An Alcaligenes Species with Distinctive Properties
Isolated from Human Sources

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SUMMARY

The distinctive properties of an Alcaligenes-like organism isolated from human pathological material are described. The organism is readily recognizable by its characteristic colonial appearances, fruity smell and greening of blood agar. It resembles to a considerable extent Alcaligenes faecalis, ‘Bacterium alcali-aromaticum’, and A. odorans; the name A. odorans var. viridans is proposed for the organism. It appears to be non-pathogenic, but may be confused with Pseudomonas aeruginosa.

INTRODUCTION

This communication describes a member of the genus Alcaligenes, occasionally found in mixed bacterial cultures from human pathological material, which does not appear to be identical with any organism previously described. It has very distinctive properties which should make for instant recognition in medical bacteriology.

METHODS

An investigation of this organism was first undertaken in this laboratory in 1952 based on a study of 20 strains (17 from urine, 3 from wound infections). Similar strains were isolated subsequently, but were not fully examined until the present work was undertaken with a collection of 27 strains isolated from routine specimens in 1963 and 1964. These 27 organisms came from different patients, and were found in 22 urine specimens, 3 ear swabs and 2 wound swabs. Except where indicated, the results of tests given below are based on an investigation of 12 of these strains, usually incubated at 37°C.

Burdon’s (1946) method was used to stain for sudanophilic inclusions after cultivation on 2% glucose peptone agar for 3 days (Hayward & Hodgkiss, 1961).

Motility was determined by direct microscopy of overnight broth cultures, and by observation of stab cultures in tubes of 0.3% nutrient agar.

Colonial appearances were examined on nutrient agar and horse blood (5%) agar (28°C, 37°C); ox blood, sheep blood and rabbit blood agar; heated blood agar, MacConkey’s medium, and deoxycholate citrate agar (4 strains); 0.03% cetrimide agar (Lowbury & Collins, 1955). Fluorescin production was tested with ultraviolet radiation on the medium B of King, Ward & Raney (1954). Growth at 42°C was tested on nutrient agar slopes immersed in a water bath.

Final pH value in fluid culture was determined in Difco ‘Bacto’ nutrient broth incubated for 7 days, using ‘Lyphan’ multi-strip pH papers.
Carbohydrate reactions. Standard peptone water media (glucose, lactose, maltose, sucrose, salicin, mannitol and dulcitol) were incubated for 21 days. Acid production from glucose, mannose, maltose, arabinose, xylose and glycerol was also tested in the medium of Hayward & Hodgkiss (1961) incubated for 12 days (6 strains).

Lipase. Sierra's (1957) medium, incubated at 28° for 6 days.

Hydrolysis of casein and starch. The media of Hayward & Hodgkiss (1961).

Indole production was tested with Ehrlich's reagent in peptone water cultures incubated for 6 days.

H₂S production was tested in peptone water containing 0·01 % L-cysteine hydrochloride (lead acetate papers) incubated for 6 days.

Urease. Christensen's (1946) method; incubation for 21 days.


Catalase. A loopful of culture from nutrient agar was held in a drop of '10 vol.' H₂O₂ on a slide and examined macroscopically for effervescence.

Voges-Proskauer (Barritt's method; Mackie & MacCartney, 1960) and methyl red reactions. Glucose phosphate broth cultures incubated for 6 days.

Nitrate reduction. Nutrient broth containing 0·1 % (w/v) KNO₃, incubated for 5 days, then tested with Griess–Illosvay reagents, and with zinc dust for residual nitrate.

Gelatin liquefaction. Kohn's (1958) method, incubation for 14 days.

Citrate utilization. Koser's (1923) medium, incubation for 2 days.


Malonate utilization and phenylalanine deaminase activity. Combined medium of Shaw & Clarke (1955), incubation for 6 days.

Growth in KCN. Moeller's (1954) method.

Decarboxylases. Moeller's (1955) method.

Two isolates were examined by electron microscopy on nitrocellulose-coated grids, shadowed with gold-palladium at an angle of 15°; magnification × 20,000.

Antibiotic sensitivities were determined by the agar gel diffusion method, with 'Mast' antibiotic discs.

RESULTS

The organism was a Gram-negative rod, average size 1·5 μ x 0·6 μ with coccoid and elongated forms (18 hr cultures on blood agar); non-sporing, non-capsulated and not acid-fast. Scattered sudanophilic inclusions were found in 5 of 9 strains examined. It was actively motile after overnight growth in broth at 37°. Electron micrographs clearly showed a peritrichous arrangement of flagella, varying in numbers up to about 12 per organism.

After incubation on blood agar at 37° for 24 hr, two distinct types of colony were recognizable, with a range of intermediate forms. The usual and more characteristic colony was umbonate, with a central plateau raised to a button in the middle, and a thin matt spreading edge. There was a surrounding zone of bright green discoloration of the medium, visible on blood (horse, sheep, ox, rabbit) agars. Colonial size varied considerably up to 6 mm. in diameter. The elevation of the colonies varied from flat-topped to almost conical. Frequently there was no central button. In areas of confluent growth, adjacent colonies were often demarcated by straight indentations, resulting in a 'paving-stone' effect, the area of growth being outlined
Distinctive *Alcaligenes* species by a skin-like fringe. The other type of colony was high-convex, greyish white, with a smooth glistening surface and a circular outline devoid of fringe. It appeared to correspond to the central button of the 'rough' type. Usually a particular culture showed a preponderance of one type, but both types might be present in the same culture. Several strains incubated simultaneously under identical conditions might show a preponderance of different types. Neither type bred true on subculture.

After incubation at $37^\circ$ for 48 hr the characteristic colonial features were no longer evident, and blood plates showed complete lysis. After incubation at $28^\circ$ for 24 hr the diameter of the colonies was similar, but the fringe was developed at the expense of the convex portion, so that the colonial mass was diminished. Cultures on blood agar nearly always had a pleasant strongly aromatic odour, resembling that of apples. This odour was very characteristic and could often be detected even when the organism was outnumbered in mixed cultures. Colonies on nutrient agar were smaller, and had a less distinctive, somewhat cheesy smell. Poor growth occurred on MacConkey's medium with an alkaline reaction and on deoxycholate citrate agar after incubation at $37^\circ$ for 24 hr. Growth did not occur at $42^\circ$ nor on solid media incubated anaerobically at $37^\circ$ for 7 days. Fluorescin was not produced on the medium B of King *et al.* (1954). Discoloration was not produced on heated blood agar. Appreciable growth occurred on 0.08% cetrimide agar, although the size of colonies was then much decreased as compared with a control strain of *Pseudomonas aeruginosa* which grew freely. Cultures in nutrient broth showed a uniform turbidity with an occasional surface pellicle, and reached pH 8.1 after 7 days. Litmus milk cultures turned blue after 4 days, and were bleached subsequently.

The following tests were always positive: catalase, oxidase, $\text{H}_2\text{S}$ production, citrate and malonate utilization.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Moderately sensitive</th>
<th>Resistant</th>
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<tr>
<td>Penicillin</td>
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<td>17</td>
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<tr>
<td>Methicillin</td>
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<td>Nitrofurantoin</td>
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<td>Bacitracin</td>
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Table 1. *Antibiotic sensitivities of 17 of the strains of Alcaligenes odorans var. viridans examined*
There was no visible growth in presence of KCN (Moeller, 1954) at 24 hr, but there was at 48 hr.

The following tests were negative: lipase, casein and starch hydrolysis, gelatinase, V.P. and M.R., indole, urease, nitrate reduction, gluconate oxidation, phenylalanine deaminase; arginine, lysine and ornithine decarboxylases. No carbohydrates were attacked in the medium of Hayward & Hodgkiss (1961).

**DISCUSSION**

Records of the previous collection of 20 strains examined 12 years ago showed that, although several recently introduced tests were not then performed, the organisms were almost certainly the same as are now reported upon, with the same characteristic aromatic smell, colonial appearances and discoloration of blood.

This organism belongs to the genus *Alcaligenes* as defined in *Bergey's Manual* (1957), being a Gram-negative rod, motile by peritrichous flagella, producing an alkaline reaction in litmus milk and not attacking carbohydrates. The genus has aroused controversy since Petruschky’s (1896) original description of ‘Bacterium alcaligenes faecalis’, which was stated to possess peritrichous flagella. The existence of such strains was doubted for a time, but several subsequent workers, notably Leifson (1960), Thibault (1961), and Hugh & Ryschenkow (1961) have maintained that the genus *Alcaligenes* should include only organisms motile by peritrichous flagella; we have followed them in this. The present organism differs from the type species *A. faecalis* Castellani & Chambers (1919) in possessing a strong fruity odour and producing greening of blood agar. A search of the literature has not revealed a description of any organism which exactly corresponds to our own, but three papers have described similar or possibly related organisms.

Stutzer (1924) described an organism found in the stools of patients with cholera and dysentery which he called ‘Bacterium faecalis aromaticum’. This was a small Gram-negative rod, $1 \mu \times 0.8-0.5 \mu$, with similar colonial appearances to those of our strains. One type of colony was matt flat-conical in elevation with radial furrows and a polyhedral outline, reaching 3-4 mm. in diameter after incubation for 24 hr. The other type had a moist glistening surface, and had an intense agreeable aromatic odour. Unlike our strains, it was non-motile and liquefied coagulated serum and gelatin; greening of blood agar was not mentioned.

Berlin (1927) isolated from human faeces an organism which he called ‘Bacterium alcali-aromaticum’. This was a Gram-negative rod, motile by peritrichous flagella. Colonies showed a raised greyish white centre and an irregular plateau, which later became cone-shaped, with regular peripheral radial furrows and a transparent edge. At 16–18° the colonies had an intense fruity odour which disappeared after a week, to be replaced by a cheesy odour; the fruity odour was not apparent at 37°. On sheep blood agar, weak haemolysis was evident after 3–4 days. Growth in broth at 37° was described as granular, reaching pH 8.0 after 7–10 days. It was indole negative, H$_2$S negative, did not liquefy serum or gelatin or reduce nitrate, and did not attack a wide range of carbohydrates. This organism showed only minor differences from our strains but lacked the intense fruity odour at 37° and did not produce greening of blood agar after overnight incubation.

Málek, Radochová & Lysenko (1963) studied 4 strains of an organism previously
Distinctive Alcaligenes species

Described as *Pseudomonas odorans* (Málek & Kazdová-Kožišková, 1946) and re-allocated them to the genus *Alcaligenes*. They were Gram-negative bacilli, motile by peritrichous flagella as shown by electron microscopy. On meat peptone agar after incubation for 2 days at 28°, colonies were either flat and spreading with irregular edges, or convex, and round with entire edges. No haemolysis occurred on blood agar. Young cultures had an odour of jasmine or strawberries, replaced by that of ammonia in older cultures. Unlike our strains, their organisms were non-haemolytic on blood agar, and differed in their antibiotic sensitivities, being resistant to tetracycline and polymixin, and only slightly sensitive to streptomycin and chloramphenicol.

Our strains resemble *A. odorans* too closely to be regarded as a separate species, and it is proposed that they be distinguished as *A. odorans* var. *viridans*. The type strain has been deposited in the National Collection of Type Cultures as NCTC 10388.

The resemblances to *Pseudomonas aeruginosa* are only superficial; but confusion may arise in clinical bacteriology because of the colonial appearances, obligate aerobic growth, aromatic odour, green discoloration of medium, positive oxidase reaction, appreciable growth on 0.03% cetrimide agar, and marked sensitivity to polymixins. Our strains have been isolated only in mixed cultures, with other Gram-negative bacilli. They have not been shown to have a pathogenic role. The widely differing antibiotic sensitivity patterns of the strains are strong evidence that the organism was not recurring as a laboratory contaminant.

Our thanks are due to Professor W. A. Gillespie, under whose direction this work was done, for his invaluable advice and encouragement. We are greatly indebted to the late Dr K. J. Steel for considerable help in revising the manuscript, and to C. A. Bassett of the Department of Physics, University of Bristol, for making electron micrographs of the organism.

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