MORPHOLOGICAL AND BIOCHEMICAL
CHARACTERIZATION OF ALCALIGENES ODORANS
VAR. VIRIDANS

Gerald L. Gilardi and Simon Hirschl

Division of Laboratories, Hospital for Joint Diseases
and Medical Center, New York City

ABSTRACT. The morphological and physiological characteristics of 14 strains of Alcaligenes odorans var. viridans Mitchell and Clarke (1965), are described and more than 65 characters of the strains recorded. Peritrichous flagellation was demonstrated. These strains produce a distinct aromatic odor on culture media and a dark green discoloration of blood agar. Growth occurs in a basal mineral medium containing inorganic nitrogen as the nitrogen source supplemented by a suitable single carbon compound. The physiological differences between these strains and the type strain (NCTC 10388) of Mitchell and Clarke are pointed out.

- - - - -

INTRODUCTION

Mitchell and Clarke (1965) assigned the name Alcaligenes odorans var. viridans to an unusual Gram-negative bacillus having the general characteristics of the genus Alcaligenes but in addition producing an aromatic odor on culture media and a green discoloration of blood agar. Gilardi (1967) described 6 strains of a peritrichous bacillus isolated from clinical materials with similar characteristics and has since recovered an additional 8 strains. The purpose of this report is to describe additional morphological and physiological characteristics of these strains and to point out their differences from the type strain (NCTC 10388) of Mitchell and Clarke.

MATERIALS AND METHODS

The 14 strains of Alcaligenes odorans var. viridans were isolated from wound, urine, and sputum specimens between 1966 and 1968. Deoxyribonuclease activity was determined using DNase Test Medium (Difco). The measurements of the organism and the demonstration of the flagella were done by means of the RCA EMU-3M electron microscope. Negative staining with phosphotungstic acid was used. All other procedures employed in this study have previously been described (Gilardi 1967;1968a;1968b).

RESULTS

Morphology: Gram-negative, asporogenous, noncapsulated, motile bacilli. They occurred singly and in coccoid forms and demonstrated pleomorphism in the form of elongated cells. Under the electron microscope the bacilli measured 0.3 to 0.8 by 1.2 to 2.8 μ in size. They showed peritrichous flagella which ranged in number from 2 to 8 per cell (Figures 1 and 2). The flagella had a wave length of approximately 2 μ .

Colonies about 1 mm in diameter developed on trypticase soy agar, MacConkey agar, and blood agar plates. Poor growth on S S agar. Surrounding the colonies on blood agar plates was a zone of dark green discoloration which completely covered the plates after 48 hours. Cultures on all the above mentioned media had a strong aromatic odor resembling that of fruit. The aromatic odor persisted on all media through approximately 7 days of incubation and then was gradually replaced by an odor of ammonia. Two distinct types of colonies with intermediate forms developed on trypticase soy agar and blood agar plates. One was umbonate with a thin, spreading, irregular edge and the other was convex and circular with an entire edge. Analogous morphological types were observed on MacConkey agar but were slower in developing. Both morphological types were present in the same culture, and on subculture neither type of colony would breed true.

Physiology: The strains were nutritionally nonexacting, growing in a basal mineral medium containing ammonium as the nitrogen source supplemented with a suitable single organic carbon compound. The obligate aerobes grew well at 37°C but did not grow at 4°C. All but 1 strain grew at 42°C. No distinct pigment was produced on any media in-

Figure 1. Electron micrograph of Alcaligenes odorans var. viridans showing peritrichous cells with 5 flagella. X 16,000. The bar represents 0.5 μ .

Figure 2. Electron micrograph of A. odorans var. viridans showing 2 flagella. X 70,000. The bar represents 0.5 μ .

cluding Sellers¹ medium, pseudomonas agar F or pseudomonas agar P.

An alkaline reaction was produced in OF basal medium containing the following carbon compounds: glucose, fructose, galactose, mannose, lactose, sucrose, maltose, rhamnose, and mannitol. Indole, urease, Voges-Proskauer, methyl red, gelatin, oxidation of 10% lactose, gluconate oxidation, lysine and ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, nitrate to nitrite, hemolytic activity, deoxyribonuclease activity, and sensitivity to penicillin (2 units) were negative. The following carbon sources were not utilized as the sole sources of carbon: glucose, L-arabinose, D-xylose, sucrose, maltose, D-trehalose, inositol, D-mannitol, L-arginine, beta-alanine, DL-serine, L-lysine, DL-valine, adipate, and suberate.

Simmons' citrate, catalase, oxidase, malonate utilization (Difco), growth on cetrinide, and sensitivity to polymyxin (50 units) were positive in all strains. Nitrate reduction to nitrogen gas was detected in all but 2 strains. Weak hydrogen sulfide production in Kligler iron agar was detected in 3 strains after several days of incubation. The following carbon sources were utilized as the sole source of carbon and energy by all strains: asparagine, DL-aspartate, glycine, L-glutamate, DL-methionine, citrate, acetate, propionate, D-malate, succinate, fumarate, pyruvate, and DL-lactate. Butyrate was assimilated by 4 strains.

DISCUSSION

Several reports in the literature describe Gram-negative bacilli which produced a characteristic fruity odor on culture media and may be similar to the strains under discussion: Stutzer (1924) recovered small Gram-negative bacilli from feces which he designated Bacterium faecale aromaticum; Berlin (1927) recovered Gram-negative bacilli from stool cultures which he referred to as Bacterium alcaliaromaticum; Málek, Radochová, and Lysenko (1963) found that a Gram-negative bacillus, Pseudomonas odorans Málek and Kazdová-Kožisková 1946, belonged to the genus Alcaligenes, and designated it Alcaligenes odorans. Their 4 isolates showed similar characteristics to the strains under discussion, but the former demonstrated variable gelatinase-, urease-, oxidase-, and arginine dihydrolase-activity, and variable reduction of nitrate to nitrite. None of their strains reduced nitrate to nitrogen gas or produced greening of blood agar, and they were resistant to polymyxin.

Mitchell and Clarke (1965) recovered 47 strains of a Gram-negative bacillus which, in addition to the aromatic odor, produced a green discoloration of blood agar. Because of the latter characteristics, they proposed the name A. odorans var. viridans and deposited the type strain in the National Collection of Type Cultures as NCTC 10388. The strains under discussion resembled the description by Mitchell and Clarke in most respects but there were a few differences. The present strains produced nitrogen gas from nitrate and grew at 42°C, but did not produce hemolysis around the discrete colonies of human, rabbit, or sheep blood agar. The present study also demonstrated the strains to be capable of growing in a basal mineral medium containing inorganic nitrogen as the nitrogen source supplemented with a complex organic compound such as acetate.

REFERENCES

- Berlin, A. L. 1927. Bacterium alcaliaromaticum. Vestn. Mikrobiol. Epidemiol. Parazitol. 6:402-413.
- Gilardi, G. L. 1967. Characteristics of Alcaligenes odorans var. viridans isolated from human sources. Canad. J. Microbiol. 13:895-900.
- _____. 1968a. Morphological and biochemical differentiation of Achromobacter and Moraxella (DeBord's tribe Mimeae). Appl. Microbiol. 16:33-38.
- _____. 1968b. Diagnostic criteria for differentiation of pseudomonads pathogenic for man. Appl. Microbiol. 16:1498-1502.
- Málek, I., M. Radachová, and O. Lysenko. 1963. Taxonomy of the species Pseudomonas odorans. J. Gen. Microbiol. 33:349-355.
- Mitchell, R. G. and S. K. R. Clarke. 1965. An Alcaligenes species with distinctive properties isolated from human sources. J. Gen. Microbiol. 40:343-348.
- Stutzer, M. J. 1924. Zur Frage über die Fäulnisbakterien im Darm. Arch. Hyg. Bakt. 91:87-90.