Rapid Screening of *Veillonella* by Ultraviolet Fluorescence

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Among 51 strains of anaerobic gram-negative cocci belonging to the family *Veillonellaceae*, all strains of *Veillonella* (*V. parvula* and *V. alcalescens*) displayed red fluorescence under long-wave (366 nm) ultraviolet light, whereas no *Acidaminococcus* or *Megasphaera* demonstrated fluorescence. In contrast to *Bacteroides melaninogenicus*, growth of *Veillonella* does not require hemin and menadione, and fluorescence is rapidly lost upon exposure to air. The fluorescent component of a strain of *V. parvula* examined could not be extracted in solution with water, ether, methanol, or chloroform, but was readily extracted with 0.4 N NaOH. Spectrophotofluorometrically, the fluorescence maximum of this extract was 660 nm with an excitation maximum of 300 nm, when measured at pH 7.2 and 25 C. Coupled with the Gram stain, ultraviolet fluorescence may be a useful tool for rapid screening of *Veillonella* and is particularly helpful for detection and, isolation of this organism from mixed culture.

Detection of red fluorescence in colonies of anaerobic, gram-negative, nonsporeforming bacilli by ultraviolet (UV) light is a useful technique for rapid presumptive identification of *Bacteroides melaninogenicus* (1). During routine screening of all anaerobic clinical isolates by UV fluorescence, we observed that, in addition to *B. melaninogenicus*, anaerobic gram-negative cocci of the genus *Veillonella* regularly display red fluorescence. Furthermore, in contrast to *B. melaninogenicus*, the fluorescence of *Veillonella* is rapidly lost after exposure to air. Therefore, the specificity and nature of UV fluorescence in *Veillonella* was further investigated.

MATERIALS AND METHODS

Test organisms. Fifty-one clinical isolates belonging to the family *Veillonellaceae* (*Veillonella*, *Acidaminococcus*, and *Megasphaera*) that were recovered from inpatients at Harbor General Hospital during 1971 to 1976 were examined for UV fluorescence. All were obligately anaerobic, gram-negative cocci, identified and speciated in prereduced anaerobically sterilized (PRAS) differential media according to the method of Holdeman and Moore (2). A pH meter was used for recording carbohydrate fermentation reactions, and the AnaBac chromatograph was used for fatty acid analysis of fermentation end products. Each organism was subcultured on solid media, either in roll tubes containing PRAS brain heart infusion agar supplemented with cysteine and yeast extract with or without menadione and hemin (Scott Laboratories, Fiskeville, R. I.), or

on brain heart infusion (Difco) agar plates enriched with 5% sheep blood incubated in vented GasPak jars. Anaerobiosis was achieved by flushing roll tubes, or evacuation and replacement of GasPak jars, with an oxygen-free gas mixture containing 80% N₂, 10% CO₂, and 10% H₂.

Examination for UV fluorescence. Fresh colonies in roll tubes, or on agar plates immediately removed from GasPak jars, were examined for red fluorescence in a dark room with a hand-held long-wave (366 nm) UV light (model SL-3660 Spectroline, Black Light Eastern Corp., Westbury, N.Y.).

Fluorescence under aerobic and anaerobic conditions. A fresh subculture of *Veillonella parvula* (strain 2186N) was inoculated under oxygen-free gases (80% N₂, 10% CO₂, 10% H₂) in two roll tubes containing PRAS brain heart infusion agar. Both were incubated at 37 C until luxuriant growth appeared and strong UV fluorescence was observed. One roll tube was then exposed to air by removing the stopper, whereas the other was maintained under anaerobic conditions. Both tubes were then serially and simultaneously examined for UV fluorescence at regular intervals.

Fluorescence photomicrography. A Nikon F camera (135 mm, F/2.8) with two M rings (Micro-nikor lens) and two barrier filters (Kodak Wratten no. 6 and no. 2E, 400 nm) attached was used for recording UV fluorescence. Kodak Ektachrome high-speed daylight film (EH 135, 160 ASA) was used and exposure time was set at 25 s.

Fluorescence spectrometry. The fluorescence spectrum of *V. parvula* (strain 2186N) was measured with an Aminco-Bowman spectrophotofluorometer (American Instrument Co. Inc., Silver Spring, Md.) (3). A 48-h subculture of the organism in PRAS pep-

tone yeast glucose broth (BBL) was centrifuged at 2,000 rpm for 20 min at 20 C. The pellet was removed, extracted with 0.4 N NaOH under N₂ in an ice-bath, and recentrifuged. The supernatant was checked for red fluorescence under UV light and was then dialyzed (membrane pore radius 2.4 nm, Union Carbide, Chicago, Ill.) against 0.1 M Na₃PO₄ buffer, pH 7.2. All reagents were previously deoxygenated by vacuum suction and flushing with N₂, and all preparatory procedures were performed anaerobically under N₂ in an ice-bath. The final dialysate was analyzed spectrophotofluorometrically under aerobic conditions within 3 h after the extraction.

RESULTS

Among 51 strains of *Veillonellaceae* examined, 25 were *V. parvula*, five were *V. alcalescens*, nine were *Acidaminococcus fermentans*, and 12 were *Megasphaera elsdenii* (Table 1). All strains of *Veillonella* displayed red fluorescence under long-wave UV light, whereas *Acidaminococcus* or *Megasphaera* demonstrated no fluorescence. The fluorescence of *Veillonella* was pink to red in color and was often indistinguishable from that of *Bacteroides melaninogenicus* (Fig. 1). However, in contrast to *B. melaninogenicus*, fluorescence of *Veillonella* faded rapidly upon exposure to air. Fading of

TABLE 1. Fluorescence of *Veillonellaceae* by long-wave (366 nm) UV light

Organism	No. tested	No. fluorescent
<i>Veillonella parvula</i>	25	25
<i>V. alcalescens</i>	5	5
<i>Acidaminococcus fermentans</i>	9	0
<i>Megasphaera elsdenii</i>	12	0

of fluorescent colonies on blood agar plates was noticeable within 15 min of exposure (Fig. 1), and fluorescence generally could no longer be detected after 2 h. On the other hand, colonies cultivated in PRAS media in roll tubes maintained strong fluorescence after 45 min of exposure and faded rapidly thereafter, whereas colonies kept anaerobically continued to fluoresce after 102 h. Fluorescence persisted after repeated freeze-thawing, as long as air was excluded. Growth and fluorescence of *Veillonella* did not require supplementation with menadione and hemin.

The fluorescent component of *V. parvula* (strain 2186N) could not be extracted in solution with water, ether, methanol, or chloroform, but

FIG. 1. Appearance of *Veillonella parvula* and *Bacteroides melaninogenicus* subsp. *asaccharolyticus* under visible light (A and D, respectively) and long wave UV light (B and E, respectively). Rapid fading of fluorescence was noted for *V. parvula* after exposure to air (C).

was readily extracted with 0.4 N NaOH at 4 C under N₂. However, fluorescence could not be preserved beyond 18 h in this form. The fluorescence spectrum of this extract is depicted in Fig. 2. The fluorescence maximum was 660 nm when measured at pH 7.2 and 25 C, with 300 nm as the excitation maximum.

DISCUSSION

These studies demonstrate that, in addition to *B. melaninogenicus*, *Veillonella* species also display red fluorescence under UV light. In contrast to *B. melaninogenicus*, growth of *Veillonella* does not require hemin and menadione, and colonies do not turn jet black on laked blood agar after 4 to 5 days of incubation. In

addition, fluorescence of *Veillonella* is rapidly lost upon exposure to air, whereas that of *B. melaninogenicus* is stable. The biochemical nature of the fluorescent component in *Veillonella* remains to be investigated. Regardless of its exact biochemical nature, it appears to be specific for *Veillonella* but absent among other anaerobic gram-negative cocci of the *Veillonellaceae* family. Coupled with the Gram stain, UV fluorescence may be a useful tool for rapid screening of *Veillonella* and is particularly helpful for detection and isolation of this organism from mixed culture. It should be emphasized, however, that cells of many *B. melaninogenicus* may be cocco-bacillary on Gram stain and thus may be confused with *Veillonella*. Reincubation for observation of jet black pigmentation may aid in differentiation from the latter. Definitive identification and speciation, however, should be confirmed by biochemical testing and gas-liquid chromatography in all cases.

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FIG. 2. Fluorescence spectrum of *Veillonella parvula* (strain 2186N) after extraction with 0.4 N NaOH. Excitation maximum 300 nm, pH 7.2, 25 C.