STREPTOCOCCUS FECALIS

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In some respects *Streptococcus fecalis* (Andrewes and Horder, 1906) might be considered one of the better established species of the streptococci, and certainly some of the rather unique characteristics of this organism, or the general group to which it belongs, are commonly known by bacteriologists. However, much concerning this species is not clear. As the fermentation tests have made up the major portion of the criteria used by many investigators of this organism, and since these tests are extremely variable within this particular species, there is considerable confusion concerning the boundaries of the group and whether one or more species are involved. On the one hand, we have those who would give this group of organisms separate generic standing (*Enterococcus*) quite outside of the streptococci, but many of whom would at the same time lump together a rather heterogeneous mixture of fecal streptococci, as if only one species were contained in this special genus. On the other hand, among those who group these organisms as streptococci, are some who would classify as separate species variants within that rather homogeneous group which is more specifically known as *S. fecalis*. There are, therefore, many points on which additional information is needed. And in passing it might be noted that so long as we are fated to see new papers purporting to show the identity of *S. fecalis* and *Streptococcus lactis*, and to read in papers on the enterococcus that this organism probably includes "the '*S. lactis*' of dairy workers," no apology need be made for the presentation of a paper which contributes, however slightly, to a clearer understanding of this group of organisms.

The present study, though not a continuous one, has extended over several years and has covered 434 cultures which have been identified as *S. fecalis*. All of these cultures were tested for their minimum and maximum temperatures of growth, for action on milk and reducing action on litmus, hemolysis of blood, final pH in glucose broth, production of ammonia from peptone, hydrolysis of starch, liquefaction of gelatin, and ability to ferment each of the ten test substances used. All except 128 of the cultures were tested for ability to hydrolyze esculin and sodium hippurate, and for the production of acetyl-methyl-carbinol. The thermal resistance of the organisms was not tested on the entire collection, but this well known characteristic of the group was checked on 132 cultures. The more recently suggested "tolerance tests"—the ability to grow in media of high alkalinity, and in the presence of relatively high concentrations of sodium chloride and methylene blue—were used on only a few of the cultures of this collection, but we have had sufficient experience with these tests on other cultures to feel sure of their value in studies of this group of the streptococci (Sherman and Stark, 1934; Sherman, Stark and Mauer, 1937).

This study is limited to non-hemolytic and non-proteolytic cultures. The hemolytic and proteolytic members of the "enterococcus group"—*Streptococcus zymogenes* and its relatives—are dealt with in another paper.

THE VALUE OF CERTAIN BASIC TESTS

In a number of papers from this laboratory we have emphasized the value of minimum and maximum temperatures of growth in the study of the streptococci. All of our strains of *Streptococcus fecalis* grew at 10°C. and at 45°C. Some cultures were able to grow at 50°C. and all except five grew at 5°C. Indeed, Foter and Rahn (1936) have shown that at least one of the strains in this collection was able to grow at 0°C.

Taking 10°C. and 45°C. as the test temperatures, we have a rather striking combination for the characterization of *S. fecalis* and its relatives in the enterococcus group—*S. zymogenes* and *Streptococcus liquefaciens*. While not the exclusive property of

these organisms, this combination of temperature tolerance for growth is not found among any of the better known and adequately described species of the streptococci. It is not true of any of the following species, or their varieties: *Streptococcus pyogenes, Streptococcus mastitidis, Streptococcus lactis, Streptococcus salivarius, Streptococcus bovis, Streptococcus inulinaceus, Streptococcus equinus,* and *Streptococcus thermophilus* (Sherman and Albus, 1918; Sherman and Stark, 1931 and 1934; Sherman and Wing, 1935; Hansen, 1935; Safford, Sherman and Hodge, 1937; Hodge and Sherman, 1937). As indicated above, other streptococci are known which can grow at both of these temperatures. Sherman and Wing (1935) studied a hemolytic and non-reducing streptococcus from milk powder, and Mauer (1934) found another hemolytic and non-reducing type in feces, which grew both at 10°C. and at 45°C.

In this connection we beg to be pardoned for mentioning a hoary subject: Including many cultures of *S. fecalis* in addition to the 434 used in this study, we have not yet had a strain of this organism which has failed to grow at 45°C., while among a larger number of *S. lactis* we have not encountered one which grows at that temperature. We do not mean to imply that the temperature limits of growth do not vary as do other characteristics of bacteria. In fact, we have recently pointed out the variation which may occur in this respect in certain lactobacilli (Sherman and Hodge, 1936). It so happens, however, that in the streptococci the normal temperature limits are sufficiently different between certain groups to make these tests of paramount importance.

Another important test in the study of streptococci is one for the ability to cause a complete reduction of litmus in milk before curdling. So far as present information goes, the only streptococci which have this property are those of the "enterococcus group" (*S. fecalis, S. zymogenes* and *S. liquefaciens*) and those of the "lactic group" (*S. lactis* and its relatives). It is true that *Streptococcus apis* has also been described as having this characteristic, but the work of Hucker (1932) indicates that this organism and *S. liquefaciens* are identical. All of our cultures

of *S. fecalis* gave a prompt and complete reduction of litmus in milk, with the exception, of course, of the narrow zone which is exposed to the air at the surface. Most of the cultures reduced before curdling in the typical way, but in a few the reduction was completed after curdling. In these cultures, however, the reduction was complete and prompt, and could not be confused by an observant worker with the marked, but not quite complete, reduction after curdling which is given by such organisms as *S. salivarius* and *S. mastitidis*.

The production of ammonia from peptone (Ayers, Johnson and Mudge, 1921) is a valuable, though unexploited, characteristic of certain streptococci. All of our cultures were found to produce ammonia. The ability to hydrolyze esculin has been considered a special characteristic of the enterococcus group (Meyer and Schönfeld, 1926), and none of our cultures of *S. fecalis* failed to attack this substance.

What may eventually prove to be highly specific tests for streptococci belonging to the enterococcus group are those for the ability to grow in broth containing 6.5 per cent of sodium chloride, and in broth adjusted to a pH of 9.6 (Sherman and Stark, 1934; Sherman, Stark and Mauer, 1937). Although these tests have been tried on only 24 of the cultures of *S. fecalis* contained in this collection, all of them grew actively. (In making these tests the sodium chloride and the alkali were added to the previously sterilized 0.5 per cent glucose broths immediately before use.)

The ability to grow in skimmed milk containing 0.1 per cent medicinal methylene blue is a property which is limited to the "enterococcus" and "lactic" groups of the streptococci, in so far as present knowledge extends (Sherman, Stark and Mauer, 1937). The much-used and uncritically-applied test of Sherman and Albus (1918), involving more dilute methylene blue, was devised for another purpose and is of course much less inhibitory. *S. fecalis* grows actively in the presence of 0.1 per cent methylene blue.

DIVERSITY OF FERMENTATION REACTIONS

Although the fermentation tests are known to show considerable diversity in different strains of *S. fecalis*, the extent of

this variability within the species has not been fully appreciated. All of the 434 cultures studied by us fermented glucose, maltose, lactose and salicin. On the other hand, fermenting and non-fermenting strains were found with arabinose, sucrose, raffinose, inulin, glycerol and mannitol. Some strains fermented none of these six substances, while a few strains fermented them all; and it might be added that the collection contained a goodly proportion of the theoretically possible 64 strains obtainable with six diverse characteristics. This in itself would indicate that no species differentiation based on fermentation tests would be permissible within the group, but perhaps it is better to be more specific.

The fermentation of mannitol has long been recognized as especially characteristic of *S. fecalis* (Andrewes and Horder, 1906; Winslow and Palmer, 1910; and many subsequent investigators). Our results confirm these findings in a broad sense, but we had 13 strains out of the entire collection which failed to ferment mannitol. That these mannitol-negative strains do not constitute a definite and clear-cut type is shown by the fact that among this small number were fermenting and non-fermenting strains on arabinose, sucrose, raffinose, and glycerol. In this connection it should be recalled that Dible (1921) also found a variant form of the "enterococcus" which failed to ferment mannitol. *S. fecalis* is usually described as not fermenting inulin. This also is generally true, but 11 of our strains fermented this substance; and among these atypical cultures were strains which fermented, and others which did not ferment, arabinose, raffinose, and glycerol. Again, considering the radical fermentative diversity within such a small number of cultures, the recognition of a type which ferments inulin would not be justifiable on the basis of present knowledge. The same is true with glycerol: Among the glycerol-positive and glycerol-negative cultures were strains which fermented, and others which did not ferment arabinose, sucrose, raffinose, inulin, and mannitol.

This emphasis on the diversity of *S. fecalis* in the fermentation tests should not be interpreted as implying that they are without value in the study of the streptococci. In some groups, certain of these tests are of very great importance; and when applied

in their proper subsidiary rôle they have an important function in the characterization of all of the groups within the genus.

THE STREPTOCOCCUS GLYCERINACEUS OF ORLA-JENSEN

Orla-Jensen (1919) has divided the streptococci of this group, which do not liquefy gelatin, into two species, *Streptococcus faecium* and *Streptococcus glycerinaceus*, based principally upon the fermentation of glycerol. The rather slender basis upon which this differentiation rests has been previously noted (Sherman and Stark, 1931). *S. faecium* has been generally recognized as identical with *S. fecalis*, but the name *S. glycerinaceus* has, to some extent, become attached to the literature. So far as can be told from Orla-Jensen's data, *S. glycerinaceus* appears very definitely to belong in the "enterococcus group." From a study of his titration figures, it also appears that a number of his cultures of *S. faecium* fermented glycerol to some extent, though *S. glycerinaceus* caused a more vigorous fermentation of this substance.

In view of what has been said in the preceding section, it seems scarcely necessary to pursue this question further, but since a separate species has been proposed on the basis of glycerol fermentation, and since *S. fecalis* has frequently been described as not fermenting raffinose and always fermenting mannitol, we have subjected our data to detailed analysis. With these three substances, if the fermentation of each of them is an inconstant characteristic within the species, there would be as possibilities eight combinations of characteristics from (raffinose +, mannitol +, glycerol +) to (raffinose −, mannitol −, glycerol −). Our collection contained seven of these eight possible strains. When sucrose is added to these three substances, the possible number of different combinations becomes 16 and our collection contained 12 of the strains. This would appear sufficient to show that the various reactions on these tests are random ones, and that no additional species can be established in the group, based upon the fermentation or non-fermentation of any one of these substances.

One should not take issue lightly with the magnificent work of

Orla-Jensen. The improbable possibility should still be admitted that his organism is something quite outside of the enterococcus group. If this be the case, his species has not yet been properly defined.

THE CHARACTERISTICS OF STREPTOCOCCUS FECALIS

General characteristics

The characteristic grouping is in pairs and to a less extent in short chains. The organism is hardy and grows well in laboratory media. Blood is not hemolyzed, and gelatin is not liquefied. Milk is acidulated and curdled without digestion of the casein.

Characteristics of primary differential value

Growth takes place at 10°C. and at 45°C. At 5°C. only a few cultures fail to grow, while at 50°C. growth may or may not take place but more often does not.

Litmus in milk cultures is completely reduced, and with relatively few exceptions the reduction takes place before the milk is curdled. In the few strains which reduce after curdling, the reduction of the litmus is prompt and complete below the surface layer, and is in marked contrast with the picture produced by those streptococci which cause a fairly strong but not quite complete reduction after curdling.

Growth takes place in media having an initial pH value of 9.6; in the presence of 6.5 per cent of sodium chloride; and in the presence of 0.1 per cent of medicinal methylene blue in skimmed milk.

Characteristics of secondary differential value

Ammonia is produced in 4 per cent peptone; esculin is hydrolyzed; and a heat treatment of 62.8°C. for 30 minutes, in skimmed milk, is survived. In glucose broth final pH values of 4.4 to 4.0 are obtained; starch is not hydrolyzed; while sodium hippurate may or may not be hydrolyzed, as revealed by the conventional test. Acetyl-methyl-carbinol is produced in skimmed milk cultures.

Fermentation characteristics

The fermentation tests are diverse within the species and these characteristics are regarded as of minor importance in this group.

Glucose, maltose, lactose, and salicin are fermented. Arabinose, sucrose, raffinose, inulin, glycerol, and mannitol may or may not be fermented. Inulin, however, is rarely fermented (11 of 434 cultures), while only a few cultures fail to ferment mannitol (13 of 434 cultures).

SUMMARY

A study was made of 434 cultures of *Streptococcus fecalis* with the application of a more extensive series of tests than has heretofore been used in its characterization. Especial attention was paid to certain basic tests of primary differential value which are not in general use by students of the streptococci. The fermentation tests are diverse within this species and are regarded as of minor value in its description. On the basis of the more fundamental tests, the species is made up of a very homogeneous group of organisms, and no justification was found for its subdivision on the basis of the fermentation tests.

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