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Two Redundant Sodium-Driven Stator Motor Proteins Are Involved in *Aeromonas hydrophila* Polar Flagellum Rotation▽

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Motility is an essential characteristic for mesophilic *Aeromonas* strains. We identified a new polar flagellum region (region 6) in the *A. hydrophila* AH-3 (serotype O34) chromosome that contained two additional polar stator genes, named *pomA*$_2$ and *pomB*$_2$. *A. hydrophila* PomA$_2$ and PomB$_2$ are highly homologous to other sodium-conducting polar flagellum stator motors as well as to the previously described *A. hydrophila* AH-3 PomA and PomB. *pomAB* and *pomA*$_2$*B*$_2$ were present in all the mesophilic *Aeromonas* strains tested and were independent of the strains' ability to produce lateral flagella. Unlike MotX, which is a stator protein that is essential for polar flagellum rotation, here we demonstrate that PomAB and PomA$_2$B$_2$ are redundant sets of proteins, as neither set on its own is essential for polar flagellum motility in either aqueous or high-viscosity environments. Both PomAB and PomA$_2$B$_2$ are sodium-coupled stator complexes, although PomA$_2$B$_2$ is more sensitive to low concentrations of sodium than PomAB. Furthermore, the level of transcription in aqueous and high-viscosity environments of *pomA*$_2$*B*$_2$ is reduced compared to that of *pomAB*. The *A. hydrophila* AH-3 polar flagellum is the first case described in which two redundant sodium-driven stator motor proteins (PomAB and PomA$_2$B$_2$) are found.

The bacterial flagellum usually is powered by a reversible rotator motor at the base of the flagellum structure, which uses energy from either the proton or sodium ion gradient to drive the rotation of the flagellum filament. The flagellum motor is divided into two substructures: the stator and the rotor. The rotor is composed of the FliM and FliN proteins, which form the C ring structure, and the FliG protein. These three proteins act as a switch that controls the direction of flagellum rotation, clockwise or counterclockwise. The stator is the stationary component of the motor and consists of different proteins surrounding the rotor, which constitute proton or sodium ion channels that couple the flow of ions to flagellum rotation (8, 10). In the proton-driven motor of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, the stator is composed of two integral membrane proteins, MotA and MotB (9, 31, 45). MotA has four transmembrane domains, whereas MotB has one transmembrane domain as well as a peptidoglycan-binding motif at its C terminus (17, 51). These two proteins form a complex, in a ratio of four MotA to two MotB, that conducts protons across the inner membrane (29). However, the stator of the *Vibrio parahaemolyticus* proton-driven lateral flagellum motor (MotA$_L$ [LafT] and MotB$_L$ [LafU]) requires an additional protein, which has a peptidoglycan-binding domain, for motor function called MotY (LafY) (44). Torque generation in the proton-driven motors is obtained through the electrostatic interaction between conserved charges on the cytoplasmic domains of the FliG and MotA proteins (28).

In the sodium-driven motor of alkaliphilic *Bacillus* species, the stators require two proteins, MotsP and MotS (24), whereas the polar flagellum stators of *Vibrio* species, such as *V. alginolyticus* and *V. parahaemolyticus*, require four proteins: PomA, PomB, MotX, and MotY (3, 34, 48). MotP/PomA and MotS/PomB proteins are homologous to the proton-driven MotA and MotB, respectively. MotX and MotY do not have paralogous proteins in *E. coli* and are components of the T ring (46), which is located beneath the P ring of the polar flagellum basal body in *Vibrio* species. In *V. alginolyticus*, MotX and MotY are required for the assembly of the PomAB complex in the polar flagellum motor (30). MotY has an N-terminal region that is essential for the association of the stator unit around the rotor (30) and, like MotB and PomB, has a peptidoglycan-binding motif in its C-terminal region (33). It is believed that torque generation in sodium-driven motors is obtained in a manner similar to that of proton-driven motors (4, 21), although charged residues that are critical for flagellum rotation appear to be different or additional charged residues may be required (19, 49).

Mesophilic *Aeromonas* species are ubiquitous waterborne bacteria and pathogens of reptiles, amphibians, and fish (6). In humans, *Aeromonas hydrophila*, belonging to hybridization groups 1 and 3 (HG1 and HG3), *A. veronii* biovar *sobria* (HG8/HG10), and *A. caviae* (HG4) have been associated with gastrointestinal and extraintestinal diseases such as wound infections of healthy humans and, less commonly, with septicemias of immunocompromised patients (25). The swimming motility of all mesophilic aeromonads has been linked to a single polar unsheathed flagellum, expressed constitutively, that is required for the adherence and invasion of human and fish cell lines (13, 36, 40). Certain strains also are able to express many un-

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description[a]	Reference or source
Strains		
A. hydrophila		
AH-3	*A. hydrophila* wild type, serogroup O:34	35
AH-405	AH-3, spontaneously Rif^r	1
AH-4442	AH-405, *flhA*::Km^r	13
AH-4444	AH-405, *pomB*::Km^r	13
AH-4448	In-frame AH-405Δ*pomAB*	This work
AH-4449	AH-405Δ*pomAB*, *pomB*$_2$::miniTn5Km-1, Km^r	This work
AH-4450	AH-405Δ*pomAB*, Δ*lafTU*::Km^r	This work
AH-4452	AH-405Δ*pomAB*, *pomB*$_2$::Cm^r	This work
AH-4461	AH-405, *motX*::Km^r	13
AH-4470	AH-405, *pomB*$_2$::Cm^r	This work
AH-4471	AH-405Δ*pomA*$_2$*B*$_2$	This work
AH-4472	AH-405, *pomB*::Km^r, *pomB*$_2$::Cm^r	This work
AH-4473	AH-405Δ*pomAB*, Δ*pomA*$_2$*B*$_2$	This work
AH-5503	AH-405, *lafK*::Km^r	14
AH-5510	AH-405Δ*lafTU*::Km^r	This work
AH-5511	AH-405, *lafK*::Km^r, Δ*pomAB*	This work
AH-5512	AH-405, *lafK*::Km^r, Δ *pomA*$_2$*B*$_2$	This work
E. coli		
DH5α	F$^-$ *endA hdsR17*(r_K^- m_K^+) *supE44 thi-1 recA1 gyr-A96* ϕ80*lacZ*	22
XL1-Blue	*endA1 recA1 hsdR17 supE44 thi-1 gyrA96 relA1 lac* (F′ *proAB lacIZ*ΔM15 Tn*10*)	Stratagene
S17-1(λ*pir*)	*thi thr1 leu tonA lacY supE recA*::*RP4-2* (*Tc*::*Mu*) *Km*^{r} λ*pir* with miniTn5Km1	16
MC1061(λ*pir*)	*thi thr1 leu6 proA2 his4 argE2 lacY1 galK2 ara14 xyl5 supE44* λ*pir*	41
Plasmids		
pGEMT	Cloning vector, Ap^r	Promega
pBCSK	Cloning vector with *lacZ* gene, Cm^r	Stratagene
pRK2073	Helper plasmid, Sp^r	41
pCM100	pGP704 suicide plasmid, *pir* dependent, Cm^r	50
pUC4-KIXX	Plasmid carrying 1.6-kb *aph* cassette, Ap^r, Km^r	Pharmacia
pDM4	Suicide plasmid, *pir* dependent with *sacAB* genes, oriR6K, Cm^r	37
pACYC184	Plasmid vector, Cm^r, Tc^r	42
pACYC-POMAB	pACYC184 with *pomAB* genes, Tc^r	This work
pACYC-POMA$_2$B$_2$	pACYC184 with *pomA*$_2$*B*$_2$ genes, Tc^r	This work
pCM-POMB$_2$	pCM100 with a internal fragment of AH-3 *pomB*$_2$ gene, Cm^r	This work
pDM-LAFTU	pDM4 with AH-3 *lafTU*::Km, Cm^r, Km^r	This work
pDM-POMA$_2$B$_2$	pDM4 with AH-3Δ *pomA*$_2$*B*$_2$, Cm^r	This work
pDM-POMAB	pDM4 with AH-3Δ*pomAB*, Cm^r	This work

[a] Tc^r, tetracycline resistant; Km^r, kanamycin resistant; Ap^r, ampicillin resistant; Rif^r, rifampin resistant; Cm^r, chloramphenicol resistant; Sp^r, spectinomycin resistant.

sheathed peritrichous lateral flagellum when grown in viscous environments or on surfaces that increase bacterial adherence and are required for swarming motility and biofilm formation (14, 20). The expression of two distinct flagellum systems is relatively uncommon, although it has been observed in *V. parahaemolyticus* (32), *Azospirillum brasilense* (38), *Rhodospirillum centenum* (26), and *Plesiomonas shigelloides* (23).

Recently, we reported the genes involved in *A. hydrophila* lateral and polar flagellum formation, and surprisingly we found that PomB mutations do not affect swimming (13, 14), in contrast to the similar mutants described for *V. cholerae*, *V. alginolyticus*, and *V. parahaemolyticus* (11, 21). In this work, we describe a new *A. hydrophila* AH-3 *pomAB*-like locus (*pomCD*) and its implication in polar flagellum motility. Furthermore, we investigated the distribution of these genes among mesophilic *Aeromonas* species.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were grown on Luria-Bertani (LB) Miller broth and LB Miller agar at 37°C, and *Aeromonas* strains were grown in tryptic soy broth (TSB) or agar (TSA) at 30°C. When required, ampicillin (50 μg/ml), kanamycin (50 μg/ml), chloramphenicol (25 μg/ml), rifampin (rifampicin; 100 μg/ml), spectinomycin (50 μg/ml), and tetracycline (20 μg/ml) were added to the different media.

Motility assays (swarming and swimming). Freshly grown bacterial colonies were transferred with a sterile toothpick into the center of swarm agar (1% tryptone, 0.5% NaCl, 0.5% agar) or swim agar (1% tryptone, 0.5% NaCl, 0.25% agar). The plates were incubated face up for 24 to 48 h at 25°C, and motility was assessed by examining the migration of bacteria through the agar from the center toward the periphery of the plate. Moreover, swimming motility was assessed by light microscopy observations in liquid media. When required, the specific inhibitor for the sodium-driven flagellar motors, amiloride (Sigma), was added at 0.5 to 2 mM to the motility assay media, or motility assay media with different sodium chloride concentrations (0 to 100 mM) were used.

TEM. For transmission electron microscopy (TEM), bacterial suspensions were placed on Formvar-coated grids and negatively stained with a 2% solution of uranyl acetate, pH 4.1. Preparations were observed on a Hitachi 600 transmission electron microscope.

MiniTn5Km-1 mutagenesis. The conjugal transfer of transposition element miniTn5Km-1 from *E. coli* S17-1λ*pir*Km-1 (16) to *A. hydrophila* AH-4448 (AH405Δ*pomAB*) was carried out in a conjugal drop incubated for 6 h at 30°C with the ratio 1:5:1, corresponding to *E. coli* S17-1λ*pir*Km-1, *A. hydrophila* AH-4448, and *A. hydrophila* HB101/pRK2073 (helper plasmid), respectively. Serial dilutions of the mating mix were plated on TSA supplemented with rifampin and kanamycin in order to select mutants.

DNA techniques. DNA manipulations were carried out essentially as previously described (42). DNA restriction endonucleases, T4 DNA ligase, *E. coli*

TABLE 2. Primers used in the construction of chromosomal in-frame *pomAB* and $pomA_2B_2$2 deletion mutants

Primer	Sequence[a]	Amplified fragment
pomAB mutant		
A primer	5′-CGCGGATCCACAGCGGGTCAAGGAAATA-3′	AB fragment
B primer	5′-CCCATCCACTAAAC TTAAACACAGGATCAGGCCAAACAT-3′	
C primer	5′-TGTTTAAGTTTAGTGGATGGGGTGCAACAGGCGGTAGAG-3′	CD fragment
D primer	5′-CGCGGATCCACGCTTGTCAAACATGGTG-3′	
$pomA_2B_2$2 mutant		
$POMA_2$-F	5′-CGCGGATCCATGGTTTCCAGCTCTTCCA-3′	$pomA_2B_2$2 fragment
$POMB_2$-R	5′-CGCGGATCCTGGTGACATCGATCACCTG-3′	

[a] Underlined letters show overlapping regions; double-underlined letters show BamHI restriction site.

DNA polymerase Klenow fragment, and alkaline phosphatase were used as recommended by the suppliers. PCR was performed using *Taq* DNA polymerase (Invitrogen) in a gene amplifier PCR system 2400 thermal cycler (Perkin Elmer). Colony hybridizations were carried out by colony transfer onto positive nylon membranes (Roche) and then lysed according to the manufacturer's instructions. Probe labeling with digoxigenin, hybridization, and detection (GE Healthcare) were carried out as recommended by the suppliers.

RT-PCR. For reverse transcription-PCR (RT-PCR), total RNA was isolated from *A. hydrophila* AH-3 grown at 25°C in liquid media (TSB) by RNA Protect bacterial reagent (Qiagen) and an RNeasy mini kit (Qiagen). To ensure that RNA was devoid of contaminating DNA, the preparation was treated with RNase-free TurboDNase I (Ambion). First-strand cDNA synthesis was carried out using the Thermoscript RT-PCR system (Invitrogen) and random primers on 3 μg of total DNase-digested RNA according to the manufacturer's instructions. PCR without reverse transcriptase was performed to confirm the absence of contaminating DNA in the RNA sample. Semiquantitative PCR is an image estimation of the size of the amplified bands before they reach the plateau. For semiquantitative PCR, second-strand synthesis and subsequent DNA amplification were carried out using Accuprime TaqDNA polymerase (Invitrogene) and one pair of oligonucleotides, 5′-ATCCAGGCCATGTTCCATC-3′ and 5′-CAACCGCCGTTCAACCTG-3′, to amplify the *pomA* DNA fragment of 227 bp, and another pair of oligonucleotides, 5′-CGCGGATCCGTATGATAA-3′ and 5′-CAAGAGCGAAGACAAGCTG-3′, to amplify the $pomB_2$ DNA fragment of 170 bp; the oligonucleotides were designed using the Prime program from the Genetics Computer Group package (Madison, WI). To analyze the amount of cDNA template, 15-μl aliquots were removed for each PCR sample every five cycles, starting at cycle 15 and ending at cycle 35. Amplicons at each time point were analyzed by agarose gel electrophoresis with ethidium bromide staining. *A. hydrophila* ribosomal 16S primers were used as a control for the amount of cDNA template.

Cloning of DNA flanking miniTn5Km-1 insertions. Chromosomal DNA of miniTn5Km-1 mutants was digested with EcoRI, PstI, and EcoRV, purified, ligated into the vector pBCSK (Stratagene), and introduced into *E. coli* XL1-Blue. Recombinant plasmids containing the transposon with flanking insertions were selected on LB plates supplemented with kanamycin and chloramphenicol. The miniTn5Km-1 flanking sequences were obtained by using specific primers to the I and O end of miniTn5Km-1 (5′-AGATCTGATCAAGAGACAG-3′ and 5′-ACTTGTGTATAAGAGTCAG-3′, respectively) as well as M13for and T3 primers.

Nucleotide sequencing and computer sequence analysis. Plasmid DNA for sequencing was isolated by a Qiagen plasmid purification kit (Qiagen, Inc., Ltd.) as recommended by the supplier. In some cases, inverse PCR was used to amplify a chromosomal DNA fragment for sequencing, as described previously (14). Double-stranded DNA sequencing was performed by using the Sanger dideoxy chain termination method (43) with an ABI Prism dye terminator cycle-sequencing kit (Perkin-Elmer). Custom-designed primers used for DNA sequencing were purchased from Isogen Life Sciences.

The DNA sequence was translated in all six frames, and their deduced amino acid sequences were inspected in the GenBank, EMBL, and SwissProt databases by using the BLASTX, BLASTP, or PSI-BLAST network service at the NCBI (2). The protein family profiling was performed using the protein family database Pfam at the Sanger Center (7). The determination of possible terminator sequences was done by using the Terminator program from the Genetics Com-

TABLE 3. Motility phenotypes of *A. hydrophila* AH-3 in-frame and defined insertion mutants and in *trans*-complemented mutants

Strain and type of mutant	Gene defect interval or plasmid used	Motility		Flagellation type[c]	
		Swarming[a]	Swimming[b]	Lateral	Polar
In frame and defined insertion					
AH-3	Wild type	3.7 ± 0.2	+	+	+
AH-5503	*lafK*	0.6 ± 0.1	+	−	+
AH-5510	*lafTU*	0.6 ± 0.1	+	+	+
AH-4444	*pomB*	3.4 ± 0.1	+	+	+
AH-4448	*pomAB*	3.5 ± 0.2	+	+	+
AH-4470	$pomB_2$	3.4 ± 0.3	+	+	+
AH-4471	$pomA_2B_2$2	3.4 ± 0.1	+	+	+
AH-4472	*pomB-*B_2	1.1 ± 0.1	−	+	+
AH-4473	*pomAB-*$A_2B_2$2	1.2 ± 0.2	−	+	+
AH-4452	*pomAB-*B_2	1.1 ± 0.1	−	+	+
AH-4449					
AH-4450	*lafTU-pomAB*	0.5 ± 0.1	+	+	+
AH-5511	*lafK-pomAB*	0.6 ± 0.1	+	−	+
AH-5512	*lafK-* $pomA_2B_2$2	0.5 ± 0.1	+	−	+
In *trans* complemented					
AH-4472	pACYC-POMAB	3.3 ± 0.2	+	+	+
	pACYC-$POMA_2B_2$	3.4 ± 0.2			
AH-4473	pACYC-POMAB	3.2 ± 0.1	+	+	+
	pACYC- $POMA_2B_2$	3.4 ± 0.2			
AH-4452	pACYC-POMAB	3.2 ± 0.2	+	+	+
	pACYC- $POMA_2B_2$	3.1 ± 0.1			

[a] Migration (in centimeters) of bacteria through the swarm agar plates from the center toward the periphery of the plate.
[b] −, no motility in liquid media; +, motility in liquid media.
[c] −, absence of flagella; +, flagella equivalent to those of the wild type.

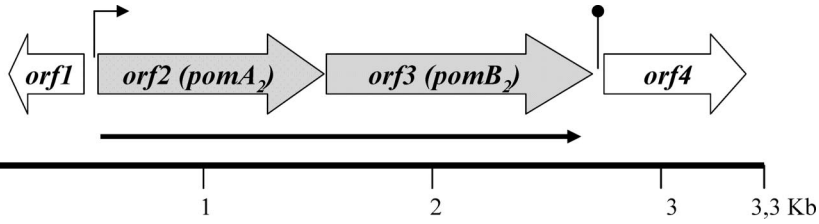


FIG. 1. Genetic organization of *A. hydrophila* AH-3 polar flagellum region 6. ORFs and their transcriptional directions are indicated by block arrows. The black arrow indicates the transcriptional unit. Small horizontal arrows indicate the location of putative promoter sequences. Lollipop structures depict the approximate position of the putative transcriptional rho-independent terminators.

puter Group package (Madison, WI). Other online sequence analysis services also were used.

Construction of defined mutants. The chromosomal in-frame *pomAB* strain and $pomA_2B_2$ deletion mutant, *A. hydrophila* AH-4448 and AH-4471, respectively, were constructed by allelic exchange as described by Milton et al. (37). Briefly, DNA regions upstream (fragment AB) and downstream (fragment CD) of *pomAB* were amplified using two sets of asymmetric PCRs. DNA fragment AB contains 750 bp upstream of *pomA* and the first eight codons of *pomA*. DNA fragment CD contains the first base in codon 276 of *pomB* to 577 bp downstream of *pomB*. DNA fragments AB and CD were annealed at the overlapping regions provided by the primers B and C and amplified as a single fragment using primers A and D (Table 2). For the $pomA_2B_2$ mutant construction, a 2,318-bp DNA fragment containing $pomA_2B_2$ was amplified by PCR using the primer pair $POMA_2$-F and $POMB_2$-R (Table 2). The amplified fragment was digested with SacI to make a $pomA_2B_2$ deletion. Two DNA fragments containing 682 bp upstream of $pomA_2$ and 601 bp downstream of $pomB_2$, respectively, were recovered and ligated. The fusion product was amplified as a single fragment of 1,283 bp using $POMA_2$-F and $POMB_2$-R primers. The *pomAB* and $pomA_2B_2$ fusion products were purified and sequenced, BamHI digested (the BamHI site is present in primers), ligated into BglII-digested and phosphatase-treated pDM4 vector (37), electroporated into *E. coli* MC1061, and plated on chloramphenicol plates at 30°C to obtain pDM-POMAB and pDM-$POMA_2B_2$ plasmids, respectively. The introduction of the plasmids into rifampin-resistant (Rif^r) *A. hydrophila* AH-405 was performed as previously described (13). Transconjugants were selected on plates containing chloramphenicol and rifampin. PCR analysis confirmed that the vector had integrated correctly into the chromosomal DNA. After sucrose treatment, transformants that were rifampin resistant (Rif^r) and chloramphenicol sensitive (Cm^s) were chosen and confirmed by PCR.

The defined insertion $pomB_2$ mutants were obtained using a method based on suicide plasmid pCM100 (50). Briefly, an internal fragment of the selected gene was amplified by PCR, ligated into pGEM-Teasy (Promega), and transformed into *E. coli* XL1-Blue. The DNA insert was sequenced, recovered by EcoRI restriction digestion, blunt ended, and ligated into EcoRV-digested and phosphatase-treated pCM100. Recombinant pCM-$POMB_2$ plasmid was transformed into *E. coli* MC1061(λ*pir*) and selected for chloramphenicol resistance (Cm^r). Triparental mating with the mobilizing strain HB101/pRK2073 was used to transfer the recombinant plasmid into the *A. hydrophila* AH-405 rifampin-resistant strain to obtain the defined insertion mutant AH-4470, selecting for Rif^r and Cm^r. The correct construction was verified by Southern blot hybridization.

The defined *A. hydrophila lafTU* mutant AH-5510 was constructed by the PCR amplification of *lafTU* internal gene fragments. The PCR product was sequenced and ligated into the vector pGEMTeasy (Promega) and transformed into *E. coli* XL1-Blue. A recombinant plasmid containing *lafTU* genes was EcoRV digested to make a *lafTU* deletion, and fragment deletion was performed instead of using the SmaI-digested kanamycin-resistant cassette from pUC4-KIXX. The inserted cassette contains an outward-reading promoter that ensures the expression of downstream genes when inserted in the correct orientation (12); however, such an insertion will alter the regulation of such genes. The presence of a single HindIII restriction site in the SmaI-digested cassette allowed its orientation to be determined. The DNA fragments containing *lafTU* with the kanamycin-resistant cassette were recovered and ligated into suicide vector pDM4 (37) to construct the pDM-LAFTU plasmid. The recombinant plasmid was electroporated into *E. coli* MC1061(λ*pir*) and plated on chloramphenicol and kanamycin plates at 30°C. The plasmids with mutated genes were transferred into rifampin-resistant *A. hydrophila* AH-405 by triparental mating using *E. coli* MC1061(λ*pir*) (containing the insertion construct) and the mobilizing strain HB101/pRK2073. Transconjugants were selected on plates containing chloramphenicol, kanamycin, and rifampin. PCR analysis confirmed that the vector had integrated correctly into the chromosomal DNA. To complete the allelic exchange, the integrated suicide plasmid was forced to recombine out of the chromosome by adding 5% sucrose to the agar plates. The pDM4 vector contains *sacB*, which produces an enzyme that converts sucrose into a product that is toxic to gram-negative bacteria. Transconjugants surviving on plates with 5% sucrose that were rifampin resistant, kanamycin resistant, and chloramphenicol sensitive were chosen and confirmed by PCR. The *A. hydrophila pomAB-lafTU* double mutant AH-4450 was obtained by the introduction of the suicide plasmid pDM-LAFTU into the *A. hydrophila pomAB* mutant AH-4448 using the method previously described.

Plasmid construction for complementation studies. Plasmid pACYC-POMAB, containing the complete *pomAB* genes, and the plasmid pACYC-$POMA_2B_2$, containing $pomA_2B_2$ genes from *A. hydrophila* AH-3, were obtained by the PCR amplification of genomic DNA using oligonucleotides 5′-GCATC GCCACTGAGTCAC-3′ and 5′-ATACCGGCTAACGAGACCA-3′, to generate a band of 1,800 bp, and 5′-TGGCCGATAATAAGCCATC-3′ and 5′-AGC TCTTGACGCAGCTTTT-3′, to generate a band of 1,976 bp. The amplified band was sequenced, ligated into pGEM-Teasy (Promega), and transformed into *E. coli* XL1-Blue. The DNA insert was recovered by EcoRI restriction digestion, ligated into EcoRI phosphatase-treated pACYC184 vector (42), and introduced

TABLE 4. Characteristics of the *A. hydrophila* AH-3 polar flagella region 6

ORF no.	Nucleotide position	Protein size (bp)	Molecular weight	pI	Predicted function	Homologous gene	%Identity/ %similarity
1	684–433	83	9.3	4.5	Exonuclease VII	SamaDRAFT_0389 of *Shewanella amazonensis*	64/83
						xseB of *Vibrio fischeri*	59/78
						xseB of *Haemophilus influenzae*	57/72
2	905–1663	252	27.0	5.1	Flagella motor protein	AHA-3318 of *A. hydrophila* $ATCC7966^T$	94/98
						pomA of *Vibrio cholerae*	66/81
						motA of *Vibrio parahaemolyticus*	64/80
3	1669–2562	297	32.5	5.0	Flagella motor protein	AHA-3317 of *A. hydrophila* $ATCC7966^T$	94/96
						motB of *Vibrio parahaemolyticus*	59/74
						pomB of *Vibrio cholerae*	57/72
4	2707–3183	158	17.5	4.7	Transcription elongation factor	*greA* of *Salmonella enterica* serovar Typhi	77/87
						greA of *Yersinia pestis*	75/85

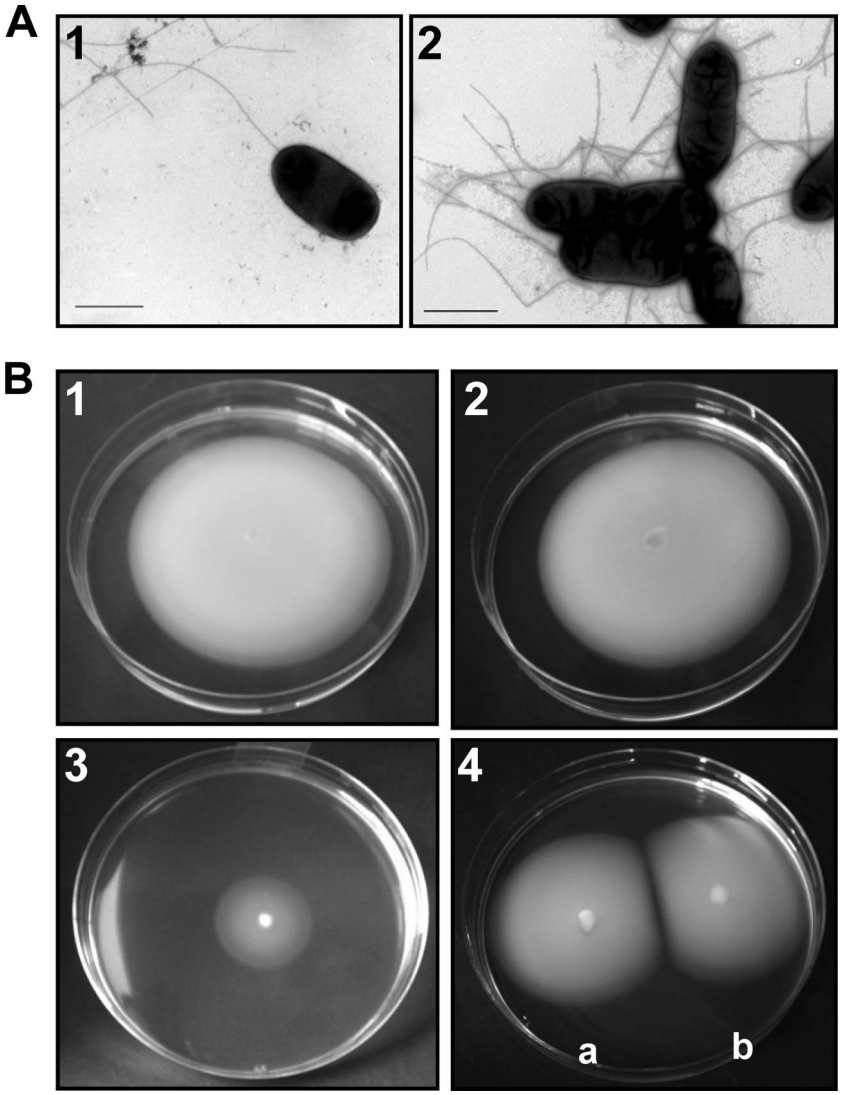


FIG. 2. (A) TEM of an *A. hydrophila* AH-3 defined mutant in $pomB_2$ (AH-4470) grown at 25°C on liquid medium (1) and grown at 25°C on swarm agar (2). Bacteria were gently placed onto Fonvard-coated copper grids and negatively stained using 2% uranyl acetate. Bar, 1 μm. (B) Swarming motility observed for *A. hydrophila* AH-3 (1), $pomB_2$ mutant AH-4470 (2), $pomB$-$pomB_2$ double mutant AH-4472 (3), and double mutant AH-4472 complemented with plasmid pACYC-POMAB (4a) or pACYC-$POMA_2B_2$ (4b) independently. The $pomA_2B_2$ double mutant AH-4471 shows the same TEM result and swarming phenotype as $pomB_2$ mutant AH-4470. The double mutants AH-4473 ($pomAB$-A_2B_2) and AH-4452 ($pomAB$-B_2) show the same swarming phenotype as the AH-4472 mutant ($pomB$-B_2).

into *E. coli* DH5α. Both inserted fragment orientations were determined by restriction analysis and sequencing.

Nucleotide sequence accession number. The nucleotide sequences reported in this paper have been submitted to the GenBank/EMBL database under accession number FJ409648.

RESULTS

LafTU and PomAB function in *A. hydrophila* AH-3 polar flagellum motility. Previous work demonstrated that the *A. hydrophila pomB* defined insertion mutant, AH-4444, is able to swim and swarm as well as wild-type *A. hydrophila* AH-3. In contrast, the *A. hydrophila motX* mutant, AH-4461, is unable to swim but still is able to swarm (13). In this work, we constructed an in-frame *pomAB* mutant, AH-4448, and analyzed its ability to swim and swarm. As previously described for AH-4444 (*pomB*), the *A. hydrophila* AH-4448 (*pomAB*) mutant was able to swarm and swim as well as the wild-type strain (Table 3). In order to explore whether the lateral flagellum stator proteins LafTU were involved in polar flagellum motility, we constructed *A. hydrophila* AH-5510, with a defined mutant in the *lafTU* genes, and analyzed its abilities to swim and swarm. The AH-5510 (*lafTU*) mutant strain showed an 80% decrease in radial expansion on swarm agar plates, which is similar to that observed for *A. hydrophila* AH-3 structural lateral flagellum mutants previously (14). The swimming motility of the AH-5510 (*lafTU*) mutant was identical to that of wild-type *A. hydrophila* AH-3 (Table 3), and TEM showed both lateral and polar flagella (data not shown).

Since neither *lafTU* nor *pomAB* mutations affect *A. hydrophila* AH-3 swimming motility, we constructed an *A. hy-*

drophila strain mutant in both *lafTU* and *pomAB* genes (AH-4450). Mutant strain AH-4450 showed reduced swarming motility, similar to that of the AH-5510 (*lafTU*) mutant; however, its swimming motility was not affected, being similar to that observed for the *A. hydrophila* AH-4448 (*pomAB*) single mutant and AH-3 wild-type strain (Table 3). These results suggested that *lafTU*-encoded proteins are involved only in lateral flagellum motility and do not participate in polar flagellum rotation.

Identification of a new locus of polar flagellum motor in *A. hydrophila* AH-3. We performed a miniTn5Km-1 mutagenesis using the *A. hydrophila* AH-4448 mutant (AH-405Δ*pomAB*) as a recipient strain to find a second polar stator locus of *A. hydrophila* AH-3 that is involved in polar flagellum motility. Transconjugants were screened for greatly reduced or null swimming motility in swim agar and the inability to move in liquid media, as determined by light microscopy. Fifteen transposon insertion mutants unable to swim were analyzed by TEM after growth in liquid media. Only the *A. hydrophila* AH-4449 transposon insertion mutant was unable to swim (Table 3), but it did produce polar flagella. As no EcoRV restriction sites were present in the transposon, the altered-motility mutant was analyzed for the presence of the transposon by the Southern hybridization of EcoRV-digested chromosomal DNA. A single band was detected in the mutant, indicating that the mutant had a single copy of the minitransposon in its genome.

The DNA flanking the transposon in the *A. hydrophila* AH-4449 insertion mutant was cloned into pBCSK as described in Materials and Methods. The nucleotide sequencing of the cloned fragment revealed an open reading frame (ORF) whose predicted amino acid sequence shared homology with different sodium-driven flagellum motor proteins, such as the *V. parahaemolyticus* PomB-homologous protein, MotB, and PomB of *V. alginolyticus*, *V. cholerae*, and *Shewanella oneidensis*. Since a PomB protein already was described as a polar flagellum motor protein in *A. hydrophila* AH-3 polar flagellum region 3 (13), we named this new flagellum motor protein $PomB_2$.

Organization of *A. hydrophila* AH-3 polar flagellum region 6 ($pomA_2B_2$ loci). To amplify and sequence the *A. hydrophila* AH-3 genomic region that contains the $pomB_2$ gene, progressive inverse PCR with specific oligonucleotides was performed as described in Materials and Methods. The sequence analysis of 3,399 bp obtained from the amplified fragments revealed four complete ORFs. ORF2 to ORF4 are transcribed in the same direction, and ORF1 is transcribed in the opposite direction (Fig. 1). The start codon of ORF2 was located 221 bp upstream of ORF1, the ORF3 start was 5 bp downstream of ORF2, and ORF4 was separated from ORF3 by 145 bp. Sequence analysis in silico showed putative ribosome-binding sites upstream of each of the ORF start codons, a putative σ^{28} promoter sequence (TAAA-N14-GCCGATAA) upstream of the ORF2 start codon, and a transcriptional terminator rho-independent sequence downstream of ORF3 (Fig. 1).

The characteristics of the individual proteins and their protein homologies were analyzed using the BLASTP program (2) of the NCBI database and are shown in Table 4. ORF1 was homologous to the exonuclease VII small subunit of different bacteria, such as *Vibrio* spp. and *Shewanella* spp. (50 to 64% identity). ORF2 and ORF3 are related to polar flagellum stator proteins that are involved in the formation of a sodium-conducting channel to generate rotational motion in the sodium-type flagellum motor (25). The ORF2-derived amino acid sequence was homologous to the flagellum motor protein ASA-0993 of *A. salmonicida* and protein AHA-3318 of *A. hydrophila* ATCC7966^T (96 to 94% identity, respectively), as well as to the sodium-driven flagellum motor proteins MotA (a PomA homologue) of *V. parahaemolyticus* and PomA of *Vibrio* and *Shewanella* species (62 to 63% identity). ORF3 was homologous to the flagellum motor protein AHA-3317 of *A. hydrophila* ATCC7966 (94% identity), MotB of *V. parahaemolyticus* (a PomB homologue), and PomB of different *Vibrio* and *Shewanella* species (60 to 62% identity). The ORF4-derived amino acid sequence was homologous to the transcription elongation factor GreA from different bacteria, such as *Salmonella* spp. and *Yersinia* spp.

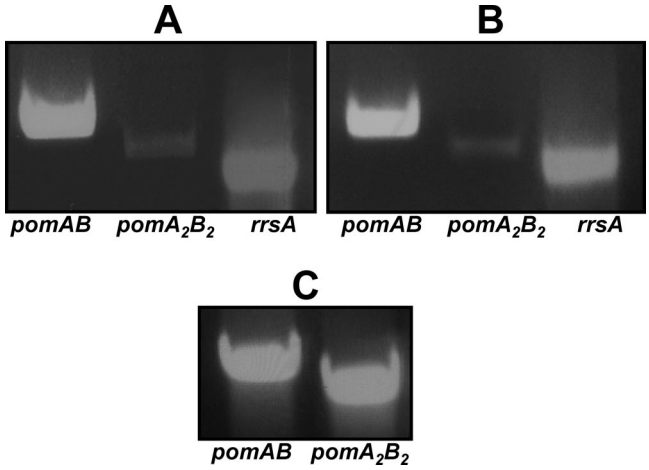


FIG. 3. RT-PCR cDNA fragments obtained from the *pomAB* (*pomA* internal fragment), $pomA_2B_2$ ($pomB_2$ internal fragment), and *rrsA* (ribosomal 16S internal fragment) genes of *A. hydrophila* AH-3 total RNA isolated when the strain was grown at 25°C in liquid (A) or solid medium (B). (C) PCR was performed with the *pomAB* and $pomA_2B_2$ genes using 100 μg of *A. hydrophila* AH-3 genomic DNA as the control primer.

Since *A. hydrophila* AH-3 PomA and PomB already have been described as two polar flagellum motor proteins encoded by genes located in the *A. hydrophila* AH-3 polar flagellum region 3 (13), we named these newly discovered polar flagellum stator proteins $PomA_2$ and $PomB_2$, respectively, and designated these polar flagellar loci region 6.

Distribution of the $pomA_2B_2$ and *pomAB* genes in mesophilic *Aeromonas* strains. The distribution of $pomA_2B_2$ and *pomAB* genes was analyzed in mesophilic *Aeromonas* strains ($n = 50$) by dot blot hybridization experiments against total genomic DNA using independent PCR probes. The distribution of these two sets of polar stator genes were performed using two PCR probes for *pomAB* and $pomA_2B_2$. These two probes hybridized to the chromosomal DNA of all mesophilic *Aeromonas* strains tested, whether the strains were able to produce lateral flagella or not.

Analysis of *A. hydrophila* $pomA_2B_2$ loci defined mutants and complementation studies. We constructed *A. hydrophila* AH-3 defined mutants in $pomB_2$ (AH-4470) and $pomA_2B_2$ (AH-4471), and we analyzed their ability to form polar and lateral flagella

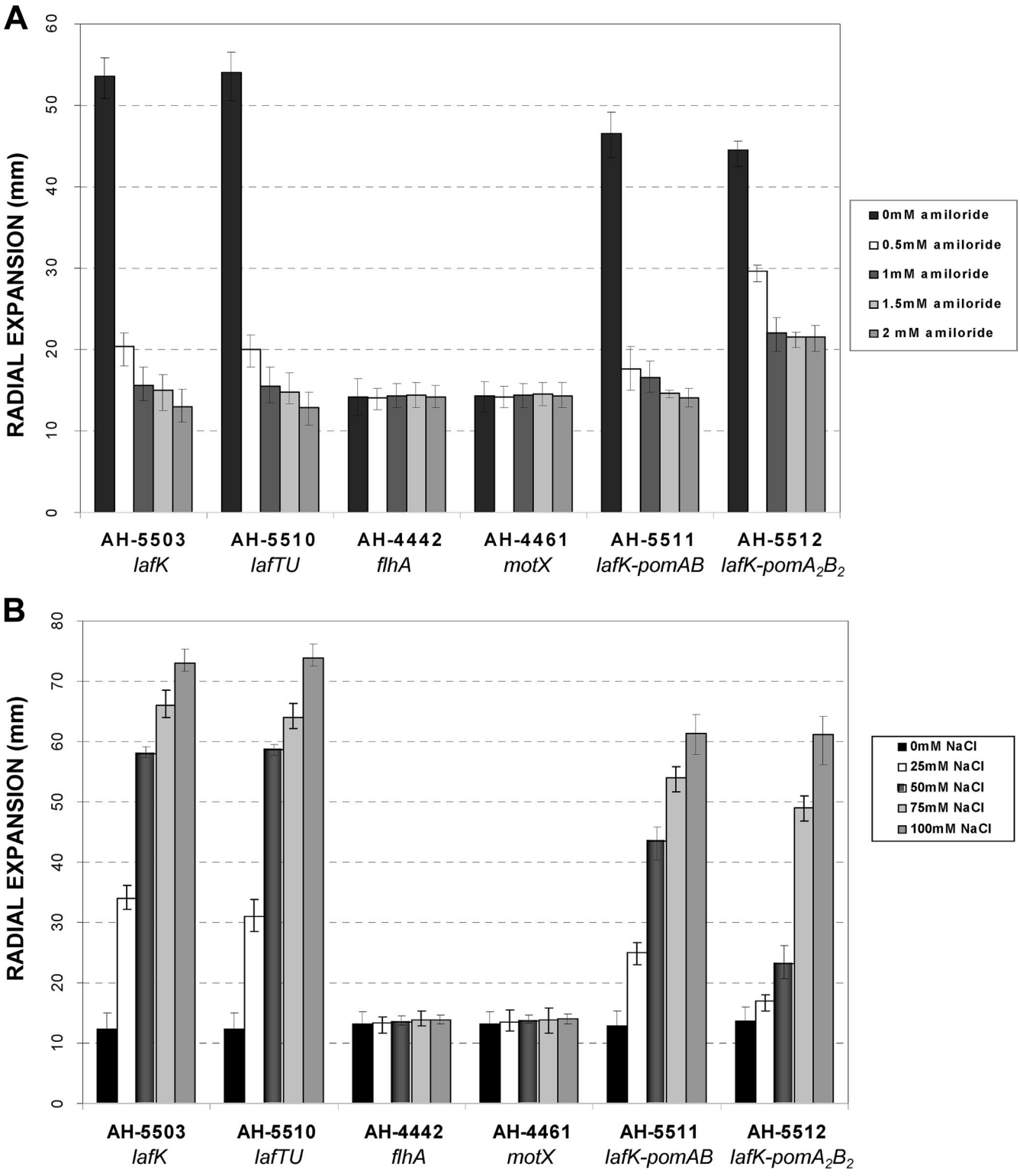


FIG. 4. Radial expansion at 25°C in swim agar plates containing no amiloride or 0.5, 1, 1.5, and 2 mM of amiloride (A) and containing no NaCl or 25, 50, 75, and 100 mM of NaCl (B) of *A. hydrophila* AH-3 structural flagellum mutants AH-5503 and AH-4442 (lateral and polar mutants, respectively), lateral stator mutant AH-5510 (*lafTU*), polar stator mutant AH-4461 (*motX*), and double mutants AH-5511 (*lafK-pomAB*) and AH-5512 (*lafK-pomA*$_2$*B*$_2$). Motility was determined by measuring the diameter (in millimeters) of the zone of expansion from the point of inoculation after 48 h of growth. The standard deviations were determined from five different experiments.

as well as their motility phenotype. TEM analyses showed that AH-4470 ($pomB_2$) and AH-4471 ($pomA_2B_2$) mutants are able to form both flagellum types (polar and lateral) (Fig. 2A). Motility assays showed that both mutants are able to swim in liquid media (examined by light microscopy) and swarm on swarming motility agar as well as wild-type *A. hydrophila* AH-3 (Table 3, Fig. 2B). These results are identical to the ones obtained for the *A. hydrophila pomB* (AH-4444) and *pomAB* (AH-4448) mutants.

Since *pomAB* or $pomA_2B_2$ disruptions do not abolish *A. hydrophila* AH-3 polar flagellum motility, we constructed mutants in both loci and analyzed their swimming and swarming phenotype. In order to disrupt the $pomB_2$ gene in either the *pomAB* or *pomB* mutant background (AH-4448 and AH-4444, respectively), we introduced the suicide plasmid pCM-$POMB_2$ into each one of these mutants. We also disrupted the $pomA_2B_2$ genes in the *pomAB* mutant strain (AH-4448) background by the introduction of the suicide plasmid pDM-$POMA_2B_2$ into this mutant strain. These three double mutants were constructed as described in Materials and Methods. Motility assays showed that AH-4473 (*pomAB*-A_2B_2), AH-4452 (*pomAB*-B_2), and AH-4472 (*pomB*-B_2) mutants were unable to swim (examined by light microscopy) and showed a decreased radial expansion (74% reduction) in swarm agar similar to that of the AH-4449 (*pomAB*-B_2) transposon insertion mutant rather than to that of wild-type *A. hydrophila* AH-3 (Table 3, Fig. 2B), since polar flagella contribute to the swarming motility.

Complementation studies were undertaken to determine if wild-type polar flagellum motility could be restored to the mutants by providing *pomAB* or $pomA_2B_2$ genes in *trans*. Plasmids pACYC-POMAB and pACYC-$POMA_2B_2$, containing the *A. hydrophila* AH-3 *pomAB* and $pomA_2B_2$ genes, respectively, were introduced independently by triparental mating into the AH-4473 (*pomAB*-A_2B_2), AH-4452 (*pomAB*-B_2), and AH-4472 (*pomB*-B_2) mutant strains. These three mutants were able to swim and swarm as well as the *A. hydrophila* AH-3 wild type when one of the recombinant plasmids was introduced into them (Fig. 2B).

Transcription of *pomAB* and $pomA_2B_2$. Previous studies demonstrated that *A. hydrophila* AH-3 *pomAB*, in polar flagellum region 3, constitute an independent transcriptional unit (13). To assure the cotranscription of genes in polar flagellum region 6, primer pairs that overlapped $pomA_2B_2$ and $pomB_2$-*orf4* were designed near the 3′ end of the upstream gene and near the 5′ end of the downstream gene. An RT-PCR product of the expected size was detected only for $pomA_2B_2$ amplification (Fig. 1). To analyze the level of transcription of *A. hydrophila* AH-3 *pomAB* and $pomA_2B_2$ in liquid and solid media at 25°C, semiquantitative RT-PCR assays were carried out as described in Materials and Methods. These assays show significantly higher levels of *pomAB* transcription than those of $pomA_2B_2$ genes in both media (Fig. 3), suggesting that *pomAB* is the prevalent polar flagellum stator.

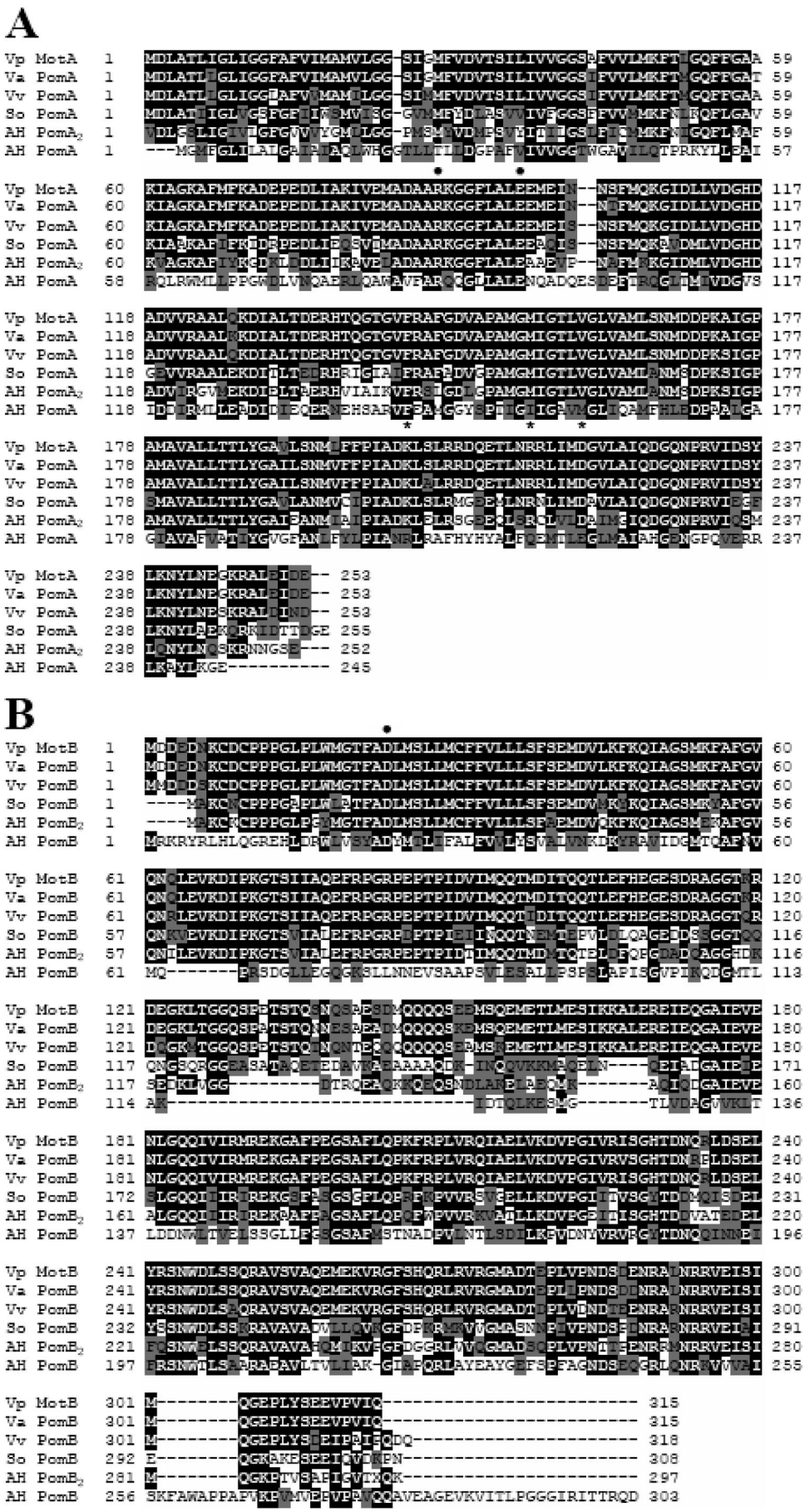
***A. hydrophila* polar flagellum energy source.** The structural lateral flagellum AH-5503 mutant (*lafK*), which was previously described (14), as well as the lateral flagellar stator motor mutant AH-5510 (*lafTU*) were used to determine if the *A. hydrophila* polar flagellum stator motor employed sodium ions as an energy source to rotate. We measured the radial expansion of these mutants after growth at 25°C in swim agar plates containing 0 to 2 mM amiloride dissolved in dimethyl sulfoxide, a potent inhibitor of Na^+ channels in different organisms (6), and plates containing 0 to 100 mM NaCl. In both mutants, the radial expansion decreased 63, 71, 73, and 76% in swim agar plates with 0.5, 1, 1.5, and 2 mM of amiloride, respectively (Fig. 4A). Both mutants showed a radial expansion of around 2.7, 4.7, 5.3, and 5.9 times higher in swim agar plates with 25, 50, 75, and 100 mM NaCl, respectively, than on the same kind of plates without NaCl (Fig. 4B). Swimming motility was completely inhibited in liquid media with 2 mM amiloride, and the amount of motile fraction increased when the NaCl concentration increased (Table 5). The motile fraction does not increase with different KCl concentrations (data not show). Furthermore, we measured the radial expansion of the structural polar flagellum mutant AH-4442 (*flhA*) as well as the polar stator *A. hydrophila motX* mutant (AH-4461), which was previously described (13), in swim and swarm agar plates containing 0 to 2 mM amiloride and plates containing 0 to 100 mM NaCl. These two mutants, which possess only functional lateral flagella, showed no difference in their radial expansion in the presence of amiloride or different concentrations of sodium ions (Fig. 4). These results suggest that only the polar flagellum motor is inhibited by the sodium channel inhibitor amiloride, and its rotation is sodium ion dependent.

TABLE 5. Effect of amiloride and NaCl in liquid media

Substance and concn. (mM)	Motile fraction (motile cells/total no. of cells) of mutant:			
	AH-5503 (*lafK*)	AH-5510 (*lafTU*)	AH-5511 (*lafK-pomAB*)	AH-5512 (*lafK-pomA*$_2$*B*$_2$*2*)
Amiloride				
0	0.66	0.65	0.67	0.64
0.5	0.58	0.56	0.58	0.6
1	0.42	0.44	0.39	0.47
1.5	0.29	0.30	0.28	0.35
2	0.03	0.05	0.03	0.05
NaCl				
0	0.06	0.03	0.05	0.08
25	0.42	0.46	0.44	0.35
50	0.65	0.67	0.62	0.51
75	0.69	0.70	0.68	0.61
100	0.78	0.77	0.76	0.75

[a] The motile fraction was determined by counting approximately the number of motile cells out of the total number of cells according to Fukuoka et al. (19).

Since both flagellum types contribute to motility in viscous media and in order to independently analyze the *pomAB* and $pomA_2B_2$ energy source, we constructed double mutants that were mutated in lateral flagellum system genes (*lafK*) and the polar flagellum stator motor genes *pomAB* and $pomA_2B_2$, as described above. Double mutant strains AH-5511 (*lafK-pomAB*) and AH-5512 (*lafK*-$pomA_2B_2$) were confirmed by PCR. Motility assays of the *A. hydrophila* AH-5511 (*lafK-pomAB*) mutant showed 63, 65, 69, and 70% radial expansion reduction, and the AH-5512 (*lafK*-$pomA_2B_2$) mutant showed 34, 51, 52, and 52% radial expansion reduction in swimming agar containing 0.5, 1, 1.5, and 2 mM amiloride, respectively (Fig. 4A). Both mutants also showed an increase in radial expansion corresponding to an increase in sodium ion concentrations. The radial expansion of mutant AH-5511 (*lafK-*



pomAB) was 1.9, 3.4, 4.2, and 4.8 times higher and that of mutant AH-5512 (*lafK-pomA*$_2$*B*$_2$) was 1.2, 1.7, 3.6, and 4.5 times higher in swim agar with 25, 50, 75, and 100 mM NaCl, respectively, than those for the mutants in swim agar without NaCl (Fig. 4B). Furthermore, we analyzed the ability of these two mutants (AH-5511 and AH-5512) as well as the lateral flagellum mutant AH-5503 to move in swarm agar plates, and all of them showed around 0.5 to 0.6 cm radial expansion (Table 3).

DISCUSSION

Mesophilic *Aeromonas* species have a constitutive single polar flagellum that propels the bacterium in aqueous environments and contributes to adhesion and biofilm formation (13, 27). Recently, five *A. hydrophila* AH-3 chromosomal regions involved in the formation and function of the polar flagellum have been described; two of them (regions 3 and 4) contain genes whose encoded proteins show high homology to different polar flagellum stator motor proteins (13). The *A. hydrophila* polar flagellar region 3 contains the *pomA* and *pomB* genes, which encode proteins that are homologous to *Pseudomonas* MotA and MotB, respectively (15), and region 4 contains *motX*, which encodes a protein homologous to the sodium-driven motor MotX of *V. parahaemolyticus* (33). MotX is involved in *A. hydrophila* polar flagellum rotation, because its mutation abolishes polar flagellum motility. Nevertheless, a PomB (13) or PomAB mutation does not abolish polar flagellum motility in liquid media, which is similar to the result observed in *Pseudomonas aeruginosa* MotAB mutants (18, 47) and in contrast to those for *V. cholerae*, *V. alginolyticus*, and *V. parahaemolyticus* PomAB mutants (11, 21).

These results suggested two possibilities about *A. hydrophila* AH-3 polar flagellum motility: the lateral flagellum stator LafTU can supply PomAB function, and *A. hydrophila* AH-3 has a second polar stator that is involved in polar flagellum motility. To explore the first possibility, we constructed a defined *A. hydrophila* AH-3 mutant in *lafTU* and a double mutant in both *lafTU* and *pomAB*. Analyses of these mutants showed that both have lateral and polar flagella, are able to swim in liquid media, and show an 80% decrease in radial expansion in swarm agar plates compared to results for the wild type at 25°C (Table 3). The fact that this double mutant does not abolish polar flagellum motility suggests the second possibility.

To investigate this hypothesis, we performed transposon insertion mutagenesis using the *A. hydrophila* AH-405Δ*pomAB* mutant as the recipient strain and isolated the mutant AH-4449, which is unable to swim in liquid media but still is able to produce polar flagella. The mutant allowed us to find *pomA*$_2$ and *pomB*$_2$. In contrast to the previously described *A. hydrophila* AH-3 *pomAB* loci, *pomA*$_2$*B*$_2$ loci are located in a separate chromosomal region, as described for the *V. parahaemolyticus* sodium-type flagellum stator genes *motAB* (34). However, *P. aeruginosa* also has shown redundant polar flagellar stator genes, *motAB* and *motCD* (18), whose chromosomal distribution is similar to those of *A. hydrophila pomAB* and *pomA*$_2$*B*$_2$, respectively. Also, *A. hydrophila* ATCC7966^T has *pomA*$_2$*B*$_2$ genes homologous to AHA-3318 and AH-3317 in a chromosomal location identical to that for *A. hydrophila* AH-3. Sequence alignments showed that *A. hydrophila* PomA$_2$ and PomB$_2$ display higher homology with sodium-conducting polar flagellum stator motors than do *A. hydrophila* AH-3 PomA and PomB (Fig. 5). Analyses of charged residues showed that *E. coli* MotA-charged residues R90 and E98, involved in torque generation, are conserved in *A. hydrophila* PomA$_2$ and PomA, as well as in *Vibrio* spp. PomA (MotA) as residues R88 and E96. The C-terminal region of *A. hydrophila* PomA$_2$ and of *Vibrio* spp. PomA show three important charged residues (K203, R215, and D220) that are essential for *Vibrio* sodium-dependent motor function (39) that are not present in either *E. coli* MotA or in *A. hydrophila* PomA. The D32 *E. coli* MotB residue and D24 *Vibrio* spp. PomB (MotB) residue, which play critical roles in ion flux and energy conversion, are conserved in *A. hydrophila* PomB$_2$ (D20) and PomB (D24). Furthermore, the analysis of different mutants with single and multiple lesions in *pomAB* and/or *pomA*$_2$*B*$_2$ suggest that *A. hydrophila* PomAB and PomA$_2$B$_2$ are able to compensate for the loss of each other and are a redundant set of proteins that do not have differential motility roles, and the single loss of PomAB or PomA$_2$B$_2$ is not essential in polar flagellum motility in aqueous or in viscous environments. In contrast, in *Pseudomonas aeruginosa* either stator (MotAB or MotCD) is sufficient for swimming, but both are necessary for swarming motility (47). Semiquantitative RT-PCR assays of *A. hydrophila pomAB* and *pomA*$_2$*B*$_2$ after growth in liquid media and on solid plates showed a transcription level of *pomAB* genes at least 10 times higher than that of *pomA*$_2$*B*$_2$ genes in both media. These results suggest that although both sets of proteins are involved in polar flagellum motility, *pomAB* is prevalent in liquid and viscous media.

Swimming motility assays in the presence of different amiloride concentrations, as well as in different NaCl concentrations, indicated that only the *A. hydrophila* polar flagellum stator is sodium dependent, whereas the lateral flagellar stator is not. To analyze the *pomAB* and *pomA*$_2$*B*$_2$ energy source independently, we performed amiloride swimming inhibition assays with AH-5511 (*lafK-pomAB*) and AH-5512 (*lafK-pomA*$_2$*B*$_2$), as well as swimming assays with different NaCl concentrations. These assays showed that the radial expansion of the lateral flagellum mutant (*lafK*) without amiloride or with 25 to 100 mM NaCl is higher than the radial expansion of the

FIG. 5. (A) Sequence alignment of a *Vibrio parahaemolyticus* PomA homologue (Vp MotA), *Vibrio alginolyticus* PomA (Va PomA), *Vibrio vulnificus* PomA (Vv PomA), *Shewanella oneidensis* PomA (So PomA), *A. hydrophila* AH-3 PomA$_2$ (AH PomA$_2$), and PomA (AH PomA). (B) Sequence alignment of a *V. parahaemolyticus* PomB homologue (Vp MotB), *V. alginolyticus* PomB (Va PomB), *V. vulnificus* PomB (Vv PomB), *S. oneidensis* PomB (So PomB), and *A. hydrophila* AH-3 PomB$_2$ (AH PomB$_2$) and PomB (AH PomB). The deduced amino acid sequences were aligned using Clustal W. White letters with a black background indicate identical amino acid residues, and black letters with a gray background indicate similar amino acid residues. The black circles over the residues show conserved charged residues in proton and sodium motors, and the asterisks show charged residues essential in *Vibrio* sodium-dependent motor function.

lafK-pomAB or *lafK-pomA*$_2$*B*$_2$ polar flagellum stator mutant, suggesting that both polar flagellum stators contribute to motility in viscous media, as is the case with the swim agar plates. Furthermore, both polar flagellum stator mutants showed a reduction in radial expansion when grown in the presence of amiloride, as well as increased radial expansion when the sodium ion concentration was increased in the medium. However, the AH-5512 (*lafK-pomA*$_2$*B*$_2$) mutant responds to sodium at a higher concentration than the AH-5511 (*lafK-pomAB*) mutant. These results lead us to the conclusion that PomAB and PomA$_2$B$_2$ constitute redundant polar flagellum stators that are sodium ion dependent and are used for swimming motility only, but PomA$_2$B$_2$ is more sensitive to low-level sodium ion variations than PomAB, and PomAB seems to be specialized to function at higher sodium levels.

Both *A. hydrophila* PomB and PomB$_2$ possess one transmembrane domain at their N termini and an OmpA domain at their C-terminal sequences that probably are involved in peptidoglycan interaction (17) and may be responsible for anchoring the force generator. *A. hydrophila* PomA and PomA$_2$ possess four transmembrane domains and the flagellar motor protein MotA family signature A-[LMF]-x-[GAT]-T-[LIVMF]-x-G-x-[LIVMF]-x(7)-P. A similar situation is described for *P. aeruginosa*, because there are two sets of *motAB*-like genes, *motAB* and *motCD*, as well as another gene, *motY*, which contributes to proton-driven flagellum motility. The loss of either *motAB*-like gene still resulted in motile bacteria in aqueous environments, *motCD* disruption abolished polar flagellum motility in viscous environments (15% Ficoll), and only mutations of both sets of genes abrogated polar flagellum motility in aqueous solutions (47). In *Pseudomonas*, MotCD is essential for the polar flagellum proton motor rotation in viscous media, but in *Aeromonas*, for which polar flagellum stator motor is sodium dependent, neither PomAB nor PomA$_2$B$_2$ are essential for polar flagellum rotation in viscous media. Most aeromonads have an entirely distinct lateral flagellum system for motility in highly viscous media or over surfaces.

The *A. hydrophila* polar flagellum is the first case in which two redundant sodium-driven stator motor proteins (PomAB and PomA$_2$B$_2$) are found. The redundancy is based on the fact that neither set on its own is essential for polar flagellum motility in either aqueous or high-viscosity environments.

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