isolated and examined. Three strains were motile. Sixty-six strains were enterococci and one was classified as *Str. bovis*. Four strains were beta-haemolytic on horse blood agar but only one of them was beta-haemolytic on sheep blood agar.

Six strains of enterococci and the *Str. bovis* strain had little or no tyrosine decarboxylase activity.

I. INTRODUCTION

Lancefield (1933) described serological Group D streptococci after examination of eight strains of haemolytic streptococci isolated from cheese. Subsequently Lancefield (quoted by Sherman 1937) and Sherman (1937) noted that strains of enterococci such as *Streptococcus faecalis* and *Str. liquefaciens* reacted with Group D antisera.

In 1937, Sherman divided the streptococci into four groups on physiological characteristics and his criteria for the enterococci were: growth at 10°C and 45°C, in the presence of 6·5 per cent. NaCl, at pH 9·6, in 0·1 per cent. methylene blue milk, strong reduction of litmus milk, survival at 60°C for 30 min, and production of ammonia from peptone. The species included in the enterococci by Sherman were *Str. faecalis, Str. liquefaciens, Str. zymogenes,* and *Str. durans.*

In 1949, Shattock produced evidence that *Str. bovis* belonged to Lancefield Group D, although Sherman (1937) had included this organism in his viridans group. *Str. bovis* differed from the enterococci in that it did not grow in 0·1 per cent. methylene blue milk, at 10°C, in 6·5 per cent. NaCl, at pH 9·6, did not strongly reduce litmus milk, or produce ammonia from peptone. Sharpe (1948) differentiated the enterococci from *Str. bovis* on the basis of the lack of tyrosine decarboxylase activity in the latter organism.

This paper describes the results of haemolytic activity, growth at 45°C, in 6·5 per cent. NaCl, at pH 9·6, reduction of litmus milk, survival after heating at 60°C for 30 min, tyrosine decarboxylase activity, motility, and serological grouping of 67 strains of streptococci.

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In view of the reports of motile Group D streptococci (Hugh 1959; Langston, Gutierrez, and Bouma 1960), an investigation of the motility of the Queensland isolates was included in this study.

II. MATERIALS AND METHODS

(i) *Source of Strains.*—Fifty-nine strains were isolated on 10 per cent. sheep blood agar during the routine examination of specimens submitted to this Institute. Thirty-two strains were isolated from fowls, 15 from pigs, 6 from cattle, 3 from birds other than fowls, 2 from sheep and 1 from a goat. In addition, eight human strains were obtained from the Pathology Department, Brisbane General Hospital. The tissues from which the 67 strains were isolated are given in Table 1.

(ii) *Serological Methods.*—The strains were identified as Lancefield Group D by Miss S. McLean, University of Adelaide, and later these results were confirmed using a Group D antiserum prepared at this Institute as described by McLean (1955), but because rabbits were found to produce unsatisfactory antiserum, sheep were used. This antiserum was prepared against strains CN2530, CN2625 and CN2626 (obtained from Streptococcal Reference Laboratory, Colindale, England), and tested against the same three strains and in addition strain C1 originating from Dr. R. Lancefield, Rockefeller Institute of Medical Research.

The precipitin test was done in tubes with an internal diameter of 3 mm, using extracts prepared by concentrating the polysaccharide as recommended by Shattock (1949).

In addition, the 67 strains were tested against sera of Groups A, B, C, E, F, G, H, K, L, M, N and O.

(iii) *Cultural Methods.*—The strains were kept in tryptone soya broth (Oxoid) at 4°C after incubation for 24 hr at 37°C. These broth cultures were subinoculated into tryptone soya broths which were used for inoculation of test media. All liquid media were inoculated with a loopful of a 24-hr culture and solid media were inoculated by streaking out a loopful of the broth culture onto the surface.

Motility was determined both by dark-ground examination at intervals up to 48 hours' growth at room temperature (25°–28°C), 31°C and 37°C in tryptone soya broth, and also by use of semi-solid medium (Edwards and Ewing 1955).

Morphology-Gram stains were done. The flagella stain used was the method of Leifson (1951).

Haemolysis was determined on 10 per cent. blood agar, using both sheep and horse blood. A thin layer of blood agar was poured on top of a layer of salt agar. The cultures were incubated under anaerobic conditions in a McIntosh and Fildes jar and examined after 24 hr.

Growth at 45°C was determined by the method of Barnes, Ingram, and Ingram (1956); growth in 6·5 per cent. NaCl by the method of Barnes, Ingram, and Ingram (1956); and growth at pH 9·6 by the method of Shattock and Hirsch (1947).

For the reduction of litmus milk, Litmus milk (Baltimore–Biological Laboratory) was used and the result recorded after 24 hours' incubation.

Survival after 60°C for 30 min was measured by the method of Barnes, Ingram, and Ingram (1956), and tyrosine decarboxylase activity by the method of Sharpe (1948).

III. RESULTS

(i) *Serology.*—All strains gave a precipitate with Group D antiserum and not with antisera for other groups.

TABLE 1

STRAINS OF STREPTOCOCCI ISOLATED

Animal	Tissue	Strain Numbers
Fowl	Liver	1, 5, 7, 13, 23, 26, 29, 30
	Joint	2, 3, 4, 8, 9, 10, 11, 15, 16
	Eye	6, 18, 31
	Lung	12, 14, 19, 25, 28
	Mandible abscess	17
	Trachea	20
	Heart	22, 24, 27, 33
	Intestine	32
Budgerigar	Liver	88
	Lung	89
Turkey	Lung	87
Pig	Liver	41, 47
	Spleen	42
	Pus	43
	Pleural fluid	44, 48
	Heart blood	45
	Heart	46
	Kidney	49, 53
	Stomach	50
	Semen	51, 52
	Lung	54, 55
Cattle	Spleen	60
	Myocardium	61
	Kidney	62
	Blood	63
	Pus	64
	Kidney	65
Human	Faeces	70, 71, 72, 73, 74, 75, 76, 77
Sheep	Hock	80, 81
Goat	Liver	84

(ii) *Motility.*—All strains, except 42 (pig, spleen), 54 (pig, lung) and 77 (human, faeces), were non-motile. These motile strains produced diffuse growth in semi-solid medium and at a critical point in their growth cycle were clearly motile by dark-ground examination. Motility was seen in cultures held at room temperature, 31°C and 37°C, the streptococci usually being motile when turbidity was first noticed. This occurred at 4-18 hr, depending on the temperature of incubation, with earlier appearance at higher temperatures.

(iii) *Morphology.*—All were Gram-positive cocci occurring in pairs or in short chains. One or two flagella per coccus were seen in preparations made from cultures of the motile streptococci.

(iv) *Haemolysis.*—Four strains (27, 28, 60 and 88) produced beta-haemolysis on horse blood agar but only one (60) produced beta-haemolysis on sheep blood agar. With these exceptions the strains were alpha-haemolytic.

(v) *Growth at* 45°*C.*—All strains except one (64) grew.

(vi) *Growth in* 6·5 *per cent. NaCl.*—All strains except one (64) grew.

(vii) *Growth at pH* 9·6.—All strains except one (64) grew.

(viii) *Litmus Milk.*—Under the test condition it was difficult to assess reaction as strong or weak reduction. Twenty-seven strains produced a clot (1, 2, 4, 5, 6, 7, 11, 15, 16, 19, 20, 22, 23, 24, 26, 30, 31, 32, 42, 44, 47, 50, 53, 65, 76, 77 and 88).

(ix) *Survival After Heating at* 60°*C for* 30 *Minutes.*—All strains survived.

(x) *Tyrosine Decarboxylase.*—All were positive except porcine strains 48, 51, 53, and 54, bovine strain 62 and human strain 77.

IV. DISCUSSION

All strains belonged to Lancefield Group D. All strains except strain 64, which did not grow at 45°C, at pH 9·6 and in 6·5 per cent. NaCl, can be considered as belonging to the Sherman group of enterococci. Strain 64 has the characteristics of *Str. bovis* (Sherman 1937). However, this strain did produce tyrosine decarboxylase, although Sharpe (1948) stated that *Str. bovis* had little or no tyrosine decarboxylase activity.

Four strains produced beta-haemolysis on horse blood although only one did so on sheep blood agar. As horse blood is recognized as the standard type of blood for determining haemolytic activity of streptococci (Wilson and Miles 1955), the two fowl strains and the single bovine and budgerigar strains may be classed as beta-haemolytic.

As six strains (48, 51, 53, 62 and 77) did not produce a positive tyrosine decarboxylase result even though they could be classed as enterococci, it would appear that this test has limited value for distinguishing *Str. bovis* from enterococci.

The discovery of three motile strains in such a small group of strains is surprising, although reports of motile streptococci are by no means rare (Hugh 1959; Langston, Gutierrez, and Bouma 1960). It is possible that they are overlooked because motility is only apparent during a short phase of their growth. The use of semi-solid medium simplifies greatly the testing for this characteristic.

The great diversity of body tissues harbouring enterococci presents a problem in the selection of strains to be identified if these organisms are potential pathogens. At present they are looked upon as relatively harmless bacteria but this may not necessarily be true (Evans 1957; Ihlenburg 1960).

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