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***Aerococcus*, a New Bacterial Genus**

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SUMMARY: The generic name *Aerococcus* is proposed for a group of aerobic Gram-positive cocci that are commonly found in the air of occupied places and in dust. The most characteristic features of the organisms are ability to grow in the presence of 40 % bile and to produce greening on blood agar. They are catalase-negative, and do not show chain-formation in culture.

For the collection and recognition of mouth streptococci from air a selective culture medium was devised (Williams & Hirsch, 1950) which had the composition: serum agar, 100 ml.; sucrose, 5 g.; potassium tellurite, 1.0 mg.; crystal violet, 0.25 mg. This selective medium inhibits the growth of diphtheroid and coliform organisms and practically all strains of those Gram-positive cocci that would ordinarily be regarded as staphylococci. It permits good growth of most streptococci, including *Streptococcus pyogenes*, viridans-type streptococci and enterococci. However, in air samples from occupied rooms, about 70 % of the colonies are not of typical streptococci, but are of a Gram-positive coccus that does not appear to have been adequately described in the literature. We consider that this organism has sufficient points of difference from both streptococci and staphylococci to justify its being placed in a new genus for which we propose the name *Aerococcus*.

DESCRIPTION OF *AEROCOCCUS*

Cultural and morphological characteristics

After 18-24 hr. incubation at 37° on blood agar the colonies of *Aerococcus* are 0.5-2.0 mm. in diameter, semi-transparent, white or grey, and surrounded by a wide or narrow zone of greening (Pl. 1, fig. 1); with some strains the centre of the area of greening may be very dark. The organisms grow well on nutrient agar with or without 0.5 % glucose, and on serum agar, chocolate agar and blood agar. The colonies are larger on blood agar than on the other media; the addition of serum or glucose to nutrient agar results in only slight increase in colony size. Growth is not enhanced, and may be slightly decreased, by incubation anaerobically or in air with 30 % carbon dioxide. The green discoloration round colonies on blood agar or chocolate agar is much decreased in depth and area when the cultures are incubated anaerobically. On all these media growth takes place at 22°, the colonies taking about 42 hr. to reach the size reached in 18 hr. at 37°. There is no growth on potato. In nutrient broth or peptone water growth is very poor, but it is considerably increased by the addition of 0.5 % glucose.

Microscopically the organisms are round Gram-positive cocci 1-2 μ . in

diam., usually staining deeply, arranged in pairs or irregular clusters (Pl. 1, fig. 2); different strains vary considerably in the size of the cells and in their arrangement. Chains are not formed on solid or in fluid media and the paired cocci do not show elongation along the axis joining their centres, as is seen with pneumococci.

The biochemical activities of twelve representative strains of these organisms are given in Table 1.

Table 1. *Cultural characteristics of aerococci*

	<i>Aerococcus viridans:</i> NCTC 8251	No. positive of 12 strains tested
Greening on blood agar	+	12
Growth in the presence of 40 % bile	+	12
Growth in the presence of 1/2500 potassium tellurite on the medium of Anderson <i>et al.</i> (1931)	—	0
Growth at pH 9·6	+	11
Growth at 45°	—	0
Resistance to 60° for 30 min.	+	12
Reduction of 0·1 % methylene blue in milk	—	0
Acid but no clot in litmus milk	+	12
Liquefaction of gelatin	—	0
Hydrolysis of: Arginine	—	0
Aesculin (in solid medium)*	±	12
Starch 1 % (in solid medium)	—	0
Acid produced from: Glucose	+	12
Maltose	+	12
Lactose	+	10
Mannitol	+	5
Sucrose	+	12
Raffinose	—	4
Final pH value in 1 % glucose broth	5·5–5·8	12
Catalase production	—	0
Coagulase production	—	0

* 0·10 % aesculin was incorporated in the 40 % bile agar with 0·05 % ferric citrate as indicator. After 24 hr. incubation the medium was unchanged, but after another day on the bench some hydrolysis of the aesculin, indicated by blackening of the plate, had occurred.

Acid and formamide extracts of many strains have been tested for precipitinogen reacting with antisera to Lancefield Group D streptococci but none has been found, even after ethanol precipitation (Shattock, 1949). An attempt was made to prepare an antiserum against one typical strain but it did not react with extracts of the vaccine strain, other aerococci, or enterococci. The organisms tend to give rather granular suspensions in saline or broth; none of six strains giving smooth suspensions was agglutinated by antisera to various Lancefield groups of streptococci or to *Staphylococcus aureus*.

In many respects the organisms resemble streptococci, particularly enterococci (see Table 2). Thus they either fail to produce catalase or produce only the merest trace of it; they are benzidine-positive; they flourish on serum agar containing 40 % bile and on agar containing 1/400,000 crystal violet and 1/100,000 potassium tellurite.

On the other hand, microscopically the organisms do not resemble streptococci. Although it is true that some strains of undoubted streptococci show few or no chains, we do not think that one could ever examine preparations from several thousand streptococci without seeing any with definite chain formation. But this is the case with the aerococci.

Table 2. *Comparison of biochemical reactions of Aerococcus viridans| with those of Streptococcus faecalis and Str. bovis*

	<i>Aerococcus viridans</i>	<i>Str. faecalis</i> and variants	<i>Str. bovis</i>
Growth on 40 % bile	+	+	+
Resistance to 60° for 30 min.	+	+	—
Growth at pH 9·6	+	+	—
Growth at 45°	—	+	+
Growth on tellurite medium	—	±	—
Hydrolysis of arginine	—	+	—
Reduction of 0·10 % methylene blue in milk	—	+	—
Litmus milk	Slight acid	Reduction	Acid
Final pH value in 1 % glucose broth	5·5–5·8	4·0–4·8	4·0–4·8

Shaw, Stitt & Cowan (1951) have already pointed out that the aerococci, which they referred to as their 'α-group', seem to be distinguished from the staphylococci by the absence or extreme poverty of catalase production, by the fact that the colonies are semi-transparent, and by the fact that on blood agar they are surrounded by a definite zone of green coloration. We consider, therefore, that they constitute a distinct genus in many ways intermediate between *Staphylococcus* and *Streptococcus*; and from the source in which we first observed them we propose as the generic name, *Aerococcus*. The genus is probably more closely related to *Streptococcus* than to *Staphylococcus*, and should be placed in the same family as *Streptococcus*.

The genus is defined as follows:

Aerococcus. *Constant characteristics*. Gram-positive, non-motile cocci occurring in pairs or irregular clusters, usually small. Aerobic and facultatively anaerobic; growth occurs on solid media at 22° almost as well as at 37°, but not at 45°. Colonies on blood agar incubated aerobically at 37° for 18–24 hr. are semi-transparent and surrounded by a zone of green colour. Growth is not inhibited by 40 % bile, nor by 1/400,000 crystal violet. Arginine is not hydrolysed. The organisms survive heating to 60° for 30 min. and grow at pH 9·6. Acid and formamide extracts do not react with sera prepared against Lancefield Group D streptococci.

Variable characteristics. Table 1 indicates that some of the carbohydrate-fermentation activities vary from strain to strain. A representative strain isolated from the air of an occupied room has been deposited in the National Collection of Type Cultures as the type strain (NCTC 8251), with the name *Aerococcus viridans* n.sp. Its characteristics are included separately in Table 1.

Ecology

Our attention was first drawn to these organisms by the fact that they confused our search for air-borne viridans streptococci; they constituted the great majority of the colonies on plates of the crystal-violet potassium tellurite medium that was employed. The following figures indicate their general prevalence in the air of occupied places, as determined by counting plates exposed in a slit-sampler (Bourdillon, Lidwell & Thomas, 1941):

	Colonies per cu.ft. air		No. of observations
	Aerococci	Total streptococci	
Occupied schoolrooms	6.92	1.18	763
Boot and shoe factories	2.00	0.52	23
Large clerical offices	0.83	0.15	90

The organisms are also very common in floor-dust from occupied places, on clothing, and in dust from yards and streets in London. In all these sites it is probable that they constitute some 5–10 % of the total aerobic flora that will grow on ordinary nutrient media at 37°. In the schoolrooms we have been able to show that the count of the aerococci in the air varies to some extent with the amount of activity by the occupants.

We have not been able to discover any obvious human source of the aerococci found in the environment. Organisms of this sort are present only in very small numbers, if at all, in the upper respiratory tract of normal persons, and they are by no means common in faeces. They may be present in rather small numbers on normal skin. We have not made any systematic search for aerococci on animals, but their frequency in occupied rooms from which animals are largely excluded, and on clothing, suggests that this is not a likely source.

DISCUSSION

Despite the large numbers of the organisms which we describe as aerococci that are present in the environment, they do not appear to have been well described in the literature. Buchbinder, Solowey & Solotorovsky (1938), who studied bacteria from air samples in occupied places, described what we presume to be the same organisms as 'putative streptococci', largely because of the similarity of their fermentation reactions to those of typical streptococci. On the other hand, Rabl & Seelemann (1949) described similar organisms from various lesions in humans as 'diplokokken', and seem to have regarded them as distinct from streptococci. Duguid & Wallace (1948) noted α -haemolytic micrococci as common in bacteriological samples from clothing, and Bourdillon, Lidwell & Lovelock (1948) found micrococci on crystal-violet blood agar plates, which were a source of confusion in a search for α -haemolytic streptococci in air samples. A number of similar organisms were deposited in the National Collection of Type Cultures by Dr C. L. Hannay, who had isolated them from dairy equipment and had noted their extreme resistance to disinfection by hypochlorite.

Several extensive studies of enterococci have been reported (e.g. Houston, 1905; Dible, 1921), and it might be thought that aerococci would have been included in these descriptions. However, we can find no direct reference to organisms with the characteristics that we describe. This is partly due to the fact that many of the tests by which we distinguish aerococci from enterococci are of relatively recent introduction. Moreover, in faeces, which were the source of most of the strains studied by Houston and by Dible, aerococci are not common, and any worker studying streptococci would tend to discard organisms that, microscopically, resembled micrococci rather than streptococci. The organisms were brought to our notice when we were studying the flora of the air in which they are common, and were employing a particular selective medium. We feel that when aerococci have been noticed in the past, they have probably been regarded as morphologically atypical enterococci. It is the fact of having handled so many cultures that gives us some confidence in describing them as different from enterococci.

The reasons for excluding the aerococci from the genus *Staphylococcus* were given by Shaw *et al.* (1951). The problem is to decide whether they are sufficiently closely related to the streptococci, particularly enterococci, to be classed with them. The absence of catalase production would suggest that they might be streptococci, but morphologically they do not resemble streptococci. The morphological criterion is admittedly unsatisfactory and could not be given much weight had our experience of the organisms been limited to the examination of a few strains, or of old laboratory strains. But during the last few years we have examined many thousands of films made from colonies, and many hundreds of blood agar cultures. We have no doubt that the aerococci form a group worthy of separate recognition, and we do not feel that they resemble the streptococci sufficiently to be classed with them.

It remains to be shown how the aerococci differ from species of *Pediococcus* Balcke, which Shimwell (1948) regarded as streptococci. Pederson (1949) characterizes pediococci as Gram-positive, non-motile, catalase-negative cocci which tend to form packets of four but which may occur as single or paired cells. They are microaerophilic, produce a final pH value of 3.25–3.4 in glucose broth, do not reduce nitrates to nitrites or liquefy gelatin. Aerococci differ from pediococci in that they do not normally form packets, they are not microaerophilic, and they do not form so much acid in glucose broth (final pH value 5.0–5.6). Pederson's cultures of pediococci seldom utilized mannitol; Shaw *et al.* found 69% of their α -group (aerococci) fermented this sugar, as did five of the twelve strains recorded in Table 1. Raffinose is fermented by nearly all pediococci but by only a third of aerococci. In addition aerococci tolerate crystal violet and potassium tellurite, which pediococci do not, and they produce marked greening on blood agar.

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EXPLANATION OF PLATE

Fig. 1. Colonies of aerococci on blood agar after 24 hr. incubation at 37° (×4).

Fig. 2. Aerococci grown in nutrient broth for 24 hr. at 37° and stained by Gram's method (×2000).

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Fig. 1

Fig. 2

R. E. O. WILLIAMS, A. HIRCH & S. T. COWAN—*AEROCOCCUS*, A NEW BACTERIAL GENUS. PLATE 1