mannitol, breakdown of inositol, and oxidase activity (table 1). Differential characteristics for the motile aeromonads include esculin hydrolysis, growth in KCN broth, L-histidine and L-arginine utilization, L-arabinose utilization, fermentation of salicin, gas from glucose, and H_2S from cysteine (table 2).

DNA analyses

DNA hybridization studies have verified the distinction of the three species, *A. hydrophila, A. sobria* and *A. caviae*. Within these species there are at least seven DNA hybridization groups, three in *A. hydrophila*, two in *A. caviae*, and at least two in *A. sobria*. Thus far these DNA-related new groups have not been distinguished phenotypically one from the other[8].

Similarly, Farmer et al.[4], using DNA hybridization (hydroxyapatite, ^{32}P, 60C), found only 5 of 60 *Aeromonas* strains to be highly related to the type strain of *A. hydrophila* (70% or greater). Similarly, the type strain of *A. sobria* was highly related to only two other strains, suggesting that many strains of the motile, mesophilic group of *Aeromonas* belong to species other than *A. hydrophila, A. sobria,* and *A. caviae*. There appear to be nine to twelve different DNA hybridization groups.

Table 1. Identifying characteristics for motile aeromonads

Catalase	+
Oxidase	+
Motility	+
Morphology	Rods in singles and pairs
Growth in nutrient broth at 37 °C	+
Arginine dihydrolase	+
Ornithine decarboxylase	−
Indole production	+
Fermentation:	
sucrose and mannitol	+
dulcitol, rhamnose, xylose,	−
raffinose, inositol, and adonitol	−
Breakdown of inositol	−
NO_3 reduction to NO_2	+
Growth in peptone H_2O without NaCl	+
0/129 resistance	+
Starch, gelatin, ONPG, RNA and DNA hydrolysis	+
Tween 80 esterase	+

Table 2. Minimal identifying characteristics for the motile aeromonads

Characteristics	*A. hydrophila*	*A. sobria*	*A. caviae*
Esculin hydrolysis	+	−	+
Growth in KCN broth	+	−	+
L-histidine utilization	+	−	+
L-arginine utilization	+	−	+
L-arabinose utilization	+	−	+
Fermentation of salicin	+	−	+
Gas from glucose	+	+	−
H_2S from cysteine	+	+	−

5S RNA sequence analysis

Results of 5S RNA sequence analysis showed that *Aeromonas* spp. should be included in a family, Aeromonadaceae, separate from the Enterobacteriaceae and the Vibrionaceae[1]. Molecular genetic information compiled to date, including results of 16S ribosomal ribonucleic acid cataloging and 5S ribosomal ribonucleic acid sequence analyses, suggest that *Aeromonas* demonstrate an evolutionary divergence which is significantly at variance with that of other members of the Vibrionaceae[6]. An indication of the phylogenetic diversity among vibrios, aeromonads, and enterics can be gleaned from the figure.

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Plesiomonas: Taxonomy, classification and enterotoxin production

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Key words. Plesiomonas taxonomy; *Plesiomonas* classification; *Plesiomonas* enterotoxin; *Aeromonas/Plesiomonas.*

Encountered in clinical specimens and the natural environment, *Plesiomonas shigelloides* has been implicated as a potential diarrheal agent in man, but has been overshadowed in importance by related bacteria in the genera *Aeromonas* and *Vibrio*, resulting in its neglect. Consequently, a good deal of uncertainty remains with regard to the taxonomy, ecology and pathogenicity of this member of the family Vibrionaceae. In fact, recent phylogenetic data, based on 5S rRNA sequencing, suggest a closer relationship of *P. shigelloides* with the Enterobacteriaceae rather than the Vibrionaceae. The closest relationship amongst the genera of the Enterobacteriaceae for *P. shigelloides* is with *Proteus mirabilis*[2,3]. Furthermore, a serological relationship between *P. shigelloides* and *Shigella* has been reported[6]. To clarify the taxonomy of *P. shigelloides*, phenotypic characterization was carried out, emphasizing extracellular enzyme profiles. Several investigators have reported production of enterotoxin(s) by *P. shigelloides*[1,4]. Enterotoxin of *P. shigelloides* is not believed to be related immunologically to cholera toxin (CT). We have deter-

mined antigenic and biological similarities between *P. shigelloides* cholera toxin-like protein and *Vibrio cholerae* enterotoxin and report these results here.

5S rRNA analysis. Results of 5S rRNA sequence analysis showed that *P. shigelloides* was much more closely related to the Enterobacteriaceae. The sequence for *P. shigelloides* is given in the figure.

Identification and conventional characterization tests. Results for most of the identification tests used in this study confirm that the strains were *P. shigelloides* and correlated well with data in the literature[5,7]. Interestingly, the majority of strains examined gave only a weak methyl red reaction, even after incubation for 7 days at 25 °C and 37 °C. Also, the *P. shigelloides* strains showed a good degree of uniformity in their reactivities. Furthermore, the results for aesculinase, amylase, gelatinase, lipase (Tween 80) and urease agreed well with the results obtained for these enzymes in other studies[5,7].

Decarboxylase activity was detected for L-arginine, L-lysine, L-ornithine, L-histidine, four of the 15 amino acid substrates examined. L-arginine, L-lysine, and L-orithine decarboxylase have been used previously in the presumptive diagnosis of *P. shigelloides*. L-histidine decarboxylase may also be useful in the diagnosis of this species. Preliminary results showed that selected strains of *Aeromonas hydrophila, A. caviae* and *A. sobria* and some *Vibrio* spp. were negative for this enzyme.

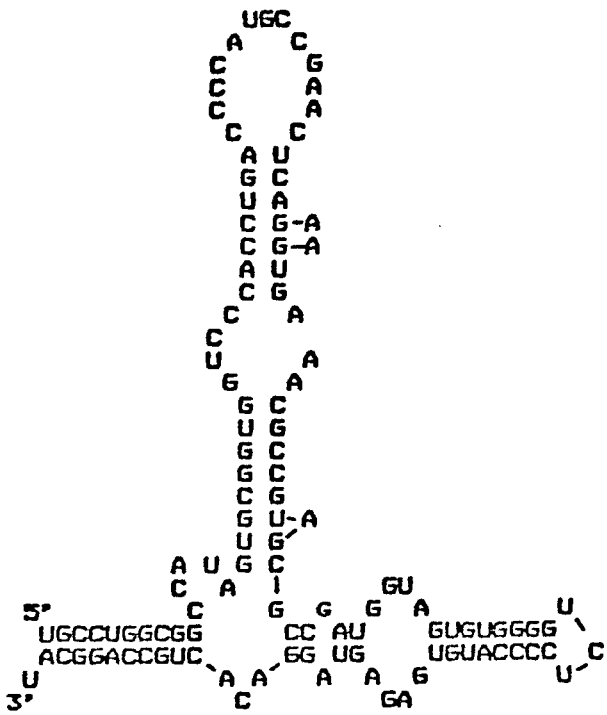
All 22 strains examined were positive for deoxyribonuclease (DNase) activity by the HCl method and most (91%) were positive using the toluidine blue 0 method described by Waller et al.[8]. Prolonged incubation (> 3 days) was usually necessary to obtain a positive reaction by either method.

Rapid characterization tests. A good correlation was observed for those enzymes tested using both 4-MUCS and API enzyme test kits. All 22 strains tested gave very strong N-acetyl-β-D-glucosaminidase activity. Similar results were obtained for this enzyme when tested using the equivalent 4-MUCS substrate and in API oxidase. This enzyme may be a potentially useful diagnostic feature for *P. shigelloides*. Most strains also gave strongly positive reactions for acid phosphatase and positive-to-strongly-positive reactions for alkaline phosphatase with API ZYM.

The overall conclusion from the results is that *P. shigelloides* is much more active enzymatically than has been reported[6,7].

CT-like and cytotoxic activity of P. shigelloides culture supernatants. The Y-1 mouse adrenal cell assay was used to measure CT-like and cytotoxic activity of culture supernatants. CT-like activity was detected in tryptic soy-inositol culture supernatants of all strains tested, with titers ranging from 4 to 16. Only ATCC 14029 and 11A produced detectable levels of CT in heart infusion broth. No activity was detected in syncase, CAYEG, or heart infusion-inositol media.

In conclusion, the data suggest a position intermediate between the Vibrionaceae and the Enterobacteriaceae for *P. shigelloides*. Furthermore, the unexpected finding of a CT-like factor produced by *P. shigelloides* suggests some interesting molecular genetic relationships of *P. shigelloides* yet to be explored.



Sequence of 5S rRNA of *P. shigelloides* ATCC 14029^T.

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Ecology of aeromonads and isolation from environmental samples

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Key words. Aeromonas; environmental samples.

Our knowledge of the ecology of the aeromonads has made little progress over the past ten years because systematics and procedures of isolation of these bacteria are still unsatisfactory.

General problems

The first problem is that aeromonads have until now been identified on a routine basis by phenotyping only. Ecological results,