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Unusual Fermentative, Gram-Negative Bacilli Isolated from Clinical Specimens

II. Characterization of *Aeromonas* Species

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Morphological and physiological characterization of 12 *Aeromonas* strains isolated from clinical specimens demonstrated several important diagnostic features. These included polar arrangement of the flagella; production of oxidase, deoxyribonuclease, amylase, gelatinase, and lipase; lack of ornithine decarboxylase; and sensitivity to polymyxin. Awareness of these features should result in more frequent differentiation of aeromonads from the physiologically similar members of the genus *Plesiomonas* and the late- or non-lactose-fermenting species of *Enterobacteriaceae*.

Members of the genus *Aeromonas*, family *Pseudomonadaceae*, are known to inhabit soil and water and to be pathogenic to marine and fresh water animals (*Bergey's Manual of Determinative Bacteriology*, 7th ed.). Infections of humans with aeromonads are rare occurrences. However, aeromonads have been recovered from human sources as early as 1937 (16), and recent reports (2, 24) have shown an association between them and infection.

Since the classification of the aeromonads in *Bergey's Manual* has been termed inadequate, several proposals have been made for the reorganization of the genus, the most recent being that of Schubert (20-22). This report discusses the morphological and physiological characters of 12 strains of aeromonads isolated from clinical materials, in terms of the taxonomy and nomenclature recommended by Schubert for the revision of the genus *Aeromonas*.

MATERIALS AND METHODS

The 12 strains examined represent isolates recovered from clinical specimens between November 1965 and January 1970. The methods for morphological and physiological studies have previously been described (12).

RESULTS

All strains were facultatively anaerobic, gram-negative motile bacilli, occurring singly and in pairs, with a single, polar flagellum. Colonies on TSBA [Trypticase Soy Agar (TSA, BBL) with 5% defribrinated rabbit blood] were 1 to 3 mm in diameter, circular, smooth, convex, and grayish-white, with marked *beta*-hemolysis. After 3 to 5 days of incubation, the growth became dark green on TSBA plates and a light beige on TSA plates. Good growth was obtained on TSBA, TSA, MacConkey Agar (Difco), SS Agar (Difco), and Desoxycholate Agar (DC Agar, Difco) at room temperature and 37 C. All but two strains grew at 42 C. In KIA, an acid butt with gas and an alkaline slant developed after 24 hr of incubation with 10 strains, whereas two strains produced an all-acid tube. All but one strain produced slight H_2S. The biochemical results (Tables 1 and 2) were detected after 24 hr of incubation, except that the Methyl Red-Voges Proskauer (MR-VP) tests were performed after 48 hr of incubation, and lactose fermentation required 48 hr of incubation with two strains. All strains were resistant to penicillin, novobiocin, lincomycin, and ampicillin, and sensitive to erythromycin, terramycin, chloramphenicol, streptomycin, nitrofurantoin, nitrofurazone, methenamine mandelate, nalidixic acid, neomycin, kanamycin, and polymyxin. Eleven strains were resistant and one strain was sensitive to keflin.

DISCUSSION

The aeromonads have recently been associated with diarrhea (18, 24), cellulitis (24), septicemia (2, 23, 24), and inflammation of the peritoneal wall (17), and have been recovered from a

TABLE 1. *Common characteristics of Aeromonas strains*[a]

Characteristic	Presence	Characteristic	Presence
Acid glucose (OFBM)	+	Arginine	+
Fructose	+	Ornithine	−
Galactose	+	Lipase	+
Rhamnose	−	Amylase	+
Xylose	−	Deoxyribonuclease	+
Sucrose	+	2.5% NaCl	+
Maltose	+	6.5% NaCl	−
Mannitol	+	*p*H 5.6	+
ONPG	+	Hemolysis	+
Indole	+	TTC	−
Gluconate	−	Tyrosinase	−
Nitrite	+	MacConkey Agar	+
Nitrogen gas	−	SS Agar	+
Gelatin	+	DC Agar	+
Casein	+	Assimilation	
Oxidase	+	Glucose (BMM)	+
Malonate	−	Acetate	−
		Succinate	+

[a] Abbreviations: OFBM, OF Basal Medium; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; TTC triphenyl tetrazolium chloride; BMM, basal mineral medium.

fatal infection in a patient with acute myelogenous leukemia (3).

Several strains in this study were recovered from wounds which developed after trauma or surgery or from abscesses. Strain 1 was recovered from a young male who developed a draining sinus tract of the medial side of the foot under the first metatarsal after he scraped his foot on a rock in a lake. Another strain (no. 2) was isolated from an infected laceration of the forehead of an adult male after he hit his head on a railroad tie. Strain 3 was isolated from a finger wound after a cut. Strain 4 was isolated from a postoperative wound after a radical neck dissection on an adult male who was previously diagnosed as having carcinoma of the tongue. Strains 5 and 6 were recovered, respectively, from a leg abscess and from the purulent exudate from a 12-inch gangrenous segment of the ileum. Three strains were isolated from patients presenting the diagnosis of respiratory tract infection. One strain (no. 7) was recovered from both the throat and sputum of an adult female with aspiration pneumonitis, one (no. 8) from a tracheal aspiration of an adult male with bronchopneumonia, and another (no. 9) from the throat of an infant with pneumonitis. Other strains were recovered from stool cultures (no. 10) from a young adult who was admitted for bloody diarrhea and rectal prolapse, and the urine (no. 12) of an adult female with metastatic carcinoma of the uterus, ovaries, and left ureter. Strain 11 was recovered from a nonhuman source.

Bergey's Manual describes these gram-negative bacilli as being motile by polar flagella or occasionally nonmotile, and fermenting lactose slowly or not at all. The major differences between the aeromonads and those members of the *Enterobacteriaceae* which they physiologically resemble, are the production of oxidase (4, 8) and the polar arrangement of the flagella in the former (4). The presence of deoxyribonuclease activity in the aeromonads was later reported by Bottone and Allerhand (1). They can be differentiated from the deoxyribonuclease-producing members of the *Enterobacteriaceae* (*Serratia marcescens*, *Enterobacter liquefaciens*) on the basis of the ornithine decarboxylase test, since the genus *Aeromonas* lacks this enzyme (20, 21). The polymyxin-resistant character of *S. marcescens* (7) can be used to differentiate the aeromonads from nonpigmented strains of *Serratia*. Aeromonads are differentiated from *Vibrio* species since *Vibrio* is ornithine decar-

TABLE 2. *Variable characteristics of Aeromonas strains*[a]

Characteristic	*A. hydrophila* (11 strains)	*A. punctata* subspecies *caviae*-like (1 strain)[b]
Gas		
Glucose (PBB)	+(11)	−
Glycerol	+(11)	−
Acid		
Lactose (OFBM)	+(1), +d(2), −(8)	+
Mannose	+(11)	−
10% Lactose (PAB)	+(1), −(10)	+
Motility	+(11)	−
Hydrogen sulfide	+s(11)	−
Urea	−(11)	+
Citrate (Simmons)	+(10), −(1)	+
Methyl red	−(11)	+
Voges-Proskauer	+(11)	−
Phenylalanine	+(7), −(4)	+
Lecithinase	+(11)	−
Lysine	+(11)	−
Aesculin	+(10), −(1)	−
Growth at 42 C	+(9), −(2)	+
Cetrimide	+(8), −(3)	−
Lactate assimilation	+(9), −(2)	+

[a] Symbols: parenthetical values refer to number of strains giving reaction; d, required 48 hr of incubation; s, slight reaction; PBB, Purple Broth Base; PAB, Purple Agar Base.

[b] Differs from published description by Schubert (21) in being urease-positive and lecithinase-negative.

TABLE 3. *Some differential characteristics of Aeromonas and Plesiomonas species adapted from reports in the literature*[a]

Characteristic	*A. hydrophila*	*A. punctata*	*A. salmonicida*	*P. shigelloides*
Motile	+	+	−	+
Indole	+	+	−	+
Ammonium glucose	+(−)[b]	+	−	+
Sodium citrate	+(−)	V	−	−
Gas				
Glucose[c]	+	+	+	−
Glycerol	+	−	+	−
Acid: lactose	−, +(d)	−, +(d)	−	−, +(d)
Methyl red	V	V	+	+
Voges-Proskauer	+	−	−	−
Growth at 37 C	+	+	−	+
Gelatin	+	+	+	−
Starch	+	+	+	−
Lipase	+	+	+	−
Ornithine	−	−	−	+

[a] Literature cited: Habs and Schubert (13), and Schubert, (20–22).
[b] Symbols: (−), a few strains are negative; (d), delayed.
[c] Three anaerogenic subspecies recognized by Schubert (21) are *A. hydrophila* subsp. *anaerogenes*, *A. punctata* subsp. *caviae*, and *A. salmonicida* subsp. *achromogenes*.

boxylase-positive, arginine dihydrolase-negative, and always anaerogenic (14).

Based on a review of the morphological and physiological characteristics of aeromonads, Eddy (4, 5) and Eddy and Carpenter (6) concluded that the present system of classification of *Aeromonas* was inadequate and redefined the genus. Ewing, Hugh, and Johnson (9) also proposed reorganization of the genus, and although their 1961 monograph somewhat clarified the status of the aeromonads, the taxonomy of this group was still subject to review.

Since 1960 Schubert has conducted extensive studies concerned with the taxonomy and nomenclature of the genus *Aeromonas*, which have been summarized in three recent publications (20–22). Table 3 lists some of the features used by Schubert to differentiate the three species and three subspecies included in his revision. Three taxa of anaerogenic subspecies are designated, which are physiologically analogous to the aerogenic *Aeromonas* species. He (22) also proposed that the species name *A. liquefaciens* should be rejected as unrecognizable and that the oldest specific epithet *punctata* be recognized as the type species of the genus.

A group of bacteria, similar to the aeromonads and referred to as *A. shigelloides* [C27 strains of Ferguson and Henderson (10)], have been transfered to the new genus *Plesiomonas* (13). They are distinguished from the aeromonads because of their lack of amylolytic, lipolytic, and proteolytic activity, and the presence of ornithine decarboxylase (22).

Strains 5 and 10 examined in this study were previously described by the senior author (11) as *A. punctata* (*hydrophila*, *liquefaceins*) on the basis of the classification of Eddy (4, 5) and Ewing, Hugh, and Johnson (9). Strains 1 through 11 fit the characteristics described by Schubert (20) for *A. hydrophila*. Strain 12 closely resembles Schubert's (21) description for *A. punctata* subspecies *caviae* but differs in several respects in that it is nonmotile, urease-positive, and lecithinase-negative. In an earlier publication, Schubert (19) referred to nonmotile variants of the anaerogenic subspecies. *A. hydrophila* is distinguished from *A. punctata* on the basis of gas production from glycerol and the VP test (20). Both species are differentiated from *A. salmonicida* since the latter fails to grow at 37 C (20). Gas production from glucose differentiates the aerogenic species from the anaerogenic subspecies (21).

The present study indicates that the positive identification of aeromonads relies on the performance of the oxidase test on all late- or non-lactose-fermenting species of enteric bacteria that demonstrate deoxyribonuclease activity. They can be further differentiated from the *Enterobacter-Serratia* group by performing the ornithine decarboxylase test and testing for polymyxin sensitivity. In addition, flagella staining of oxidase-positive strains is recommended. Separation from the genus *Plesiomonas* may be achieved by testing for amylolytic, proteolytic, and lipolytic activity. Differentiation of the species and subspecies according to Schubert's recom-

mendation would depend on demonstrating the presence or absence of gas production from glucose and glycerol, acetyl methyl carbinol production, and growth at 37 C.

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