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Pyrazinamidase Activity as a Phenotypic Marker for Several *Aeromonas* spp. Isolated from Clinical Specimens

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Negative pyrazinamidase activity was significantly associated with *Aeromonas sobria*, and positive pyrazinamidase activity was associated with *A. hydrophila* and *A. caviae* (χ^2, $P < 0.0001$). The absence of pyrazinamidase activity may be a potentially significant phenotypic marker for *A. sobria*.

Aeromonads are ubiquitous gram-negative, aquatic microorganisms that can cause a wide variety of diseases in humans as well as cold- and other warm-blooded animals (4). In the last decade, there has been increasing research centered on their relative pathogenicity for humans, especially with respect to species-associated virulence factors (5).

However, with *Aeromonas* taxonomy currently in a state of flux, i.e., having 12 or more DNA-DNA hybridization groups (genotypes) within the genus (G. R. Fanning, F. W. Hickman-Brenner, J. J. Farmer III, and D. J. Brenner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C116, p. 319) and 8 proposed and/or recognized species to date, virulence is difficult to assess. For example, Kuijper et al. (9) have shown that only 5 of the 12 genotypes were found in the examination of 142 diarrheal clinical strains. Further, it was shown that the current scheme of classification is not sufficiently discriminating, in that phenotypically similar *A. hydrophila* strains were genetically heterogeneous and that a suspected *A. sobria* could belong to either DNA hybridization group 8 (*A. sobria*) or group 10 (*A. veronii*).

Because we have been investigating several biochemical characteristics for phenotypic classification of the different DNA groups, we decided to examine pyrazinamidase activity, which has been extensively studied in *Mycobacterium* spp. (8) and considered a differentiating feature in the classification of *Corynebacterium* spp. (11), *Rhodococcus* spp., and *Nocardia* spp. (12) and a virulence-associated marker in the genus *Yersinia* (7).

Bacterial strains. There were 119 *Aeromonas* strains used for this study, including *A. hydrophila*, *A. sobria*, *A. caviae*, *A. veronii*, and *A. schubertii*. The strains were collected from 1985 to 1989 in several countries, including the United States ($n = 56$), Northeast Africa ($n = 45$), Bangladesh ($n = 11$), Indonesia ($n = 1$), and South America ($n = 1$), and originated primarily from various clinical sources ($n = 98$). The remaining aeromonads ($n = 21$) were from environmental, veterinary, and miscellaneous sources. The definition strains for the DNA hybridization groups 1, 4, 10, and 12 and a clinical American Type Culture Collection (Rockville, Md.) strain representing DNA hybridization group 8Y were included as controls (Table 1). The strains were maintained at −70°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.) with 10% glycerol added.

Strain characterization. Strains which were oxidase positive and resistant to Vibriostat (0/129; 150 μg/ml; Oxoid, Columbia, Md.) were presumptively placed in the *A. hydrophila* complex with the API 20E (Analytab Products, Plainview, N.Y.). Identification to the species level was accomplished by using conventional methods (2, 6, 10) and media (Remel, Lenexa, Kans.). Ornithine decarboxylase production, mannitol fermentation, and indole production were included to screen for the newer species, *A. veronii* and *A. schubertii*, respectively. All tests were incubated at 36°C ± 1°C. Pyrazinamidase activity was assayed by using a supplied medium that contained tryptic soy agar (15 g; Difco), yeast extract (1.5 g; Sigma Chemical Co., St. Louis, Mo.), and Tris-maleate (0.2 M, pH 6, 500 ml) buffer and pyrazinamide (0.5 g; Sigma) and was dispensed in 5-ml aliquots into screw-cap tubes (16 by 150 mm), autoclaved at 121°C for 15 min, and slanted (Remel). These slants were inoculated with 18- to 24-h bacterial growth taken from tryptic soy agar (Difco) and incubated for 48 h at 36°C. One milliliter of 1% (wt/vol) freshly prepared ferrous ammonium sulfate (aqueous) solution was flooded over each slant, and a positive (pinkish rust color) or negative reaction (colorless) was recorded after 15 min. Positive pyrazinamidase activity indicated the presence of pyrazinoic acid resulting from the action of the enzyme pyrazinamidase (7).

Increasing interest in the aeromonads as disease-causing microorganisms has resulted in numerous efforts to identify these microorganisms definitively (4). Because of the current state of taxonomic confusion surrounding aeromonads, we considered it imperative to start with strains that were well characterized to the species level. We screened 119 well-characterized *Aeromonas* strains representing five species from various geographical locales for pyrazinamidase activity. Of the 37 *A. hydrophila* isolates tested, 35 (95%) were positive. Both of the negative strains were environmental isolates from the United States. Similarly, 49 of 53 (93%) *A. caviae* were positive. Negative pyrazinamidase activity was detected in 23 of 24 (96%) *A. sobria* and in 2 of 2 (100%) *A. veronii* and 3 of 3 (100%) *A. schubertii* strains tested. The one positive *A. sobria* isolate and four negative *A. caviae* isolates were human fecal strains from North Africa.

Using the chi-square goodness-of-fit test, it was established that the proportion (percentage) of positives was different among the three species *A. hydrophila*, *A. sobria* and *A. caviae*. Further examination of the data implied that *A. sobria* was distinct from the other two species (χ^2, $P < 0.0001$).

Our findings suggest that pyrazinamidase activity could be a valuable phenotypic marker to assist in the differentiation of *A. sobria* from *A. hydrophila* and *A. caviae*. We propose

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TABLE 1. Sources of *Aeromonas* isolates

Organism (*n*)	No. of isolates						
	ATCC[a]	Blood	Fecal	Wound	Miscellaneous	Environmental	Veterinary
A. hydrophila (37)	1	0	15	12	2	5	2
A. sobria (24)	1	0	20	2	0	0	1
A. caviae (53)	1	1	41	4	1	2	3
A. veronii (2)	1	0	0	1	0	0	0
A. schubertii (3)	1	0	0	2	0	0	0

[a] DNA hybridization group strains used were *A. hydrophila* ATCC 7966 (group 1), *A. sobria* ATCC 9071 (group 8Y), *A. caviae* ATCC 15468 (group 4), *A. veronii* ATCC 35624 (group 10), and *A. schubertii* ATCC 43700 (group 12).

that this test should be included in future biotyping schema for mesophilic aeromonads. It is simple to perform, and positive and negative reactions are easily interpreted.

The results obtained with a limited number of *A. veronii* and *A. schubertii* strains suggest that this test may also prove to be useful for identifying these organisms. However, analysis of a larger sample of these strains is indicated before a final decision can be reached.

It has been proposed that *A. sobria* may indeed be the most virulent of the aeromonads causing human disease (1, 3). These observations, combined with the success obtained using pyrazinamidase activity as a test for virulence of clinical *Yersinia enterocolitica* strains (G. P. Carter, I. K. Wachsmuth, V. L. Miller, S. Falkow, and J. J. Farmer III, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C147, p. 356), suggest that negative pyrazinamidase activity might also be a new virulence-associated marker specific for *A. sobria* and the two most recently described human pathogens *A. veronii* and *A. schubertii*. Further studies are warranted to determine the nature of this association, i.e., whether or not negative pyrazinamidase activity is related to the presence of a virulence plasmid or some other as yet undetermined property of these species.

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