Morphological and Biochemical Characteristics of Aeromonas punctata (hydrophila, liquefaciens) Isolated from Human Sources

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The isolation of Aeromonas punctata (hydrophila, liquefaciens) from feces, throat, and sputum cultures is presented as further evidence that aeromonads are found in man. Morphological and biochemical studies of these strains indicate that the chief differences between the aeromonads and physiologically similar members of the Enterobacteriaceae are found in the polar arrangement of the flagella and in the production of oxidase by the former. The oxidase test should be performed on all paracolon-like bacteria, and a flagella stain should be employed when an oxidase-positive, gram-negative bacillus is isolated. Application of these tests will undoubtedly result in more frequent identification of Aeromonas species from human sources.

Members of the genus Aeromonas, which physiologically resemble closely certain members of the Enterobacteriaceae, have only recently become of interest to medical bacteriologists with the isolation of strains from human sources (8). The majority of aeromonads described to date are from soil and water, or are known to be pathogenic to cold-blooded animals (Bergery's Manual of Determinative Bacteriology). This report describes the morphological and biochemical characteristics of two strains of Aeromonas isolated from human sources, points out those tests which should be performed for the laboratory detection of this microorganism, and reviews the characteristics and nomenclature of the aeromonads.

MATERIALS AND METHODS

Cultures. Two isolates of one strain were recovered in November 1965 from a throat and a sputum culture from a patient with aspiration pneumonia. Two isolates of a second strain were cultured in September 1966 from two fecal specimens from a 5-year-old male with gastroenteritis.

Morphological, biochemical, and sensitivity studies. To study microscopic morphology, smears were made from Trypticase Soy Agar (TSA; BBL) cultures incubated for 24 hr at 37 C and stained by Gram's method. To study colonial morphology, isolated colonies were observed on TSA plates containing 5% defibrinated rabbit blood after incubation for 24 hr at 37 C. Motility was determined by microscopic examination of a hanging drop of a Trypticase Soy Broth (BBL) culture incubated for 24 hr at 37 C. For the demonstration of flagella, the bacteria were grown overnight on a TSA slant to which 2 ml of sterile distilled water had been added. Precleaned slides used for the flagella stain were washed in 95% ethyl alcohol, dried, and passed through a Bunsen burner. A few drops of the water from the TSA slant culture was placed at one end of the slide with a disposable capillary pipette and allowed to spread over the surface of the tilted slide; slides were allowed to dry at room temperature. Gray's method for flagella stain as outlined by Bailey and Scott (1) was used. The mordant was filtered before use.

Biochemical tests were performed, in general, by the procedures outlined by Edwards and Ewing (10). Cultures were incubated for 3 weeks at 37 C before tests were discarded as negative. The tests and media employed included: fermentation in 1% glucose, galactose, maltose, mannitol, dextrin, lactose, sucrose, rhamnose, salicin, raffinose, xylose, dulcitol, and inulin (Purple Broth Base, Difco); citrate utilization (Simmons' Citrate Agar, Difco); nitrate reduction (Trypticase Nitrate Broth, BBL); indole production (Tryptone Broth, BBL); hydrogen sulfide production (Kligler Iron Agar, Difco); urease activity (Christensen's Urea Agar, Difco); gelatinase activity (Nutrient Gelatin, Difco); oxidase reaction (Oxidase Discs, Difco); methyl red and Voges-Proskauer (VP) tests (buffered peptone-glucose broth); growth on S Agar (Difco) and MacConkey Agar (Difco); hemolysis (Trypticase soy-blood-agar plates); catalase reaction, oxygen-requirement studies, and temperature studies (TSA slants). Indole production was tested with Kovacs' reagent. After 48 hr of incubation, acetaldehyde was detected by reding-arrowing the addition to 1 ml of culture of 1 ml of 40% (w/v) KOH containing 0.3% (w/v) of creatine. Antibiotic sensitivity was studied by the disc sensitivity method (BBL Sensi-discs) with the use of TSA plates.
RESULTS

Morphological and biochemical characteristics. The strains were short, gram-negative bacilli, occurring singly and in pairs, and showing pleomorphism in the form of elongated cells. They possessed a single, polar flagellum (Fig. 1). Colonies on blood-agar were 1 to 3 mm in diameter, round, smooth, convex, moist, opaque, and grayish-white, with marked β-hemolysis. After 3 to 5 days of incubation at room temperature, the growth became dark green on blood-agar plates and a light beige on TSA plates. Good growth was obtained on all media including S S Agar and MacConkey Agar at 25 and 37 C. Slight growth occurred at 4 C after 3 to 5 days of incubation, but no growth occurred at 42 C. The biochemical results (Table 1) were detected after 24 hr with two exceptions: the methyl red and VP tests were performed after 48 hr of incubation; maltose fermentation required 48 to 72 hr of incubation with one strain.

Sensitivity patterns. Both strains were sensitive to tetracycline, chloramphenicol, furazolidine, nitrofurantoin, nitrofurazon, neomycin, polymyxin, colistin, and methenamine mandelate, and were resistant to penicillin, novobiocin, erythromycin, lincomycin, streptomycin, and triple sulfa. One strain was sensitive and the other resistant to cephalothin, ampicillin, and kanamycin.

**Table 1. Characteristics of two strains of Aeromonas punctata (hydrophila, liquefaciens) isolated from human sources**

<table>
<thead>
<tr>
<th>Test or substrate</th>
<th>Resulta</th>
<th>Test or substrate</th>
<th>Resulta</th>
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<tbody>
<tr>
<td>Motility</td>
<td>+b</td>
<td>Gas from glucose</td>
<td>+</td>
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<tr>
<td>Hydrogen sulfide</td>
<td>+</td>
<td>Glucose</td>
<td>A</td>
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<tr>
<td>Urea</td>
<td>−</td>
<td>Galactose</td>
<td>A</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>Maltose</td>
<td>A, A(s)</td>
</tr>
<tr>
<td>Indole</td>
<td>+</td>
<td>Sucrose</td>
<td>A</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>Mannitol</td>
<td>A</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>Salicin</td>
<td>NC</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>Dextrin</td>
<td>A</td>
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<tr>
<td>Nitrate</td>
<td>+</td>
<td>Lactose</td>
<td>NC</td>
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<tr>
<td>Methyl red</td>
<td>−</td>
<td>Rhamnose</td>
<td>NC</td>
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<tr>
<td>Voges-Proskauer</td>
<td>+</td>
<td>Raffinose</td>
<td>NC</td>
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<tr>
<td>Growth on Mac-</td>
<td>+</td>
<td>Xylose</td>
<td>NC</td>
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<tr>
<td>Conkey</td>
<td>+</td>
<td>Dulcitol</td>
<td>NC</td>
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<td>Growth on S S β-</td>
<td>+</td>
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<td>A</td>
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<tr>
<td>Hemolysis</td>
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<td>NC</td>
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<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>Glucose</td>
<td>+</td>
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<tr>
<td>Growth at 4 C</td>
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<td>Galactose</td>
<td>A</td>
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<tr>
<td>Growth at 25 C</td>
<td>+</td>
<td>Maltose</td>
<td>A</td>
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<tr>
<td>Growth at 37 C</td>
<td>+</td>
<td>Sucrose</td>
<td>A</td>
</tr>
<tr>
<td>Growth at 42 C</td>
<td>-</td>
<td>Mannitol</td>
<td>A</td>
</tr>
</tbody>
</table>

a Symbols: + = positive; − = negative; A = acid; NC = no change; (s) = slow.

b Monotrichous flagellation.

FIG. 1. Aeromonas punctata (hydrophila, liquefaciens) showing typical flagellation. Gray’s flagellar stain (1). X 2,500.

DISCUSSION

In 1937, the first isolation of Aeromonas from a human source was reported by Miles and Halnan (20). The organism was isolated from feces of a patient with chronic colitis, but no causal relationship was demonstrated. In 1954, Hill, Caseitz, and Moody (14) isolated a strain from a patient with metastatic myositis, and Bras et al. (3) reported the isolation of a strain from a patient with gangrene. Between 1955 and 1958, Kjems (15) reported the isolation of strains associated with septicemia, and Caseitz, Hofmann, and Martinez-Silva (4) reported the recovery of a strain from a patient with osteomyelitis. In 1963, Meeks (19) recovered strains of Aeromonas from a diarrheal stool, from a leg ulcer, and, as did Conn in 1964 (7), from the blood and ascitic fluid of cirrhotic patients. In 1964, Rosner (21) reported Aeromonas as the etiological agent of a case of severe gastroenteritis. Additional recovery has been reported from feces, blood (5, 6, 12), sputum, urine, bile (2, 5, 6), pus, spleen, pleural fluid, placenta (5, 6), skin lesion, throat, and gall bladder (12).

One strain in this study was isolated from both a throat and a sputum culture taken from an adult female, who was admitted with a fever of
unknown origin. She was later diagnosed as having aspiration bronchopneumonia. The other strain studied was isolated from two fecal specimens from a 5-year-old male, who was admitted for bloody diarrhea and rectal prolapse. Because of behavior problems, the patient was discharged before a causal relationship could be substantiated. In the investigation by Lautrop (16), seven of eight patients who yielded Aeromonas in fecal cultures had symptoms of intestinal disturbance, and he believes it possible that Aeromonas is a potential intestinal pathogen.

The 7th edition of Bergey's Manual describes these gram-negative bacilli as motile by polar flagella or occasionally nonmotile, methyl red negative, fermenting carbohydrates, with the production of H2, CO2, and 2,3-butylen glycol, and fermenting lactose slowly or not at all. The chief differences between the aeromonads and the late- or non-lactose-fermenting species of enteric bacteria are found in the production of oxidase (8, 16, 17, 19, 21) and in the polar arrangement of the flagella (8, 19) in the former. Though single, polar flagellation is typical, mixed polar and peritrichous flagellation (18, 30), as well as amphitrichous and lophotrichous flagellation (15), have been observed.

Based on his review of the literature and his biochemical and morphological examinations, Eddy (8) concluded that the present system of classification of Aeromonas is inadequate, and should include the oxidase test and the arrangement of flagella as important characteristics. He redefined the genus to include three oxidase-positive species: polar-flagellated, VP-positive strains, A. liquefaciens (A. hydrophila and A. punctata); polar-flagellated, VP-negative, anaerogenic strains, A. formicans (A. caviae); and nonmotile, VP-negative, gas-producing strains, A. salmonicida (Table 2).

Ewing, Hugh, and Johnson (12) also proposed a reorganization of the genus Aeromonas, but they concluded that A. hydrophila has priority as the type species of the genus. They stated that other specific epithets, except for A. salmonicida, should be considered synonymous with A. hydrophila. Furthermore, they concluded that the C27 bacteria, apparently first described by Ferguson and Henderson (13), should be classified in the genus Aeromonas, and that A. shigelloides has priority as the specific epithet. The C27 group resembles Shigella sonnei in that some strains have identical O antigens (13). These polar-flagellated, anaerogenic strains were described by Ewing and Johnson (11) and Ewing, Hugh, and Johnson (12) as oxidase-positive, VP-negative, and fermenting lactose slowly (Table 2).

After further examination of the literature on the genus Aeromonas, Eddy (9) in 1962 revised his earlier classification and suggested A. punctata as the correct type species because of its earlier complete description; A. punctata would therefore include A. liquefaciens and A. hydrophila. Since the epithet caviae was found to have precedence over formicans, he would rename the microorganism A. caviae. Eddy (9) would retain A. salmonicida, but Smith (29) disagrees with its inclusion in the genus Aeromonas because of its lack of vigorous gas production, motility, and 2,3-butanediol production. She believes it should be given a generic place in the family Pseudo-monadaceae.

After extensive morphological and biochemical studies, Schubert (22-26) in 1964 developed a classification of the aeromonads on the basis of the 2,3-butanedioldehydrogenase (2,3-BD) reaction (27, 28). He refers to the motile aeromonads which produce gas from carbohydrates as A. hydrophila (2,3-BD-positive) and A. punctata (2,3-BD-negative); the anaerogenic aeromonads, regardless of motility, as A. hydrophila var. anaerogenes (2,3-BD-positive) and A. punctata var. caviae (2,3-BD-negative); and the nonmotile, gas-producing aeromonads as A. hydrophila var. hydrophila (2,3-BD-negative). The latter strain was referred to by Lecleric and Buttiaux (17) as A. dourgesi.

Some of the differential characteristics of Aeromonas species, according to various investigators, are listed in Table 2. The polar-flagellated, VP-positive strains were further described by Eddy (8) as gelatinase-, indole-, nitrate-, catalase-,
and hydrogen sulfide-positive; methyl red- and citrate-variable; urease-negative; β-hemolytic; facultatively anaerobic; growing optimally at 30 C with a few strains growing at 1 C; producing acid and gas from glucose, galactose, maltose, mannitol, and dextrin; producing variable results from lactose, sucrose, rhamnose, and salicin; and producing no reaction from raffinose, xylose, dulcitol, and inulin.

The two strains in this study fit the characteristics described by Eddy (8) for the polar-flagellated, VP-positive strains, and would be classified as A. liquefaciens (8), A. hydrophila (12), or A. punctata (9). It is concluded that the oxidase test should be performed on all paracolon-like bacteria as part of the routine laboratory procedure. Although it is not practical to perform flagellar stains on a routine basis, this staining technique should be employed when an oxidase-positive, gram-negative bacillus is isolated. Awareness of the characteristics of aeromonads and the application of these tests will undoubtedly result in more frequent identification of Aeromonas from human sources.

Literature Cited

CHARACTERISTICS OF AEROMONAS PUNCTATA


