Genome Sequence of *Aeromonas hydrophila* ATCC 7966^T: Jack of All Trades[▽]

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The complete genome of *Aeromonas hydrophila* ATCC 7966^T was sequenced. *Aeromonas*, a ubiquitous water-borne bacterium, has been placed by the Environmental Protection Agency on the Contaminant Candidate List because of its potential to cause human disease. The 4.7-Mb genome of this emerging pathogen shows a physiologically adroit organism with broad metabolic capabilities and considerable virulence potential. A large array of virulence genes, including some identified in clinical isolates of *Aeromonas* spp. or *Vibrio* spp., may confer upon this organism the ability to infect a wide range of hosts. However, two recognized virulence markers, a type III secretion system and a lateral flagellum, that are reported in other *A. hydrophila* strains are not identified in the sequenced isolate, ATCC 7966^T. Given the ubiquity and free-living lifestyle of this organism, there is relatively little evidence of fluidity in terms of mobile elements in the genome of this particular strain. Notable aspects of the metabolic repertoire of *A. hydrophila* include dissimilatory sulfate reduction and resistance mechanisms (such as thiopurine reductase, arsenate reductase, and phosphonate degradation enzymes) against toxic compounds encountered in polluted waters. These enzymes may have bioremediative as well as industrial potential. Thus, the *A. hydrophila* genome sequence provides valuable insights into its ability to flourish in both aquatic and host environments.

Aeromonas spp. are ubiquitous bacteria found in a variety of aquatic environments worldwide, including bottled water, chlorinated water, well water, and heavily polluted waters (67). They cause infections in invertebrates and vertebrates, such as frogs, birds, and domestic animals (34, 36). Various fish species develop hemorrhagic disease and furunculosis resulting from infections by *Aeromonas* spp. (3, 52). While originally thought to be an opportunistic pathogen in immunocompromised humans, an increasing number of cases of intestinal and extra-intestinal disease documented worldwide suggest that it is an emerging human pathogen irrespective of the host's immune status (25). The organism is included in the Contaminant Candidate List by the Environmental Protection Agency, and U.S. water supplies are routinely examined for it (21). Elevated numbers of *Aeromonas* spp. were recorded in floodwater sam-

ples in New Orleans following hurricane Katrina (80). Moreover, *Aeromonas* spp. were the most common cause of skin and soft tissue infections among the survivors of the 2004 tsunami in southern Thailand (40). Most alarming are the associations of *Aeromonas* spp. with hemolytic uremic syndrome (8) and necrotizing fasciitis reported in an immunocompetent child (1).

Aeromonads are facultative anaerobic chemo-organotrophs capable of anaerobic respiration and dissimilatory metal reduction (67). While many virulence determinants (such as proteases, hemolysins, and enterotoxins) that bestow the ability to cause disease have been identified in *Aeromonas* spp. (29), little is known about the actual subset of virulence factors within certain strains and/or species that comprise a “virulent” aeromonad (53). Even less is known about the mechanisms that confer the metabolic versatility that allows *A. hydrophila* to persist in its aquatic habitats (including polluted waters) or that facilitate ecological interactions with other prokaryotic and eukaryotic organisms. Additionally, the roles of *Aeromonas* spp. in the nutrient cycles of the aquatic environment and any potential for bioremediation are relatively unknown.

Phylogenetic analysis of rRNA genes places *Aeromonas* spp. in a distinct family, the *Aeromonadaceae*, within the gamma-proteobacteria (19, 68). Historically, the genus *Aeromonas* has been divided into two groups: a group of nonmotile, psychrophilic species, best represented by *Aeromonas salmonicida*,

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which are generally only associated with fish disease and a second group of motile, mesophilic species associated with human disease. The type species for the genus, *A. hydrophila*, is in the latter category and is the only species known to cause disease in both fish and human populations. We report here the complete genome sequence of *A. hydrophila* ATCC 7966^T, a well-characterized type strain for the species, originally isolated from “a tin of milk with a fishy odor” (17, 93). The genome sequence divulges mechanisms contributing to virulence and metabolic fitness that allow the organism to grow in a variety of ecosystems and begins to explain how *A. hydrophila* is able to survive in polluted or oxygen-poor environments and to colonize and cause disease in humans and other hosts. Thus, the versatility of this organism merits the sobriquet “jack of all trades.”

MATERIALS AND METHODS

Sequencing and gene identification. The complete genome of *A. hydrophila* ATCC 7966^T was sequenced using the random-shotgun method described for genomes sequenced by The Institute for Genomic Research (28). The gene prediction and annotation of the genome were performed as previously described (86). Metabolic pathways and other properties were examined using the Genome Properties system (39).

Trinucleotide composition. The distributions of all 64 trinucleotides (3-mers) for each chromosome were determined, and the 3-mer distribution in 2,000-bp windows that overlapped by half their length (1,000 bp) across the genome was computed. For each window, we computed the χ^2 statistic on the difference between its 3-mer content and that of the whole chromosome. A large value for χ^2 indicated the 3-mer composition in this window was different from that of the rest of the chromosome. We interpreted high χ^2 values to be indicators of regions on the chromosome that appeared unusual and demanded further scrutiny.

Genome tree construction. The protein sequences of 31 housekeeping genes (*dnaG*, *fir*, *infC*, *nusA*, *pgk*, *pyrG*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rplS*, *rplT*, *rpmA*, *rpoB*, *rpsB*, *rpsC*, *rpsE*, *rpsI*, *rpsJ*, *rpsK*, *rpsM*, *rpsS*, *smgB*, and *tsf*) from selected genomes were aligned to predefined hidden Markov models (HMMs), and ambiguous regions were autotrimmed according to an embedded mask. Concatenated alignments were then used to build a maximum likelihood tree using phym1 (38, 107).

Nucleotide sequence accession number. The complete genome sequence of *A. hydrophila* ATCC 7966^T has been submitted to GenBank (accession number CP000462).

RESULTS AND DISCUSSION

General genome features. The complete genome of *A. hydrophila* ATCC 7966^T is comprised of a single circular 4,744,448-bp chromosome with 61.5% G+C content and encodes 5,195 predicted coding sequences (CDSs) (Table 1). It was possible to assign putative functions to 72.3% of the CDSs, while 21.5% possessed similarity to genes of unknown function, and no function could be proposed for 6.2% of the CDSs. A total of 128 tRNA genes are predicted in the genome, which is a relatively high proportion compared to most other sequenced genomes. Furthermore, in *A. hydrophila*, many of these tRNAs appear to have arisen via tandem duplication, with their anticodons predominantly corresponding to hydrophobic amino acids (e.g., Met, Gly, Leu, and Val). However, one caveat is that anticodons may undergo posttranscriptional modifications, thereby changing their amino acid specificities (92). While we noted no significant correlation between the overrepresented tRNAs and the codon frequencies of the corresponding amino acids in the genome, they may be involved in translating the most frequent codons in only highly expressed genes (46).

TABLE 1. General genome features

Parameter	Value
Size (bp)	4,744,448
G+C content (%)	61.5
Protein-coding genes	
No. (%) similar to known proteins	2,985 (72.3)
No. (%) similar to proteins of unknown function ^a	401 (9.7)
No. (%) of conserved hypotheticals ^b	486 (11.8)
No. (%) of hypotheticals ^c	256 (6.2)
Total	4,128
Average ORF size (bp)	1,004
Coding (%)	87.5
No. of rRNA genes	30
No. of tRNA genes	128
Intein no. ^d	1

^a Unknown function, significant sequence similarity to a named protein to which no specific function is currently attributed.

^b Conserved hypothetical protein with sequence similarity to a translation of an open reading frame (ORF) in another organism; however no experimental evidence for protein expression exists.

^c Hypothetical proteins with no significant similarity to any other sequenced gene.

^d A single intein-bearing CDS (AHA3057) of unknown function possesses a signal peptide sequence followed by a low-complexity region and a series of 23-amino-acid repeats in the C terminus.

Ten ribosomal-gene operons are predicted in the *A. hydrophila* ATCC 7966^T genome. The relatively high numbers of tRNAs and rRNAs may be correlated with an ability to rapidly respond to changing environmental conditions (54). Within the 23S rRNA genes, up to 0.9% sequence variation is seen; however, the 16S rRNA gene sequences differed by only 1 bp. This intragenomic heterogeneity in the 23S rRNA genes is significant, because a comparison of all of the available *Aeromonas* sp. 23S rRNA gene sequences in GenBank revealed that the species differed on average by as little as 0.6%. An examination of the aligned sequences revealed that these differences occurred at phylogenetically informative locations, and they are similar to the intragenomic heterogeneity recently reported for the 16S rRNA genes of *Aeromonas* spp. (73). While these differences could have arisen by the continued presence of different rRNA operons since speciation, a more likely scenario is the mosaic evolution of the rRNA by horizontal gene transfer of partial rRNA operon fragments (32). These results further support the notion that the ribosomal operons of *Aeromonas* spp. do not always reflect the phylogenetic history of the organism and have to be used with caution when employed to identify species.

Phylogenetic analysis of the concatenated sequences of 31 housekeeping genes of *A. hydrophila* ATCC 7966^T supports its distinct taxonomic position among gammaproteobacteria, as observed in rRNA gene-based trees (68). The genome tree suggests that it may share a relatively closer evolutionary relationship with the *Vibrionaceae* relative to the other families of the γ -proteobacteria (Fig. 1). Almost 90% of the *A. hydrophila* CDSs have matches ($P < 10^{-5}$) in one of the other

FIG. 1. Phylogenetic analysis of protein sequences of 31 concatenated housekeeping genes from *Aeromonas hydrophila* ATCC 7966^T and selected sequenced proteobacterial genomes. The protein sequences were aligned to predefined HMM models, and the concatenated alignments were then used to build a maximum likelihood tree using phym.

sequenced gammaproteobacterial genomes (*Pseudomonas* spp., *Vibrio* spp., *Escherichia coli*, *Salmonella* spp., *Yersinia pestis*, and *Shewanella oneidensis*). The greatest numbers of matches (~80%) are seen with the *Vibrio vulnificus* and *Pseudomonas profundum* genomes. An examination of best matches (Fig. 2) revealed a predominance of matches with the *Vibrio* spp., *P. profundum*, and *S. oneidensis* genomes, corre-

sponding to their relatively short evolutionary distances from *A. hydrophila* (Fig. 1). Regions unique to *A. hydrophila* ATCC 7966^T compared to sequenced gammaproteobacteria include a large phage region (AHA2017 to -2034, described below) marked by atypical trinucleotide composition and a cluster of pilus accessory genes (AHA0692 to -0696). The functions of these and other *A. hydrophila*-specific regions are difficult to discern, since they largely encode hypothetical or conserved hypothetical proteins or proteins of unknown functions.

As seen in many other genomes, the largest expansions of gene families (paralogs) in *A. hydrophila* ATCC 7966^T are those of ABC transporters, two-component signal transduction systems (TCS), transcriptional regulators, Fe-S cluster-binding proteins involved in energy transduction at the membrane, and methyl-accepting chemotaxis proteins (MCPs).

Integrated regions. A few regions with atypical trinucleotide and G+C compositions are present in the genome and might signify recently integrated regions. The most prominent of these is a large ~40-kb prophage (coordinates, ~2223600 to 2260300) encoding numerous hypothetical and conserved hypothetical proteins, DNA repair enzymes UmuDC, a phage terminase, an integrase, and a reverse transcriptase (AHA2035). Putative attachment sites are seen flanking this region. A second large region with atypical composition (coordinates, ~1150000 to 1187000) includes genes for a single phage protein (AHA1086), UV damage-induced DNA repair enzymes, regulatory proteins, several unknown and hypothetical proteins, and two putative invasins (AHA1064 and AHA1066).

A preliminary comparison of the *A. hydrophila* ATCC 7966^T genome sequence with an initial draft genome of *Aeromonas veronii* biovar sobria HM21, a symbiotic isolate from the medicinal leech (35), revealed shared contigs totaling ~200 kb. About 47.5 kb (23.75%) of *A. veronii* HM21 sequences did not have any matches in the *A. hydrophila* ATCC 7966^T genome, and the majority of these (~30.7 kb) included bacteriophage genes while the remainder included hypothetical genes (J. Graf, unpublished data). Similarly, T4-like phages have been discovered in *A. hydrophila*, and sequencing of these phage genomes has revealed a litany of genes with no matches to known T4 genes or other genes in sequence databases (Jim Karam, personal communication; <http://phage.bioc.tulane.edu/>).

Two other large regions (region I, ~3227000 to 3266000, and region 2, ~4599000 to 4622000) with atypical trinucleotide compositions primarily include lipopolysaccharide and carbohydrate synthesis or modification genes. In *Vibrio cholerae*, a cluster of carbohydrate biosynthesis genes was determined to produce an exopolysaccharide that confers chlorine resistance, rugosity, and the ability to form biofilm (108). It is tempting to speculate that components in one of these regions may confer a similar ability, particularly in light of previous reports of chlorine resistance and biofilm formation by *Aeromonas* spp. (10, 61). A smaller atypical region (2460000 to 2468000) harbors a previously unknown type 1 fimbrial gene cluster (AHA0518 to -0524) that is likely important for mediating interactions with the host.

No transposase, resolvase, or insertion sequence element was found in the *A. hydrophila* ATCC 7966^T genome. Although not unprecedented, the lack of these mobile elements in the relatively large genome of a free-living organism like *A. hydrophila* is uncommon. However, insertion sequence elements

FIG. 2. Numbers of best matches of *Aeromonas hydrophila* ATCC 7966^T CDSs in other bacterial genomes. The subset of organisms shown includes those for which >30 best matches were seen.

have been identified in other species, such as *A. salmonicida*, *Aeromonas caviae*, and *Aeromonas bestiarum*, and a recent comparative genome hybridization study revealed a high degree of variability in the numbers of transposon genes in these *Aeromonas* spp. (13, 75). Therefore, it is likely that other strains of *A. hydrophila* may possess such elements, unlike *A. hydrophila* ATCC 7966^T.

A. hydrophila ATCC 7966^T also lacks any CRISPR repeat or members of CRISPR-associated (*cas*) genes (50), a system of laterally transferred genetic elements that have the ability to form and express a panel of short RNA sequences, some captured from foreign genetic elements, such as phage, for an incompletely described set of functions that likely includes resistance to the sampled phage (64).

Virulence. Based on a study of the pathogenicities of type strains of 12 species of the genus *Aeromonas*, the most pathogenic species (by measuring 50% lethal doses in Swiss-Webster mice) was determined to be *Aeromonas jandaei* ATCC 49568^T, followed by *A. hydrophila* ATCC 7966^T (49).

The roles of several virulence determinants, such as cytotoxic enterotoxin (Act) and cytotoxic enterotoxins (heat-labile [Alt] and heat-stable [Ast]) had been described previously by developing targeted mutants in the clinical isolate *A. hydrophila* SSU (2, 88). Other virulence factors previously described in various *A. hydrophila* strains include aerolysin (14, 42), extracellular hemolysin (AHH1) (41), glucose-inhibited division protein (GidA), ferric uptake regulator (Fur) (89, 90), a surface-expressed enolase (87), DNA adenine methyltransferase (Dam) (22), ToxR-regulated lipoprotein (TagA) (78a), and quorum-sensing autoinducer synthase (AhyI) and transcriptional activator (AhyR) (98; A. K. Chopra, unpublished

data). Genes encoding aerolysin, AHH1, Act, Ast, GidA, Dam, Fur, enolase, hemolysin, TagA, AhyI, and AhyR have all been detected in the *A. hydrophila* ATCC 7966^T genome. In addition, ribosylhomocysteine lyase (AHA0700), which may be involved in the production of a second autoinducer (AI-2), as described for *Vibrio harveyi*, *Salmonella enterica* serovar Typhimurium, and *E. coli* (97), has been identified. A type II secretion system described in *Aeromonas bestiarum* (formerly published as *A. hydrophila*) (51) is present. *A. hydrophila* ATCC 7966^T also contains homologs (AHA1826 to -1848) of the *vas* (virulence-associated secretion) genes that were recently proposed to encode a prototypic type VI secretion system (T6SS) in *V. cholerae* (81).

However, genes (e.g., *ascV*, *acrV*, and *aopB*) that constitute a type III secretion system (T3SS) identified in fish isolates, as well as in *A. hydrophila* SSU (91, 104, 110), are strikingly absent in *A. hydrophila* ATCC 7966^T. An ortholog of the T3SS effector, AexT (chromosomally encoded), characterized in *A. salmonicida* (9, 11), is also missing. In *A. salmonicida*, loss of the plasmid bearing the T3SS resulted in loss of bacterial virulence (96). In *A. hydrophila* SSU and fish isolates of *A. hydrophila* (AH1 and AH3), the T3SS genes were located on the chromosome rather than on the plasmid (91), and recent studies have indicated that T3SS deletion mutants of *A. hydrophila* SSU induced greatly reduced levels of cytokines in mouse sera compared to animals infected with the wild-type SSU strain (24). These studies clearly established a role for the T3SS in the virulence of *A. hydrophila*. As mentioned above, *A. hydrophila* ATCC 7966^T was determined to be among the most pathogenic strains in mouse lethality experiments (49). In light of these observations, what does the absence of a T3SS in *A.*

hydrophila ATCC 7966^T signify? While it is possible that the T3SS in *A. hydrophila* ATCC 7966^T was borne on a plasmid that may have been lost, one may also hypothesize that the absence of the T3SS may be complemented by the presence of the aforementioned T6SS or by the flagellar secretion apparatus (see below), as described in *Yersinia enterocolitica* (109). That is, the presence of one of these secretion systems may be sufficient for *A. hydrophila* virulence. The functionality of the T6SS in ATCC 7966^T remains to be explored, since many strains of *V. cholerae* possessed nonfunctional T6SS (81).

A. hydrophila bacteria are motile by the action of a single polar flagellum. In the *A. hydrophila* ATCC 7966^T genome, the genes for the polar flagellum are found in four main clusters: CDSs AHA1698 to -1703, AHA1365 to -1391, AHA2832 to -2847, and AHA2824 to -2826. The same distribution or organization was reported for *A. hydrophila* AH-3 (12). Similar to *Pseudomonas* spp., additional copies of the flagellar *mot* (motor component) genes are scattered in the genome: AHA2642, -1783 to -1784, and -3317 to -3318. Approximately 60% of aeromonads possess a second (lateral) flagellar system for swarming motility (31) that is also suggested to play a role in virulence by enhancing adhesion to eukaryotic cell surfaces (57), but *A. hydrophila* ATCC 7966^T does not possess genes encoding a lateral flagellum. Indeed, the presence of lateral flagella does not appear to be species specific but may be strain specific (J. Shaw, unpublished data). In addition to the chemotaxis (*che*) genes associated with two of the polar-flagellum gene clusters (AHA1365 to -1391 and AHA2832 to -2847), two other clusters are present in the genome: AHA2528 to -2535 and AHA1030 to -1039, resulting in a total of 23 *che*-related genes. These, together with the 43 CDSs encoding putative MCPs, suggest an extremely complex chemotaxis system in *A. hydrophila*. Furthermore, the aeromonad polar flagellum has been shown to be glycosylated (82, 37); genes potentially responsible for this function are found in two clusters: AHA4150 to -4151 and AHA4175 to -4187.

In addition to these characterized virulence factors, new hemolysins, proteinases, pilus assembly proteins, and other putative virulence factors have been predicted from the genome sequence (Table 2). Surface structures, such as pili or fimbriae and adhesins, may be involved in attachment to the host cell or recognition of host cell receptors, thereby initiating the colonization process. In addition to the characterized type IV *Aeromonas* pilus (Tap) (78) (AHA3868 to -3871), AHA1450 to -1459, AHA0686 to -0696, AHA0518 to -0524, and AHA3190 to -3194 encode potential novel pili or fimbrial structures. Of these, AHA3190 to -3194 are similar to a characterized type IV fimbrial-gene cluster from *Pseudomonas aeruginosa* (66). AHA0062 is similar to an *E. coli* fimbriin gene (79). AHA0383 to -0399 encode a type IV pilus related to the mannose-sensitive hemagglutinin of *V. cholerae* (65). This pilus may be the one most readily expressed in *Aeromonas* spp., since the predicted MshA component closely matches sequences of purified pilin proteins from other strains (6). Earlier work had reported the presence of both mannose-sensitive and mannose-resistant pili among *Aeromonas* strains (20). AHA2697 and AHA3491 encode two putative high-molecular-weight proteins (~421 kDa and ~511 kDa, respectively) with type I secretion signals, which may serve as adhesins.

A paracrystalline surface layer (termed the S-layer or A-

layer) is important for binding host factors, as well as providing resistance for the bacterium against serum killing and protease digestion, and its loss in *A. hydrophila* strains causes some reduction in virulence (76). While sequences matching *Aeromonas* sp. S-layer proteins (74) are not seen in *A. hydrophila* ATCC 7966^T, we cannot rule out the presence of one given the diversity of *Aeromonas* S-layer protein sequences (23, 59). On the other hand, S-layers have been described only in *A. hydrophila* strains from serogroups O:11, O:14, and O:81, whereas *A. hydrophila* ATCC 7966^T belongs to serogroup O:1 (23).

Symptoms of *Aeromonas* sp. infections vary from gastroenteritis to wound infections (cellulitis, ecthyma gangrenosum, and myonecrosis) (101) and septicemia (25, 29, 48). Consequently, several corresponding putative toxins are identified in the genome (Table 2). For example, AHA1359 encodes a putative RTX toxin with an adjacent toxin activator gene (AHA1358) and toxin transporter genes (AHA1354 to -1356). The entire locus shares high amino acid identity with an uncharacterized locus in *Vibrio vulnificus*. A putative mucin-desulfating sulfatase (AHA0617) may increase the susceptibility of sulfated mucin, a structural component of the protective mucus layer at the surfaces of the gastrointestinal and respiratory tracts, to degradation (106).

It appears that *A. hydrophila* is well equipped to counter an onslaught of antibacterial factors in its environment: β -lactamases, chloramphenicol acetyltransferases, and other proteins that may confer resistance to bicyclomycin, fosmidomycin, and aminoglycosides are evident (Table 2). A peptide intake transport system (SapABCDEF; AHA1872 to -1876) may play a role in resistance to antimicrobial peptides, as in *S. enterica* serovar Typhimurium (77). Drug efflux transporters in the genome (see below) may confer further resistance to other classes of antibiotics and toxins. The presence of these factors corroborates reports of various antibiotic resistances in *Aeromonas* spp. (33, 58, 102, 103). In one particularly disturbing report, a plasmid-borne extended-spectrum β -lactamase and three chromosomal β -lactamases were isolated from a patient with necrotizing fasciitis (27). AHA2368 and -3370 encode putative phenazine (antibiotic) biosynthesis proteins, and products of AHA2416, -2375, and -1645 contain domains found in antibiotic biosynthesis monooxygenases.

Acquisition of iron by *A. hydrophila* may be an all-important function in its aquatic environment, as well as in the iron-limited environment of the host. *Aeromonas* spp. are known to produce two 2,3-dihydroxybenzoic acid-containing siderophores (high-affinity iron-chelating molecules): amonabactin and enterobactin (5, 69). A role in virulence has been proposed for amonabactin, but not for enterobactin (69). A large cluster of siderophore synthesis genes (AHA2473 to -2479) includes the characterized amonabactin synthesis gene (AHA2479) (4), and the remainder may be involved in enterobactin or other siderophore synthesis. AHA3282 and -3281 are similar to genes for siderophore (pyoverdine) synthesis, *pvcAB*, in the opportunistic human pathogen *P. aeruginosa* (95). AHA2473 encodes a putative siderophore receptor, and AHA1964 to -1970 may be involved in siderophore uptake as well.

Secretion of proteins across the inner membrane of the bacterium is achieved by both Sec and Tat (twin arginine translocation; Sec-independent) systems. Target sequences for

TABLE 2. Putative determinants of virulence

Virulence function	Locus	Common name	Comments or reference
Adhesion	AHA3868 to -3871	Tap pilus	78
	AHA1450 to -1459	Pilus biosynthesis	Similar to <i>V. vulnificus</i>
	AHA0686 to -0696	Pilus biosynthesis	
	AHA0518 to -0526	Type IV fimbria	
	AHA3190 to -3194	Type IV fimbria	Similar to <i>P. aeruginosa</i> cluster
	AHA0383 to -0399	Type-IV pilus	Related to mannose sensitive hemagglutinin (MSHA) of <i>V. cholerae</i>
	AHA0062	Fimbrillin	Similar to <i>E. coli</i> fimbrillin
	AHA3491	Adhesin	Type I secretion signal
	AHA2697	Adhesin	Type I secretion signal
Toxin	AHA1359	RTX toxin	Type I secretion signal
			High % amino acid identity to <i>V. vulnificus</i>
	AHA0804	Cytotoxic enterotoxin; Ast	Heat-stable enterotoxin; 88
	AHA0438	Cytolytic enterotoxin; Act	18, 43
	AHA3471	Metalloprotease	May cleave host antibacterial proteins; similar to <i>V. cholerae</i> immune inhibitor A
	AHA2712 to -2713	Extracellular protease; EprA1	15, 111
	AHA1512	Extracellular hemolysin	41
	AHA0229	Hemolysin	Similar to <i>V. vulnificus</i>
	AHA3493	Hemolysin	Similar to <i>V. vulnificus</i>
	AHA0104	Phospholipase A1	72
	AHA1474	Hyaluronidase	Degradation of host tissue polysaccharides
	AHA1354, 1356, 04192	Toxin transporter	
Iron acquisition	AHA2473 to -2479	Siderophore synthesis	Nonribosomal peptide synthetases
	AHA3281 to -3282	Siderophore synthesis	Similar to <i>P. aeruginosa</i> pyoverdinin synthesis
	AHA1530	Ferric uptake regulator; Fur	90
Antibiotic resistance	AHA0740	β -lactamase; CphA	70
	AHA3135	β -lactamase; CepS	105
	AHA4258	β -lactamase; AmpS	105
	AHA0037, AHA3656	Chloramphenicol acetyltransferases	
	AHA2550	Bicyclomycin resistance	MFS transporter
	AHA0056	Fosmidomycin resistance	MFS transporter
	AHA2734	Aminoglycoside acetyltransferase	Aminoglycoside resistance
	AHA0946	Aminoglycoside phosphotransferase	Aminoglycoside resistance
	AHA1872 to -1876	SapABCDF peptide intake transport	Resistance to antimicrobial peptides
Other	AHA1066	Invasin	Immunoglobulin-like fold domain
	AHA0821	Enolase	87
	AHA3186	DNA adenine methyltransferase; Dam	22
	AHA4273	Glucose-inhibited division protein; GidA	Regulator of cytotoxic enterotoxin; 89
	AHA0557	Autoinducer synthase; Ahyl	Quorum sensing; 98
	AHA0556	Quorum sensing regulon activator; AhvR	Quorum sensing; 98
	AHA0700	Ribosylhomocysteine lyase	Autoinducer 2 synthesis
	AHA0517	Collagenase	
	AHA0617	Mucin-desulfating sulfatase	May facilitate mucin degradation

the Tat system, which is used largely to convey redox-active proteins (with bound cofactor) across the inner membrane, are found on 15 CDSs. A Sec-independent type I secretion system may transport proteins across both the inner and outer membranes together in one step. Some virulence factors (adhesins) mentioned above have been identified as type I secretion targets. Secretion of periplasmic proteins (conveyed by Sec or Tat) across the outer membrane is accomplished by the type II secretion system (AHA0568 to -0579). As mentioned above, there is no evidence of a T3SS in *A. hydrophila* ATCC 7966^T.

Proportional to its genome size, numerous components of the TCS a (response regulator and histidine kinase [HK]) have been identified in the *A. hydrophila* genome, which is essential for an organism that may need to respond rapidly to many environmental fluctuations. Typically, HK components of the

TCS possess at least two transmembrane regions and function as periplasmic membrane receptors that detect extracellular or environmental signals. However, some of the *A. hydrophila* TCS (7 out of 31 CDSs) are predicted to be located within the cytoplasm (soluble), owing to the lack of transmembrane segments in the corresponding HK, and consequently may respond to intracellular stimuli. Similarly, out of 43 putative MCPs, 5 may be localized intracellularly. These soluble HKs and MCPs may monitor redox potential or levels of oxygen, nitrate, and other metabolites within the cell (84, 85, 94, 99). For example, the soluble TCS encoded by AHA0274 and -0275 may regulate the activity of nitrogen-assimilatory genes in response to nitrogen limitations. While intracellular signal transduction proteins are included in most bacterial genomes, the predominance of extracellular transducers over those in *A.*

hydrophila indicates an organism concerned primarily with sensing the external environment (or an "extrovert") (30). In the context of virulence, one may speculate that one or more of these extracellular TCS are more likely to play a role in *Aeromonas* pathogenesis by sensing and responding to signals originating in the host environment.

Metabolism. *A. hydrophila* ATCC 7966^T has a comprehensive repertoire of biosynthetic abilities. The tricarboxylic acid cycle is complete and is complemented by the glyoxylate shunt, by which means tricarboxylic acid cycle intermediates can be replenished from acetate for use in various biosynthetic reactions. The Entner-Doudoroff and glycolytic pathways are intact, while the pentose phosphate pathway appears to be missing the oxidative branch. Complete multistep pathways for synthesizing all amino acids are predicted, as are biosynthetic pathways for numerous cofactors: biotin, glutathione, ubiquinone and menaquinone, pantothenate, thiamine, riboflavin, heme, molybdopterin, iron-sulfur clusters, coenzyme A, and tetrahydrofolate. However *A. hydrophila* may import rather than synthesize vitamin B₁₂.

A. hydrophila possesses the oxygen-sensitive IscSUA-HscBA-Fdx system for the biosynthesis of iron-sulfur clusters rather than the oxygen-resistant SUF system, typically associated with aerobic and facultatively anaerobic organisms. Since aeromonads are described as being facultatively anaerobic, the lack of the SUF system is surprising and suggests that reduced oxygen (such as might be available within a biofilm or a microhabitat) promotes growth. The *cbb*₃-type cytochrome oxidase (and helper protein) system uses oxygen as the terminal electron acceptor and is characterized by high affinity for O₂, as is needed in microaerobic environments.

Under anaerobic growth conditions, two types of hydrogenase may perform hydrogen oxidation in *A. hydrophila*. An NiFe uptake hydrogenase (AHA2521 to -2526) may transfer electrons to appropriate acceptors, and immediately downstream is a putative nickel transporter, followed by various hydrogenase maturation/accessory proteins (AHA2510 to -2515). An adjoining formate hydrogenlyase (AHA2495 to -2506), comprised of a formate dehydrogenase (Fdh; AHA2495; a selenoprotein) and an NiFe hydrogenase 3 (AHA2499 to -2504), may release H₂ and CO₂ from formate (produced from pyruvate during anaerobic growth).

Nitrate is converted to nitrite and then to ammonia in the general assimilatory pathway (for biosynthetic processes), which may function aerobically or anaerobically with the aid of assimilatory nitrate (AHA3414 and AHA4118 to -4119) and nitrite (AHA3407 to -3408) reductases and a putative nitrate/nitrite transporter (AHA3409 to -3411). Nitrate may also serve as an electron acceptor for anaerobic respiration. A periplasmic nitrate reductase, Nap (AHA1584 to -1590), may be either dissimilatory (i.e., it dissipates excess reductive equivalents without the generation of a proton motive force) or indirectly respiratory by virtue of the consumption of electrons derived from NADH via the proton-translocating NADH dehydrogenase. Dissimilatory nitrate reduction activity in anaerobic river sediments has been attributed to *Aeromonas* spp. (55). An ammonia-forming formate-dependent nitrite reductase (AHA2464 to -2470) is also seen. A dissimilatory pathway for the production of molecular nitrogen (denitrification) is not apparent.

Sulfate assimilation is accomplished by reduction of sulfate to sulfide by means of the cysteine biosynthetic pathway in many gammaproteobacteria. This involves the participation of a sulfate adenylyltransferase (AHA3566 and -3567), adenylyl-sulfate kinase (AHA3564), and phosphoadenosine phosphosulfate reductase (AHA3373), which converts sulfate to sulfite. The sulfite product from the assimilation pathway is reduced by the biosynthetic sulfite reductase (AHA3371 and -3372) to sulfide for incorporation into an amino acid, peptide, protein, etc.

Additionally, *A. hydrophila* possesses a dissimilatory anaerobic sulfite reductase (AHA2575 to -2577) involved in dissimilation of oxidized anions for energy transduction. This enzyme catalyzes hydrogen sulfide production from sulfite, which is regulated by electron acceptors from hydrogen or an organic substrate, and serves as an important energy-conserving step. It also confers the ability to synthesize cysteine anaerobically. This *A. hydrophila* dissimilatory sulfite reductase is most similar to one characterized in *S. enterica* serovar Typhimurium (44) (which, however, is absent in most other gammaproteobacteria) and some clostridia. This dissimilatory sulfate reduction activity, while rare outside the sulfate-reducing bacteria, was previously reported for *Aeromonas* spp. isolated from industrial cooling water systems and implicated in microbially influenced corrosion (71). Other electron acceptor sources utilized by *A. hydrophila* (which may be deduced from the genome sequence) include tetrathionate, fumarate, and trimethyl-N-oxide.

A selenocysteine incorporation system comprised of *selABC* is present; however, only a single selenoprotein, the alpha subunit (AHA2495) of formate dehydrogenase, is predicted. This large investment strongly suggests a substantial role of formate catabolism in *Aeromonas* biology. Distinct from the selenoprotein Fdh, a putative nitrate-induced formate dehydrogenase N (AHA3061 to -3063) may oxidize formate in the periplasm (AHA3063 bears a Tat signal for periplasmic localization), transferring electrons to nitrate reductase, which can transfer electrons to nitrate in the cytoplasm during anaerobic respiration.

A. hydrophila has a polyhydroxyalkanoic acid storage granule system (AHA2421, -2274, -2269, and -1951) for nitrogen limitation-induced storage, similar to that in *V. cholerae*, and a glycogen system (AHA3614 to -3617 and AHA3804) for carbohydrate storage and mobilization. The organism does not appear to make any of the common compatible solutes (ectoine, glycine betaine, glucosylglycerol, trehalose, and mannosylglycerate) that serve as both osmoprotectants and storage molecules, although it may be able to utilize exogenous trehalose and betaine. It thus seems capable of storing carbon but not nitrogen.

Chitin is a major component of insect and crustacean exoskeletons and fungal cell walls and is abundant in the aquatic environment. Chitinolytic activity has been reported for *Aeromonas* spp. (16), probably for the assimilation of chitin as a carbon and nitrogen source. In addition to the characterized extracellular chitinase Chi192 (AHA0977) (16) and chitinase (AHA1528) (60), many other chitin-degrading enzymes are predicted in the genome of *A. hydrophila* ATCC 7966^T: AHA0979, AHA3100, AHA3098, AHA2363, and AHA3440, the last two of which possess signal peptides. A putative chitin-

FIG. 3. Distribution of transporter groups in *Aeromonas hydrophila* ATCC 7966^T.

binding protein (AHA0610) and chito-oligosaccharide ABC transporter (AHA3093 to -3097) have also been noted.

Transport. An abundance of transporters are documented in terms of the number of distinct families, as well as paralogs within the same family (Fig. 3). The transporter profile of *A. hydrophila* is comparable to those of pseudomonads and vibrios, with an abundance of amino acid and peptide transporters and relatively few sugar uptake systems. An exception is the expansion of a family of DcuC transporters (five CDSs) for the import of C₄ dicarboxylates. In *E. coli*, DcuC is induced only under anaerobic conditions and may function as a succinate efflux system during anaerobic glucose fermentation (112). Other transporters may aid in the efflux of heavy metals or toxic compounds encountered in potentially highly polluted waters. Numerous multidrug efflux and drug/metabolite transporters predominate within the drug/metabolite transport; major facilitator superfamily; and resistance, nodulation, and cell division efflux transporter categories. Members of the cation diffusion facilitator superfamily may also carry out efflux of heavy metals. AHA3929 to -3932 encode an ABC efflux transporter possibly conferring toluene tolerance, while AHA1356, -1354, and -2701 may play a role in toxin secretion. AHA0629, AHA4222, and AHA2300 are putative heavy-metal-translocating P-type ATPases, primarily responsible for translocating cadmium ions (or other closely related divalent heavy metals, such as cobalt, mercury, lead, and zinc) across biological membranes. AHA1722 to -1727 comprise a four-gene arsenical resistance (*ars*) operon that may pump arsenite or antimonite out of the cell in response to the proton motive force; it includes an arsenate reductase that converts arsenate to arsenite. AHA1678 is an additional arsenate reductase. Arsenical salts are often used as antibiotics, feed supplements, and her-

bicides and routinely find their way into watersheds and other reservoirs.

Other functions. *A. hydrophila* survives well in waters polluted by feces and other substances and is reportedly resistant to various disinfectants, insecticides, and chemicals. The purported abilities of various *Aeromonas* spp. include heavy-metal reduction, oil biodegradation (by producing surfactant to emulsify hydrocarbons, thereby facilitating degradation by a microbial consortium) (47), pesticide removal (63), and selenite reduction (45, 83). In addition to the efflux transporters mentioned above, other CDSs in the genome may encode enzymes for the alteration of toxic compounds and facilitate the environmental stability of *A. hydrophila*. A xenobiotic reductase (AHA0735), similar to flavoprotein nitroreductases from *Pseudomonas fluorescens* (7), may reduce xenobiotic compounds (e.g., glycerol trinitrate) found in agricultural chemicals, pharmaceuticals, dyes, and plastics. A putative nitroalkane dioxygenase (AHA0751) may catalyze the denitrification of nitroalkanes, while a nitroreductase (AHA1801) may be involved in reduction of nitroaromatic compounds. Thiopurine S-methyltransferase (AHA1996) may be involved in the biological cycling of selenium, an activity ascribed to *A. veronii* (83). Dimethyl sulfoxide reductase (AHA4049) may reduce various N-oxide and sulfoxide compounds, including trimethylamine N-oxide during anaerobic growth. Degradation of phosphonates is possible via 2-aminoethylphosphonate-pyruvate transaminase (AHA1938), which interconverts 2-aminoethylphosphonate to 2-phosphonoacetaldehyde, and phosphonoacetaldehyde hydrolase (AHA1940), which yields acetaldehyde plus inorganic phosphate. AHA4213 to -4215 encode a putative phosphonate uptake ABC transporter. AHA0785 to -0786 may confer tolerance of copper or other

heavy metals (26). AHA1327 to -1336 may be involved in the formation of a specific microcompartment in the cell (56), composed entirely of protein subunits, in which the metabolism of potentially toxic by-products (e.g., ethanolamine or propoanediol utilization) can take place.

Secretion of acetoin has been suggested as a mechanism for maintaining pH homeostasis (100). AHA1126 encodes a catabolic acetolactate synthase that yields acetolactate from pyruvate, while an adjacent alpha-acetolactate decarboxylase (AHA1125) may convert acetolactate into acetoin. AHA1733 and AHA3571 may be involved in acetoin catabolism as well, but an acetoin reductase (for further reduction to 2,3-butanediol) is absent.

DNA damage is likely sustained repeatedly in the aquatic (or host) environment; thus, the genome includes sets of DNA repair enzymes that are typical for the gammaproteobacteria (e.g., RecBCD and RuvABC). However, this set does not include the Ku/LigA pair for nonhomologous end joining. Stress response and detoxification are similarly important functions in the aquatic and host environments: superoxide dismutases (Mn and Fe), catalase, alkylhydroperoxide reductase, thiol peroxidase, arsenate reductase, glutathione reductase, and glutathione peroxidases are present, among others. Mn superoxide dismutase was previously reported to protect against environmental peroxide as opposed to intracellular (host-produced) peroxide (62); however, some of the other factors may serve as well within the eukaryotic host environment. For example, nitric oxide reductases (AHA0119 to -0120) may be involved in detoxification of nitric oxide that may be produced abiotically or biotically (by the host).

In summary, the 4.7-Mb genome sequence of *A. hydrophila* ATCC 7966^T reveals an extremely versatile bacterium endowed with the potential to instigate different pathogenic processes and to readily persist in the aquatic environment. Further sequencing of additional strains of *A. hydrophila*, as well as members of other species within the genus, will undoubtedly provide additional knowledge and verification of these and other abilities of this multitasking group of microorganisms. Finally, *Aeromonas* spp. represent a distinct lineage within the gammaproteobacteria, and the genome sequence serves to expand the breadth of coverage for this group.

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